

Institute for Marine and Antarctic Studies  
University of Tasmania

**Submission**  
**to the**  
**Legislative Council Government Administration**  
**Committee 'A' Inquiry into Fin Fish Farming in**  
**Tasmania**

**Contributors**  
Assoc Prof Catriona Macleod  
Dr Jeff Ross  
Prof Barbara Nowak  
Prof Chris Carter  
Prof Caleb Gardner  
Flora Bush

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## **INTRODUCTION**

This submission has been prepared by the University of Tasmania's Institute for Marine and Antarctic Studies (IMAS) in response to a request from the Legislative Council Government Administration Committee 'A' for information to address the following Terms of Reference:

*To inquire into and report on the planning, assessment, operation and regulation of finfish farming in Tasmania, with particular reference to:*

*(1) The implementation of the Sustainable Industry Growth Plan for the Salmon Industry and its impact on commercial finfish farming operations and local communities, including:*

*a. data collection and publication;*

*b. progress in the development of an industry wide biosecurity plan;*

*(2) Application of the Marine Farming Planning Act 1995 relating to:*

*a. preparation and approval process for marine farming development plans, including modifications and amendments to marine farming development plans;*

*b. allocation of leases, applications for and granting of leases;*

*c. management of finfish farming operations with respect to the prevention of environmental harm;*

*(3) Any other matter incidental thereto:*

This submission identifies the key research initiatives (past, present and proposed) undertaken by IMAS relevant to the issues identified in the Terms of Reference for this inquiry. We have sought to provide comment where appropriate on the value of IMAS research to the application of the *Marine Farming Planning Act 1995*, and how IMAS research relates to development and expectations of the Sustainable Industry Growth Plan for the Salmon Industry.

The submission also provides, as an Appendix, a comprehensive review of research undertaken over the last two decades by the University of Tasmania's Institute of Marine and Antarctic Studies and its predecessors, commonly and in collaboration with other organisations, to support sustainable production and biosecurity practices, and to inform management and monitoring of salmon farming in Tasmania.

Response to Terms of Reference:

***The implementation of the Sustainable Industry Growth Plan for the Salmon Industry and its impact on commercial finfish farming operations and local communities, including:***

***a. data collection and publication;***

The Sustainable Industry Growth Plan clearly identifies a requirement for the “*establishment of an independent web portal that will be hosted by the Institute for Marine and Antarctic Studies. The website will provide access to all environmental data and to as much production information as possible, subject only to not revealing genuine “commercial in confidence” information*”. There has been considerable discussion between industry and government regarding just what this portal might look like, and what data it should contain, as a result DPIPWE has determined that they will host a website to provide access to all regulatory compliance data, and that is currently under construction.

IMAS has a data management policy that applies to all research staff and higher degree students. This policy reflects the Australian code for responsible research conduct, and seeks, as much as possible, to ensure open data access (such that data should be easily findable, accessible, interoperable and reusable). IMAS seeks to make all of our research available to the broader research community through publication of results in peer reviewed scientific literature. We also strive to make our research findings available to broader interest groups (i.e. government, industry and the broader community) through a range of alternative publication and communication mechanisms (i.e. government advice notes, workshops and public lectures and funding agency reports) and by posting these reports (<https://www.imas.utas.edu.au/research/fisheries-and-aquaculture/publications-and-resources>), details of our research (<https://www.imas.utas.edu.au/research/fisheries-and-aquaculture/aquaculture/atlantic-salmon-aquaculture-research>) and data (<https://www.imas.utas.edu.au/data>) on our website (<https://www.imas.utas.edu.au/>). Where data have been obtained through collaboration then the resultant data and summary information can be made available on each organisation’s website, either as a report or via relevant data links. Consequently, IMAS data may also be found in reports listed on funding agency websites (e.g. FRDC

<https://www.frdc.com.au/research/final-reports>) or on research partner's websites (e.g. DPIPWE <https://dpiuwe.tas.gov.au/sea-fishing-aquaculture/marine-farming-aquaculture/marine-farming-reports-publications>, EPA, <https://epa.tas.gov.au/regulation/salmon-aquaculture>, CSIRO <https://research.csiro.au/aquaculture/> and <https://research.csiro.au/cem/>). It is important to note that some information may have limitations on access as a result of commercial confidentiality requirements.

There is a wealth of data available through the University of Tasmania. These data, where held by the University of Tasmania, are available so long as data requests meet relevant intellectual property, and ethical standards and expectations.

Finally, with respect to data collection and publication, a key issue for IMAS is the increasing requirement for a rapid turnaround in analysis and reporting, or additional interim reporting. Whilst we acknowledge that this may be informative, particularly where there is some urgency to decision making or public interest, it is important to understand the risks in doing this. There is real potential for analysis and interpretation of an incomplete dataset to be inaccurate. Research programs are purposefully designed with temporal replication required to capture the full variability in the data, hence caution needs to be taken when analysing a sub-set of the data.

***b. progress in the development of an industry wide biosecurity plan:***

The Sustainable Industry Growth Plan for the Salmon Industry identifies a number of priority actions intended to **improve the efficiency, effectiveness and transparency of the industry's environmental regulation, and the effectiveness of its biosecurity systems**. Several of these priority actions relate specifically to research areas where IMAS has provided, or is currently providing, research data and analysis.

We have outlined below the main initiatives that IMAS is involved with in the biosecurity area going forward and have attempted to summarise the key research activities in this space to date.

***Relevant Priority Actions:***

*"Analysis of existing marine farming development plan areas used for salmon*

*farming, particularly with a view to strengthening biosecurity” and “Development of an industry-wide Biosecurity Program that can be given effect through the proposed new Biosecurity Act”*

*“Continuing strong support for the State’s advanced fish health and biosecurity facilities”*

A key focus in the Sustainable Industry Growth Plan is the development of a Biosecurity Blueprint that provides a common understanding of industry compliance and regulatory obligations and area management controls to ensure effective management of both current and emerging biosecurity threats. Biosecurity is a significant threat with the potential to decimate production and prevent industry growth. Consequently, the industry in collaboration with the Tasmanian Government committed to “Develop an industry-wide Biosecurity Program” in early 2018. IMAS remains ready to support any emerging research needs for the salmon industry, and remains actively involved in salmon health research, research on fish nutrition and performance, and research to address welfare and wildlife interaction issues.

IMAS has provided targeted research support to the Tasmanian salmon industry in the areas of fish health, welfare and biosecurity for over 25 years. IMAS researchers have collaborated with industry and government scientists to address key salmon health and welfare issues (e.g. around amoebic gill disease (AGD), yersiniosis and seals in particular) and support a range of fish health improvement strategies, including; identification of potential pathogens (e.g. from Nowak and Clark (1999) to Johnson-MacKinnon et al. (2019) – AGD; from Costa et al. (2011) to Ghosh et al. (2018) - *Yersinia ruckerii*; and from Zainathan et al. (2013) to Zainathan et al. (2017) - Aquareovirus), investigation of the efficacy and administration protocols of various therapeutic treatments, and development and administration of vaccines.

A significant line of research addresses issues in fish growth performance through two lines of enquiry into nutrition as well as impacts on reproductive physiology and premature maturation. Feed development for Tasmanian conditions through more effective supply of required nutrients (e.g. Hauler and Carter (2001); Carter et al. (2002b)) and ingredient selection for suitable protein, oil and mineral sources (e.g. Carter (1998); Carter and Hauler (2000); Carter et al. (2002a); Bransden et al. (2003); Amoroso et al. (2016)). IMAS conducted some of the first research globally

on the interactions between nutrition and negative environmental effects caused by climate change (e.g. Miller et al. (2006); Carter et al. (2008), (2010); to Nuez-Ortin et al. (2018)), this work was extended to consider impacts on product quality such as fatty acid profile (Bransden et al. 2003) and colour (Grunenwald et al. 2019). Collaborative research with industry and later CSIRO established underlying endocrinology and solutions around several key production cycle bottle necks: premature maturation and led to the use of lighting systems still in use (e.g. Wilkinson et al. (2008)); more effective management of fertile males and females (e.g. Pankhurst et al. 2011). Higher water temperatures lead to decreased oxygen concentrations and detailed investigation of respiratory physiology has been done in various ways (e.g. Katersky Barnes et al. (2012); Stehfest et al. (2017)) and provided support to the whole industry via the selective breeding program (e.g. Barnes et al. (2011); Experimental Aquaculture Facility 2018-19 unpublished).

IMAS was a founding partner and integral participant in the 2018 Global Salmon Symposium (GSS) (Carter et al. 2019) and helped to co-ordinate research discussions throughout the meeting. At this symposium biosecurity was one of three critical issues which were discussed as having the potential to make or break the industry. A number of recommendations for improved biosecurity practices were identified as part of the GSS:

- Single cohort stocking and fallowing periods between cohorts;
- Fallowing protocols for the pens, sites and regions and including regulation of protocols;
- Spacing of farms with guidelines drawn from international experience, for example 5 km minimum;
- Single owners within region (i.e. company separation) or ensuring stocking is coordinated between different companies in shared region;
- Mortality removal and disposal protocols;
- Biosecurity training, including regular refreshers and updates, for all staff;
- Tracking and management of any movement between pens or sites including fish, vessels, equipment, people; and
- Management of ecological interactions with such possible vectors in seals and wild fish.



We feel that these quite clearly align with the stated aims of the Sustainable Industry Growth Plan, and as such advancement of knowledge in any area would inform management practices.

Although IMAS has not been formally involved in developing the Biosecurity Blueprint, the summary of GSS discussions collated by IMAS has played a key role in generating the commitment to that initiative.

The Atlantic salmon industry has been affected by a range of infectious diseases worldwide, many still absent from Australia. The international experience based on outbreaks of those diseases suggests that single management areas are most effective for biosecurity. Allocation of leases to more than one company in a farming area will require high level cooperation between companies, including open communication and timely exchange of information. Use of enclosed transport, for example wellboats to transfer fish between sites significantly improves biosecurity. Awareness of new pathogens, fast development of diagnostic tests and vaccines support the industry sustainability but cannot replace biosecure farm management practices.

Finally, it is worth noting that IMAS, through the Experimental Aquaculture Facility (EAF), has access to state of the art research facilities with the capacity to undertake targeted biosecurity research on a range of fish sizes. In a unique model the EAF is a partnership with two industry partners, Huon Aquaculture and Skretting Australia (aquafeed maker) which ensure rapid industry uptake of research findings. The EAF capability has an established record of successfully growth large salmon at industry equivalent growth rates and therefore provides Tasmania with some of the best research support in the world, and we would hope to collaborate with government and industry to address some of the key research issues identified in the Sustainable Industry Growth Plan. For example, the potential to increase the use of Tasmanian grown ingredients in salmon aquafeeds can be done at the EAF. With further modification the EAF can be used to further understand climate change impacts on water quality by quantifying the combined influences of sub-optimum elevated temperature and sub-optimum decreased dissolved oxygen. Research on amoebic gill disease can also be done in the EAF and in conjunction with these sub-optimum environmental conditions. IMAS facilities, including the EAF, can be modified to

explore the effects of moving salmon farming off-shore by greater focus on swimming, respiration and energy expenditure.

**2. Application of the Marine Farming Planning Act 1995 relating to:**

**a. preparation and approval process for marine farming development plans, including modifications and amendments to marine farming development plans;**

The *Marine Farming Planning Act 1995* requires the preparation of an environmental impact statement (EIS) in order to assess the suitability of proposed sites for marine farming activities in Tasmania. A similar statement is also required for modifications to existing sites. The EIS contains a range of production and environmental information including a benthic suitability assessment which contains details of the extent, depth and nature of the substrate within the proposed farming zone. It also requires environmental data on substrate type, habitat distribution, bathymetry, benthic flora and fauna to be collected, including an evaluation of the likelihood of interactions with any threatened species. IMAS has been instrumental in determining what factors should be considered in these assessments, with the requirements based on early research and baseline assessments (i.e. Barrett et al. (2001); Crawford et al. (2002); Macleod et al. (2002, 2004)).

IMAS has played a key role, through the Sustainable Marine Research Collaboration Agreement (SMRCA) with state government, in undertaking these “zone” assessments (details of these reports can be found at the end of Appendix 1). However, in recent years these environmental assessments have been conducted by proponents using specific guidelines and sampling protocols developed by the planning authority (DPIPWE) in conjunction with IMAS researchers and based on IMAS research and experience.

**b. allocation of leases, applications for and granting of leases;**

IMAS is not directly involved in the allocation of leases or in the lease application process. However, IMAS has on occasion been asked to provide advice on the information contained in environmental impact statements (EISs) or to obtain

additional data or undertake research on areas of uncertainty. This is provided for under the SMRCA.

The SMRCA is a collaborative agreement between the Tasmanian Government and the University of Tasmania (University). The SMRCA provides a mechanism for IMAS, as part of the University, to provide world class research to support the effective and sustainable management of Tasmania's living marine resources to the benefit of all Tasmanians. The SMRCA enables the government (often, but not exclusively the Department of Primary Industry, Parks, Water and Environment) to request research to support fisheries, aquaculture, and estuarine and coastal marine environmental needs; to encourage and support new industry, promote Tasmania's advantages, and ensure that Tasmania's natural resources are managed in a sustainable way now and for future generations.

The SMRCA fishery and aquaculture research program is one of Australia's largest and most successful, with activities worth more than \$10 million per annum. This comprises:

- \$5 million funded jointly by the Tasmanian State Government and the University;
- an additional \$5 million leveraged through externally funded research projects;
- a significant number of research higher degree projects; and
- research outputs that contribute to UTAS attainment of world class research status in fisheries science.

The SMRCA strategic plan (2017-21

[https://www.imas.utas.edu.au/\\_data/assets/pdf\\_file/0017/1095200/V10HighQualPrintPrintSpreads-low-res.pdf](https://www.imas.utas.edu.au/_data/assets/pdf_file/0017/1095200/V10HighQualPrintPrintSpreads-low-res.pdf)) identifies several priority research areas that closely

align with the objectives of the Sustainable Industry Growth Plan for the salmon industry, including research to support:

- sustainable development of the Tasmanian salmonid farming industry;
- management and stewardship of salmon aquaculture;

- evaluation of biosecurity risks.

In addition, IMAS has identified a number of other areas where it can provide targeted research to enhance the SMRCA objectives and further align with the Sustainable Industry Growth Plan by improving understanding of:

- environmental ecosystem changes in the coastal environment;
- temperate marine environments, their resources, and their roles in the global climate system;
- marine mammal interactions

and supporting collaborations, networking and researcher training that serves the needs of industry, government and the community.

The research undertaken through the SMRCA provides the environmental understanding to inform the assessment requirements for both the development planning and lease allocation processes. This research is outlined in detail in Appendix 1 (See section on Environmental Interactions and Management).

***c. management of finfish farming operations with respect to the prevention of environmental harm;***

Prevention of environmental harm requires a process that can predict, evaluate and respond to potential risks and threats. The Tasmanian Government's environmental monitoring and management protocols are informed by a significant body of research undertaken at UTAS/ IMAS over the last 20 years. This research has improved our understanding of the interactions between salmon farming and the environment, identifying risks and providing risk-appropriate monitoring and management strategies. The resultant research has provided baseline data and system understanding that has informed industry and government management and monitoring strategies and supports effective regulation. The research has established indicators of gradation in level of impact and also monitoring criteria for local scale organic enrichment associated with salmon farming. The Marine Farming environmental monitoring program is well established, and the environmental management protocols developed with respect to localised benthic impacts have

been shown through review to be consistent with current best practice in aquaculture management both nationally and internationally (Woods et al. (2004), Keeley et al. (2014)).

However, adaptive management requires that monitoring and assessment approaches be reviewed at regular intervals: new technology may become available, and farming practices or background environmental conditions may change, as may community expectations. In the initial studies local scale benthic impacts were the key concern, and the research was focused on developing on-farm management controls. Concern then shifted to broadscale effects of dissolved wastes and research was undertaken to characterise the risks and develop a Broadscale Environmental Monitoring Program (BEMP) for the Huon Estuary and D'Entrecasteaux Channel. The BEMP clearly reflects the transition in our understanding of the interactions of marine farming (and therefore monitoring requirements) from a need to understand local-scale impacts, to a need to define broader-scale impacts, to the current situation whereby ecosystem interactions and multiple-use management are now the focus. The clearly stated intention of the BEMP was to provide *“a monitoring program with the capacity to detect the effects of those processes judged to be most threatening to the .... ecosystem at the whole-of-ecosystem level .... to provide knowledge of how well the ecosystem is functioning with an increased nutrient load and to allow any significant temporal trend(s) in ecological indicators to be detected”*. The key focus of the BEMP was on water-column and benthic effects on soft sediments within the system, the primary concern for this program being the potential for eutrophication. This need to understand how the broader ecosystem accommodates the additional nutrient load resulting from aquaculture inputs has seen broadscale environmental monitoring introduced as a system-wide monitoring requirement within the regulatory adaptive management framework. The BEMP has since been highlighted as world's best practice; with few countries having anything comparable. It provides an important and highly reliable body of information on the conditions associated with salmon farming that has been independently authenticated and can therefore be used by regulators, industry, and other stakeholders to assess ecological condition and to support adaptive management strategies. This research is summarised in Appendix 1.

IMAS has remained engaged with research to review monitoring and management practices for salmon aquaculture as the industry has grown, and as concerns have changed. IMAS research continues to work alongside the management processes, with research focus developing in response to evolving information needs and concerns to ensure that monitoring and management practices remain “fit for purpose”. The possibility of adverse interactions between salmonid aquaculture and reef systems has been highlighted as an area of concern that is not specifically addressed by the BEMP or other existing monitoring. It is clear from discussions with different resource users that the perception of risk as a result of increased marine farming activities differs regionally. In some areas the critical issue is whether there may be adverse effects on reef health (i.e. off-site interactions) as a result of increased aquaculture activities, whilst in other areas the key concern is whether the current on-farm monitoring and local scale impact indicators do actually support sustainable management by providing an accurate understanding of sediment conditions. The research project (FRDC 2015-024 Managing ecosystem interactions across differing environments: building flexibility and risk assurance into environmental management strategies) was established to specifically determine whether the current on-farm monitoring and local scale impact indicators are “fit for purpose” in new farming regions and under contemporary farming practices. This study also looked at developing techniques that can be used to better assess and monitor interactions between salmon farming and reef communities. This research is being undertaken in collaboration with industry, regulators and other stakeholders, specifically to inform and improve existing monitoring and ecosystem based management processes. Whilst the final report for this project is still being prepared, the preliminary findings suggest that:

- current local scale monitoring practices are still “fit for purpose” albeit with the need for regional context and interpretation;
- basic hydrodynamic and connectivity modelling can provide very a useful indication of the environmental footprint of dissolved nutrients and inform risk profiles;
- there is potential for dissolved nutrients to influence the ecology of nearby reef communities, but modelling in conjunction with an understanding of the reef sensitivity and uniqueness can provide an evaluation of the actual risk level;

- reef monitoring may be warranted where modelling indicates sufficient risk;
- for maximum benefit, reef monitoring should be embedded in a program which includes baseline and targeted biodiversity assessment projects;
- there are some relatively simple indicators of reef health and sensitivity that can be incorporated into a monitoring program; and
- these recommendations will be provided to industry and government to be incorporated into the existing monitoring and adaptive management framework.

Current management and regulation require that operations be governed by the requirements of the *Living Marine Resources Management Act 1995* and the *Marine Farming Planning Act 1995*. The implementation of the management expectations of this legislation is undertaken through a combination of marine farming development plans, leases and licences, with both development plans and licences having provision for specific regulatory and management requirements.

The adaptive management framework does allow for individual licences to include specific monitoring and reporting requirements. It is also possible to seek amendments to individual development plans to address specific issues of concern; this has been done in relation to the monitoring and management of dissolved nutrients in other Plan areas. Consequently, by amending the development plan and introducing specific licence requirements for this lease to include provision for a more recent baseline and additional broadscale water quality and reef interaction monitoring, it should be possible to address the key environmental management concerns.

It is worth noting that the scientific understanding outlined in this document represents two decades of accumulated knowledge and that this has been developed through a broad range of research collaborations both with other research providers (notably CSIRO) and in collaboration with industry, government, various not for profit organisations, funding agencies (particularly FRDC, Natural Heritage Trust, National Resource Management, various CRCs), and the community. Whilst the research has progressed incrementally, with each question answered leading quite naturally to further questions, there will inevitably be gaps in our understanding,

and the need to address these will depend on the level of risk and concern associated with each particular issue. In seeking to develop farming in new areas the regulatory context needs to be open and responsive to concerns and prepared to be informed by the science as it evolves. IMAS will continue to support all parties to better understand the issue and to find effective management solutions.

There have been some significant environmental management challenges for government with respect to the salmon industry, most notably in Macquarie Harbour. IMAS has been a key collaborator in a major research program in Macquarie Harbour supported by the FRDC. The Macquarie Harbour research program is a collaboration between IMAS, CSIRO, industry and a range of Tasmanian state government agencies, but particularly the Environmental Protection Agency (EPA). The resultant research has provided significant understanding of the hydrodynamics and ecological interactions in this unique ecosystem. The findings, outlined in a series of IMAS reports (e.g. Ross et al. 2015, Ross et al. 2016, Ross & Macleod 2017b, Ross & Macleod 2017a, Ross et al. 2017, Ross & Macleod 2018b, c, Ross & Macleod 2018a, Ross et al. 2018a, Ross et al. 2018b, Ross et al. 2019), have underpinned ongoing management decisions regarding salmon farming and the potential recovery of this important water body. Macquarie Harbour has highlighted that different water bodies can have different issues and sensitivities, and that as a result research (and monitoring) in each system need to be undertaken at the level most appropriate to address the specific issues and concerns.

There are many potential interactions of salmon farming with nearby wildlife and ecosystems. As noted above some of these are being investigated through reef interaction studies, where a risk-based assessment approach is being used to identify priority interactions and develop a tiered monitoring approach. Issues with threatened and endangered species are currently addressed through zone assessments and appropriate site selection, with specific risk assessments undertaken as required. In Macquarie Harbour, interactions between farming and the endangered Maugean Skate have been assessed (Bell et al., 2016, Weltz et al. 2017, 2018). Preliminary results suggest that there are no direct interactions between farming and the endangered Maugean Skate and whilst the indirect effects of low dissolved oxygen levels may have limited impact on juveniles and adults, the situation for the well-being of the skate eggs is less certain.



In summary, research to support management of finfish farming operations with respect to the prevention of environmental harm is inherently part of the adaptive management process; with targeted research developed as required to address specific issues and concerns.

### Future IMAS Research Relevant to The Sustainable Industry Growth Plan for the Salmon Industry

The Sustainable Industry Growth Plan for the Salmon Industry identifies a number of priority actions intended to **maintain public confidence in the salmon industry.**

*“Clearly identifying the areas of Tasmania’s coastal waters where salmon will continue to be farmed, areas where further growth might be possible (subject always to careful and open planning and approval processes), and areas where salmon farming will be excluded.”*

*“Development of a “Tasmanian Salmon Industry Scorecard” that benchmarks the industry against international best practice, as applicable in the Tasmanian context, and is regularly reviewed and updated.”*

*“Encouraging relevant research and development, and the subsequent adoption of new technologies that reduce environmental impacts”*

*“Collection of a wider range of environmental information, including additional real time data, and increased public access to relevant environmental information through an independent portal hosted by the Institute for Marine and Antarctic Studies”*

*“A commitment to future expansion moving into oceanic (deeper and high-energy) waters, rather than estuarine waters”*

Whilst the Tasmanian Government has recognised the value of the salmonid aquaculture industry as a key driver for the Tasmanian economy and has signalled its support for industry expansion in the Sustainable Industry Growth Plan, it also recognises that there is a need to better understand environmental risks in new farming areas. This requires predictive tools and scientifically robust environmental information at relevant spatial and temporal scales for these systems. This

information will also support the Environment Protection Authority (EPA) in its ongoing environmental regulatory management of existing and proposed marine farming operations.

The Sustainable Industry Growth Plan for the Salmon Industry has made a commitment to supporting the salmon industry in its plans to expand into oceanic (deeper and high-energy) waters. This has been the catalyst for the development of a number of key research projects within IMAS designed to support the implementation of the proposed strategies and to help ensure the sustainable development of the salmon industry in Tasmania.

Two new research programs have been approved that will provide the information necessary for sustainable management of leases in these new areas. The first is a program of research in Storm Bay. The Storm Bay project has been instigated to support salmon farming expansion into Storm Bay. This research is being undertaken by IMAS and CSIRO in collaboration and comprises three parts that together will provide the i) predictive, ii) observational and iii) implementation understanding to sustainably manage aquaculture in Storm Bay. Work package i), is being undertaken by CSIRO and aims to develop and calibrate an integrated biogeochemical model (BGCM) for Storm Bay. Work package ii) will develop a comprehensive system-wide environmental monitoring (observational) program that both underpins the model development and is critical to ongoing regulation of operations in Storm Bay. Work package iii) will provide the governance structure to further the research, monitoring and communication objectives.

A key objective of the IMAS component of this project is to assess the monitoring requirements in this new environment. Storm Bay is a challenging environment for farming, and as such is also a challenging environment for monitoring. Given the highly dynamic nature of the water body it might be assumed that there is a lower risk of adverse interactions and that any dissolved nutrients will be rapidly diluted. However, as this ecosystem is naturally quite oligotrophic it may be relatively sensitive to change in nutrient loads, particularly bioavailable nutrients. The proposed program of research and monitoring will evaluate this and adjust the monitoring required to provide a realistic risk-based monitoring program going forward.

However, the dynamic nature of the Storm Bay environment also presents some very particular challenges for monitoring and environmental assessment, as these are not easy waters to sample. This represents an opportunity to develop innovative techniques for remote sampling and to improve current modelling tools, and IMAS is collaborating with CSIRO to develop and validate improved modelling and monitoring options. IMAS is already engaged in a number of projects, several in collaboration with CSIRO, that seek to provide a wider range of data, including real-time data to support both management decisions and modelling. Much of this research was initiated in response to the need to understand real-time changes in environmental conditions in Macquarie Harbour, particularly oxygen levels. However, the technology developed and system understanding has since been extended to other areas, including Storm Bay.

In Storm Bay, IMAS will undertake the environmental monitoring for the first 3-5 years using a combination of traditional and innovative monitoring methods across a broad range of receiving habitats. This will ensure consistency of methods and analytical approach, and compatibility of data, which will in turn enable researchers to more accurately identify redundancy in approaches and recommend the most cost-effective and risk-relevant monitoring strategies.

The Blue Economy CRC (<https://blueeconomycrc.com.au/>) is a major research collaboration hosted by UTAS that will directly address the research needs to support the salmon industry to expand into oceanic (deeper and high-energy) waters. The CRC will bring together expertise in the seafood, marine renewable energy and offshore marine engineering sectors to revolutionise how salmon farming is undertaken, not just in Tasmania or Australia, but worldwide. This CRC hopes to address many of the key environmental challenges facing offshore food and energy production, and IMAS will play a key role in that developing and addressing that research agenda.

### ***3. Any other matter incidental thereto:***

The summary of research projects undertaken to date (Appendix 1) clearly shows the extent to which IMAS has already provided research to address issues of biosecurity, and fish health and welfare; to improve fish performance, to support

sustainable farming practices and to identify and address the potential for adverse interactions.

There will inevitably be gaps in our understanding, and the ability to address these will depend on the level of risk and concern associated with each particular issue, and availability of resources. IMAS research is responsive to the concerns raised by industry, government and the community. We acknowledge that marine and coastal ecosystems are a shared resource, so collaborative research remains our focus. We are focused on partnerships that develop system understanding, bring together theoretical and empirical studies, basic and applied science, biological and socioecological research and the development of leading technology to deliver world-class science that supports sustainable aquaculture development. Our management recommendations seek to promote multi-use management solutions, and to provide advice that supports sustainable management practices for all stakeholders.

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## Appendices – Project Summaries and References

To assist the committee, a listing of the main IMAS reports and papers are provided below with abstracts or summaries where possible. We have tried to capture as much relevant literature as possible but acknowledge that there may be some omissions.

We have also included a list of key externally funded IMAS research projects and student research higher degree projects in areas relevant to salmon farming.

We are compiling a reference folder for the documents referred to in this submission, this can be found on the IMAS website ([www.imas.utas.edu.au](http://www.imas.utas.edu.au)).

### Appendix 1 - IMAS Publications Relevant to Salmon Research (Grouped by Research Area)

#### Salmon Health and Nutrition

2019

Cano I, Taylor NGH, Bayley A, Gunning S, McCullough R, Bateman K, Nowak BF, Paley RK (2019) **In vitro gill cell monolayer successfully reproduces in vivo Atlantic salmon host responses to Neoparamoeba perurans infection.** *Fish Shellfish Immunol* 86:287-300

An in vitro model to study the host response to *Neoparamoeba perurans*, the causative agent of amoebic gill disease (AGD), was evaluated. The rainbow trout gill derived cell line, RTgill-W1, was seeded onto permeable cell culture supports and maintained asymmetrically with apical seawater. Cells were inoculated with either a passage attenuated or a recent wild clone of *N. perurans*. Amoebae, loaded with phagocytosed fluorescent beads, were observed associated with host cells within 20 min post inoculation (pi). By 6 h small foci of cytopathic effect appeared and at 72 h cytolysis was observed, with total disruption of the cell monolayer at 96 h pi. Due to cell monolayer disruption, the platform could not support proliferation of amoebae, which showed a 3-log reduction in parasite 18S rRNA mRNA after 72 h (106 copies at 1 h to 103 at 72 h pi). SEM observations showed amoebae-like cells with either short pseudopodia and a malleiform shape, or, long pseudopodia embedded within the gill cells and erosion of the cell monolayer. To study the host immune response, inoculated gill cells were harvested from triplicate inserts at 0, 1, 3, 6, 24 and 48 h pi, and expression of 12 genes involved in the Atlantic salmon response to AGD was compared between infected and uninfected cells and between amoebic clones. Both clones induced similar host innate immune responses, with the up-regulation of proinflammatory cytokine IL1 $\beta$ , complement C3 and cell receptor MHC-1. The Th2 pathway was up-regulated, with increased gene expression of the transcription factor GATA3, and Th2 cytokines IL10, IL6 and IL4/13A. PCNA and AG-2 were also up-regulated. The wild clone induced significantly higher up-regulation of IL1 $\beta$ , MHC-1, PCNA, lysozyme and IL10 than the attenuated clone for at least some exposure times, but AG-2 gene expression was higher in cells inoculated with the attenuated one. A principal component analysis showed that AG-2 and IL10 were key genes in the in vitro host response to *N. perurans*. This in vitro model has proved to be a

promising tool to study host responses to amoebae and may therefore reduce the requirement for in vivo studies when evaluating alternative therapeutants to AGD control.

Gonzalez L, Taylor RS, Bridle AR, Crosbie PBB, Nowak BF (2019) **Parasitic isopods *Ceratothoa banksii* (Leach, 1818) and *Nerocila orbigny* (Guerin-Meneville, 1832) of farmed Atlantic salmon and their potential as vectors of *Neoparamoeba perurans* (Young et al. 2007) in Tasmania.** *Aquaculture* 507:28-34

Reiterative freshwater bathing is the main treatment to control amoebic gill disease (AGD) of farmed Atlantic salmon in Tasmania, Australia. Regular freshwater exposure appears to control ectoparasitic cymothoid isopods, which were only seen at high prevalence and intensity in summer when fish had not been treated for over 100 days. With the potential advent of non-freshwater AGD treatments or increased periods between freshwater bathing due to selective breeding for AGD resistance, it is possible that cymothoid parasitism will become an increasing threat on Tasmanian salmon farms. In order to establish whether isopods could be vectors of *Neoparamoeba perurans* Young et al. 2007 (the causative agent of AGD), gill isopods were collected from salmon that had not been bathed for seven months and carried a 95% prevalence of isopods, including *Ceratothoa banksii* (Leach, 1818) and *Nerocila orbigny* (Guérin-Méneville, 1832). PCR analyses of gill swabs indicated that 82% of salmon were positive for *N. perurans* while 41% of the sampled isopods were positive for *N. perurans* on external surfaces. When internal material was analysed, only 9% of the isopods were positive for the amoeba, but in very low concentration. Quantitative analysis showed no correlation between the concentrations of *N. perurans* from gill swabs and the isopods from the same individual fish. Thus, it is unlikely that these isopods act as a significant reservoir or vector for *N. perurans*.

Grunenwald M, MB, Carter CG, Nichols DS, Koppe W, Verlhac-Trichet V, Schierle J, Adams LR (2019) **Pigment-depletion in Atlantic salmon (*Salmo salar*) post-smolt starved at elevated temperature is not influenced by dietary carotenoid type and increasing alpha-tocopherol level.** *Food Chem* 299 (available online)

Pigment-depletion in the fillets of farmed Atlantic salmon (*Salmo salar*) arises after periods of elevated water temperatures with voluntary starving. This study tested the effects of dietary pre-loading with different pigment carotenoids (astaxanthin and/or canthaxanthin) combined with two alpha-tocopherol levels (normal and high: 500 and 1000 mg/kg, respectively) on pigment-depletion in vivo in Atlantic salmon after four weeks of challenge. We also tested whether oxidative stress manifested as an underlying depletion mechanism. Carotenoid levels in whole fillet homogenates were not decreased significantly post-challenge but fillet alpha-tocopherol concentrations were increased significantly in contrast to decreased oxidative stress indices. However, image analysis revealed localised fillet pigment-depletion following all dietary treatments. These data imply that localised pigment-depletion was not prevented by pre-loading of the fillet with different carotenoid-types/mixtures and increased of alpha-tocopherol levels from normal to high, respectively. Further, we suggest that oxidative stress might not facilitate pigment-depletion in vivo.

Johnson-Mackinnon JC, Crosbie PBB, Karlsbakk E, Marcos-Lopez M, Paley R, Nowak BF, Bridle AR (2019) **Multilocus Sequence Typing (MLST) and Random Polymorphic DNA (RAPD) Comparisons of Geographic Isolates of *Neoparamoeba perurans*, the Causative Agent of Amoebic Gill Disease.**

*Pathogens* 8 (available online).

*Neoparamoeba perurans*, is the aetiological agent of amoebic gill disease (AGD), a disease

that affects farmed Atlantic salmon worldwide. Multilocus sequence typing (MLST) and Random Amplified Polymorphic DNA (RAPD) are PCR-based typing methods that allow for the highly reproducible genetic analysis of population structure within microbial species. To the best of our knowledge, this study represents the first use of these typing methods applied to *N. perurans* with the objective of distinguishing geographical isolates. These analyses were applied to a total of 16 isolates from Australia, Canada, Ireland, Scotland, Norway, and the USA. All the samples from Australia came from farm sites on the island state of Tasmania. Genetic polymorphism among isolates was more evident from the RAPD analysis compared to the MLST that used conserved housekeeping genes. Both techniques consistently identified that isolates of *N. perurans* from Tasmania, Australia were more similar to each other than to the isolates from other countries. While genetic differences were identified between geographical isolates, a BURST analysis provided no evidence of a founder genotype. This suggests that emerging outbreaks of AGD are not due to rapid translocation of this important salmonid pathogen from the same area.

Oldham T, Nowak B, Hvas M, Oppedal F (2019) **Metabolic and functional impacts of hypoxia vary with size in Atlantic salmon.** *Comp Biochem Phys A* 231:30-38

The most capricious environmental variable in aquatic habitats, dissolved O<sub>2</sub>, is fundamental to the fitness and survival of fish. Using swim tunnel respirometry we test how acute exposure to reduced O<sub>2</sub> levels, similar to those commonly encountered by fish in crowded streams and on commercial aquaculture farms, affect metabolic rate and swimming performance in Atlantic salmon of three size classes: 0.2, 1.0 and 3.5 kg. Exposure to 45–55% dissolved O<sub>2</sub> saturation substantially reduced the aerobic capacity and swimming performance of salmon of all sizes. While hypoxia did not affect standard metabolic rate, it caused a significant decrease in maximum metabolic rate, resulting in reduced absolute and factorial aerobic scope. The most pronounced changes were observed in the smallest fish, where critical swimming speed was reduced from 91 to 70 cm s<sup>-1</sup> and absolute aerobic scope dropped by 62% relative to the same measurement in normoxia. In normoxia, absolute critical swimming speed (U<sub>crit</sub>) increased with size, while relative U<sub>crit</sub>, measured in body lengths s<sup>-1</sup>, was highest in the small fish (3.5) and decreased with larger size (medium=2.2). Mass specific metabolic rate and cost of transport were inversely related to size, with calculated metabolic scaling exponents of 0.65 for bSMR and 0.78 for bMMR. Metabolic O<sub>2</sub> demand increased exponentially with current speed irrespective of fish size (R<sup>2</sup>=0.97–0.99). This work demonstrates that moderate hypoxia reduces the capacity for activity and locomotion in Atlantic salmon, with smaller salmon most vulnerable to hypoxic conditions. As warm and hypoxic conditions become more prevalent in aquatic environments worldwide, understanding local O<sub>2</sub> budgets is critical to maximizing the welfare and survival of farmed and wild salmon.

2018

Ghosh B, Crosbie PBB, Nowak BF, Bridle AR (2018) **A highly sensitive, non-invasive qPCR-based strategy for direct quantification of *Yersinia ruckeri* in fish faeces.** *J Fish Dis* 41:1421-1428

Finfish with asymptomatic *Yersinia ruckeri* infections pose a major risk as they can transmit the pathogen and cause clinical outbreaks in stock populations. Current tools have insufficient quantitative ability for accurately detecting the trace levels of *Y. ruckeri* typically associated with asymptomatic infection, necessitate invasive or lethal sampling, or require

long processing times. This study presents a highly sensitive qPCR-based method, targeting part of the *Y. ruckeri* 16S rRNA sequence, that is capable of detecting extremely low levels of *Y. ruckeri* in noninvasively collected faecal samples. Quantitative precision and accuracy of faecal sample analysis was consistent, despite the complexity of the faecal matrix. The assay demonstrated linearity over a six log-wide dynamic range. Its limit of detection (LOD) and limit of quantification (LOQ) were 4 and 10 copies of the target sequence, respectively. Sensitivity of the assay was comparable to other qPCR-based methods without requiring invasive or lethal sampling. Applicability as a screening strategy was tested using passively collected faecal samples. Asymptomatic *Y. ruckeri* infection was detected in all samples, although none of the fish exhibited overt infection. This method will be beneficial for finfish disease management if developed further as a non-invasive, screening tool against asymptomatic *Y. ruckeri* infection.

Gonzalez L, Bridle A, Crosbie P, Leef M, Nowak B (2018) **Spatial and temporal distribution of *Neoparamoeba perurans* in a tank recirculation system during experimental AGD challenge.** *Aquaculture* 491:399-399

Amoebic gill disease (AGD) is the major disease negatively impacting Atlantic salmon aquaculture in Tasmania, Australia. From an epidemiological perspective, it is essential to determine the reservoirs of AGD etiological agent *Neoparamoeba perurans*. During tank-based experimental infections it was demonstrated that the concentration of *N. perurans* was significantly higher in the water column ( $13 \pm 7$  cells/L after 22 days) than on the interface surface air–water–tank ( $0.01 \pm 0.1$  cells/L) in a recirculation system with Atlantic salmon in a stocking density of 7.5 kg/m<sup>3</sup> and weekly water changes. These are lower numbers compared to those found on farmed Atlantic salmon chronically affected with AGD that can reach 100 times more cells/swab. This suggests that fish themselves and not water are reservoirs of this pathogen. A similar concentration was observed in a different system, the recirculating seawater system where *N. perurans* infection is perpetuated with 1.7 kg/m<sup>3</sup> stocking density of Atlantic salmon but no water changes. This suggests that a critical maximum concentration of the amoeba in seawater and time exposure for fish are what need to be corroborated in field studies.

Nguyen TD, Crosbie PBB, Nowak BF, Bridle AR (2018) **The effects of inactivation methods of *Yersinia ruckeri* on the efficacy of single dip vaccination in Atlantic salmon (*Salmo salar*).** *J Fish Dis* 41:1173-1176

This study aimed to compare the efficacy of *Y. ruckeri* bacterins produced using three different inactivation methods, which were formalin, ammonium sulphate followed by heat treatment and lysis by pH manipulation followed by formalin treatment. The vaccine was administered to Atlantic salmon by a single dip immersion. The vaccine efficacy was determined using the RPS, serum antibody levels against *Y. ruckeri*, serum agglutination and carrier status determined by the presence of *Y. ruckeri* in the spleen.

Nowak BF, Archibald JM (2018) **Opportunistic but Lethal: The Mystery of Paramoebae.** *Trends Parasitol* 34:404-419

Paramoebae are enigmatic single-celled eukaryotes that can be opportunistic pathogens of marine animals. For example, amoebic gill disease ravages farmed salmonids worldwide, causing tens of millions of dollars in losses annually. Although paramoebae can be found associated with animals ranging from fish and lobster to molluscs and sea urchins, how and how often they actually cause disease is unknown. Here we review recent progress towards understanding the biology and ecology of paramoebid species and the eukaryotic

endosymbionts that live inside them. Genomic and transcriptomic sequence data serve as a platform upon which future research on paramoebiasis can build.

Nuez-Ortin WG, Carter CG, Nichols PD, Cooke IR, Wilson R (2018) **Liver proteome response of pre-harvest Atlantic salmon following exposure to elevated temperature.** *BMC Genomics* 19 (available online)

Background: Atlantic salmon production in Tasmania (Southern Australia) occurs near the upper limits of the species thermal tolerance. Summer water temperatures can average over 19 degrees C over several weeks and have negative effects on performance and health. Liver tissue exerts important metabolic functions in thermal adaptation. With the aim of identifying mechanisms underlying liver plasticity in response to chronic elevated temperature in Atlantic salmon, label-free shotgun proteomics was used to explore quantitative protein changes after 43 days of exposure to elevated temperature.

Results: A total of 276 proteins were differentially (adjusted p-value < 0.05) expressed between the control (15 degrees C) and elevated (21 degrees C) temperature treatments. As identified by Ingenuity Pathway Analysis (IPA), transcription and translation mechanisms, protein degradation via the proteasome, and cytoskeletal components were down-regulated at elevated temperature. In contrast, an up-regulated response was identified for NRF2-mediated oxidative stress, endoplasmic reticulum stress, and amino acid degradation. The proteome response was paralleled by reduced fish condition factor and hepato-somatic index at elevated temperature.

Conclusions: The present study provides new evidence of the interplay among different cellular machineries in a scenario of heat-induced energy deficit and oxidative stress, and refines present understanding of how Atlantic salmon cope with chronic exposure to temperature near the upper limits of thermal tolerance.

Wright DW, Nowak B, Oppedal F, Crosbie P, Stien LH, Dempster T (2018) **Repeated sublethal freshwater exposures reduce the amoebic gill disease parasite, *Neoparamoeba perurans*, on Atlantic salmon.** *J Fish Dis* 41:1403-1410

Freshwater bathing is one of the main treatment options available against amoebic gill disease (AGD) affecting multiple fish hosts in mariculture systems. Prevailing freshwater treatments are designed to be long enough to kill *Neoparamoeba perurans*, the ectoparasite causing AGD, which may select for freshwater tolerance. Here, we tested whether using shorter, sublethal freshwater treatment durations are a viable alternative to lethal ones for *N.perurans* (2-4hr). Under invitro conditions, gill-isolated *N.perurans* attached to plastic substrate in sea water lifted off after 2min in freshwater, but survival was not impacted until 60min. In an invivo experiment, AGD-affected Atlantic salmon *Salmo salar* subjected daily to 30min (sublethal to *N.perurans*) and 120min (lethal to *N.perurans*) freshwater treatments for 6days consistently reduced *N.perurans* cell numbers on gills (based on qPCR analysis) compared to daily 3min freshwater or seawater treatments for 6days. Our results suggest that targeting cell detachment rather than cell death with repeated freshwater treatments of shorter duration than typical baths could be used in AGD management. However, the consequences of modifying the intensity of freshwater treatment regimes on freshwater tolerance evolution in *N.perurans* populations require careful consideration.

2017

Amin M, Adams M, Bolch CJS, Burke CM (2017) **In vitro screening of lactic acid bacteria isolated from gastrointestinal tract of Atlantic Salmon (*Salmo salar*) as probiont**

**candidates.** *Aquacult Int* 25:485-498

A total of 20 lactic acid bacteria (LAB) were isolated from the gastrointestinal tracts of Atlantic salmon (*Salmo salar*) and screened for antimicrobial activity against six fish pathogens using either a microtiter plate assay or a well diffusion assay. The results indicated that three LAB exhibited antimicrobial activity against at least two pathogens. Based on their partial 16S ribosomal deoxyribonucleic acid (16S rDNA) sequences, these isolates were identified as *Lactobacillus farraginis*, *Pediococcus acidilactici*, and *P. pentosaceus*. In addition, these LAB were able to tolerate the simulated gastrointestinal tract conditions (stomach and intestine), had a good adhesion capacity to intestinal mucus as well as were able to grow in intestinal mucus of Atlantic salmon. These results suggest that these LAB are potential probiotics in aquaculture. However, further studies are required to evaluate probiotic properties in aquatic animals.

Nuez-Ortin WG, Carter CG, Wilson R, Cooke IR, Amoroso G, Cobcroft JM, Nichols PD (2017) **Triploid Atlantic salmon shows similar performance, fatty acid composition and proteome response to diploids during early freshwater rearing.** *Comp Biochem Phys D* 22:67-77

There is currently renewed interest in farming triploid Atlantic salmon. Improving farming requires identifying triploid specific phenotypic and physiological traits that are uniquely derived from ploidy per se and developed under optimal growing conditions. This study investigated firstly, the impact of ploidy on growth performance and whole body composition of Atlantic salmon at different early freshwater stages [34 dph (days post-hatching) alevin, 109 dph fry, and 162 dph parr] and secondly, whether phenotypic differences at these stages were reflected in protein samples collected from whole fish, white muscle or liver tissue. Female diploid and triploid Atlantic salmon (n = 3) were first fed at 35 dph and then maintained by feeding to satiation on commercial feeds. Triploids were significantly lower in weight at the late alevin and fry stages but matched diploid weight at the parr stage. The whole-body lipid content was significantly higher for triploids at the parr stage, while the whole-body lipid class profile was broadly similar and was largely not affected by ploidy. Comparative label-free shotgun proteomic analysis did not detect significant alterations in protein expression between diploids and triploids at any growth stage. The present results indicate that ploidy under optimal growing conditions and during early freshwater stages only result in small phenotypic differences in weight and whole body lipid content that were not reflected at the proteome level. These findings suggest that optimal husbandry conditions for freshwater Atlantic salmon are similar between ploidies, at least for all-female populations.

Stehfest KM, Carter CG, McAllister JD, Ross JD, Semmens JM (2017) **Response of Atlantic salmon *Salmo salar* to temperature and dissolved oxygen extremes established using animal-borne environmental sensors.** *Sci Rep-Uk* 7 (available online)

Understanding how aquatic species respond to extremes of DO and temperature is crucial for determining how they will be affected by climate change, which is predicted to increasingly expose them to levels beyond their optima. In this study we used novel animal-borne DO, temperature and depth sensors to determine the effect of extremes of DO and temperature on the vertical habitat use of Atlantic salmon *Salmo salar* in aquaculture cages. Salmon showed a preference for temperatures around 16.5 to 17.5 degrees C, however, selection of preferred temperatures was trumped by active avoidance of low DO (< 35% saturation) at the bottom of the cage. In addition to low DO, salmon also avoided warm surface waters (> 20.1 degrees C), which led to a considerable contraction in the available

vertical habitat. Despite their avoidance behaviour, fish spent a large amount of time in waters with suboptimal DO (<60% saturation). These results show that vertical habitat contraction could likely be a significant consequence of climate change if the reduction in DO outpaces the increase in hypoxia tolerance through local adaptation. They furthermore highlight that site-specific environmental conditions and stock-specific tolerance thresholds may need to be considered when determining stocking densities.

Wright DW, Nowak B, Oppedal F, Bridle A, Dempster T (2017) **Free-living *Neoparamoeba perurans* depth distribution is mostly uniform in salmon cages, but reshaped by stratification and potentially extreme fish crowding.** *Aquaculture Environment Interactions* 9:269-279

Understanding the spatio-temporal positioning of hosts and their parasites in free-living states is useful in devising methods to diminish parasite encounters in animal production systems. We explored the potential for depth-based control methods of the amoebic gill disease (AGD) agent, *Neoparamoeba perurans*, in salmon mariculture systems by conducting: (1) depth-stratified *N. perurans* water sampling surveys in 2 years, and (2) a mensurative experiment comparing depth distributions of *N. perurans* and salmon hosts in commercial salmon sea-cages. From water sampling mostly at marine salinities, *N. perurans* abundance (quantitative PCR-derived cells l<sup>-1</sup>) varied among years, but overall, neither depth, time since freshwater bathing, temperature and salinity were predictors of *N. perurans* abundance. However, at 1 survey time, depth patterns in *N. perurans* abundance appeared during strong vertical salinity gradients following rainfall (at 1 site, salinity ranged between 14 and 35 g l<sup>-1</sup>), with greater numbers of cells below a less saline surface layer. This suggested that salinity mediates *N. perurans* depth distribution during intermittent halocline development. Fish depth distribution monitoring revealed intense fish crowding, with local swimming densities up to 5 times stocking densities, typically at the surface at night. Simultaneously collected daytime water samples during low levels of fish crowding, with stock scattered amongst upper and lower cage sections, revealed no relationship between *N. perurans* and fish depth distributions. If intense fish crowding in narrow depth bands leads to high concentrations of *N. perurans* in cage environments and increased AGD risk, behavioural manipulations that vertically spread fish could be a successful AGD mitigation strategy.

Zainathan SC, Carson J, Crane MS, Williams LM, Hoad J, Moody NJG, Gudkovs N, Crameri S, Hyatt AD, Young J, Nowak BF (2017) **Preliminary characterization of Tasmanian aquareovirus (TSRV) isolates.** *Arch Virol* 162:625-634

In an attempt to determine whether or not genetic variants of the Tasmanian strain of Atlantic salmon aquareovirus (TSRV) exist, 14 isolates of TSRV, originating from various locations in Tasmania, covering a 20-year period (1990-2010), obtained from various host species and tissues, and isolated on different cell lines, were selected for this study. Two categories, termed "typical" and "atypical", of variants of TSRV were identified based on preliminary genotypic and phenotypic characterization carried out on these 14 different isolates. In addition, electron microscopic examination indicated the existence of at least three variants based on viral particle size. Finally, this study demonstrated the existence of at least one new variant of TSRV isolates, other than the more commonly isolated typical TSRV isolates, in farmed Tasmanian Atlantic salmon.

2016

Adams M, Bridle A, Norte Dos Santos C, Pennachi Y, Nowak B (2016) **Comparative susceptibility and host responses of endemic fishes and salmonids to amoebic gill disease in Tasmania**. FRDC 2011/070 Report. Fisheries Research & Development Corporation, Launceston, Tasmania

Scientists at the Institute for Marine and Antarctic Studies have completed a two year experimental project on a globally emerging fish disease. Dr Mark Adams and co-investigators Dr Andrew Bridle and Professor Barbara Nowak investigated the comparative susceptibility and host responses of various endemic and salmonid fishes to amoebic gill disease (AGD). The research, conducted at the University of Tasmania's aquaculture research facility in Launceston, found that yellow eye mullet were able to spontaneously resolve pathological signs of AGD under experimental conditions. A tempered disease response to experimental infection with the causative agent *Neoparamoeba perurans* was demonstrated in other native species including Australian salmon, purple wrasse and southern sand flathead. Negligible differences in immune-regulatory genes were found in Atlantic salmon repeatedly infected with *N.perurans* in comparison to naïve and uninfected fish; a finding that contrasted with concomitant observations at the cellular level. AGD induced tissue remodelling of mucosa associated lymphoid tissues in Atlantic salmon gills. Hybridised Atlantic salmon and brown trout displayed lower levels of disease severity induced by experimental infection supporting recently published field observations (collaboration with Dr Ben Maynard (CSIRO - FRDC project 2011/071). The disease has affected Tasmanian salmonid aquaculture since its inception three decades ago. AGD has become an increasingly complex management issue as the industry expands and the condition has recently emerged worldwide affecting major producers in the northern hemisphere. It is anticipated that the identification of fish species resistant to or tolerant of AGD will provide insight and linkage toward alternative treatments or prophylaxis for farmed Atlantic salmon.

Amoroso G, Adams MB, Ventura T, Carter CG, Cobcroft JM (2016) **Skeletal anomaly assessment in diploid and triploid juvenile Atlantic salmon (*Salmo salar* L.) and the effect of temperature in freshwater**. *J Fish Dis* 39:449-466

Triploid Atlantic salmon tend to develop a higher prevalence of skeletal anomalies. This tendency may be exacerbated by an inadequate rearing temperature. Early juvenile all-female diploid and triploid Atlantic salmon were screened for skeletal anomalies in consecutive experiments to include two size ranges: the first tested the effect of ploidy (0.2-8g) and the second the effect of ploidy, temperature (14 degrees C and 18 degrees C) and their interaction (8-60g). The first experiment showed that ploidy had no effect on skeletal anomaly prevalence. A high prevalence of opercular shortening was observed (average prevalence in both ploidies 85.8%) and short lower jaws were common (highest prevalence observed 11.3%). In the second experiment, ploidy, but not temperature, affected the prevalence of short lower jaw (diploids > triploids) and lower jaw deformity (triploids > diploids, highest prevalence observed 11.1% triploids and 2.7% diploids) with a trend indicating a possible developmental link between the two jaw anomalies in triploids. A radiological assessment (n=240 individuals) showed that at both temperatures triploids had a significantly ( $P < 0.05$ ) lower number of vertebrae and higher prevalence of deformed individuals. These findings (second experiment) suggest ploidy was more influential than temperature in this study.



Amoroso G, Cobcroft JM, Adams MB, Ventura T, Carter CG (2016) **Concurrence of lower jaw skeletal anomalies in triploid Atlantic salmon (*Salmo salar* L.) and the effect on growth in freshwater.** *J Fish Dis* 39:1509-1521

Triploid Atlantic salmon populations are associated with higher prevalence of lower jaw skeletal anomalies affecting fish performance, welfare and value deleteriously. Anomalous lower jaw can be curved downward (LJD), shortened (SJ) or misaligned (MA). Two separate groups of triploid Atlantic salmon (similar to 12g) with either normal lower jaw (NOR) or SJ were visually assessed four times over three months for presence and concurrence of jaw anomalies (with severity classified) and opercular shortening to understand the relatedness of these anomalous developmental processes. The prevalence of jaw anomalies increased in both groups over time (NOR group - SJ, LJD and MA combined 0-24.5%; SJ group - LJD and MA combined 17-31%). SJ and LJD occurred both independently and concurrently whereas MA exclusively concurred with them. All three anomalies could be concurrent. Severity of both LJD and SJ increased in the SJ group only. Opercular shortening recovery was observed in both groups but at a slower rate in the SJ group. The SJ group specific growth rate (SGR) was significantly ( $P < 0.05$ ) lower than the NOR group. This study demonstrated the concurrence of SJ, LJD and MA and showed possible deleterious consequences deriving from the conditions.

Amoroso G, Ventura T, Cobcroft JM, Adams MB, Elizur A, Carter CG (2016) **Multigenic Delineation of Lower Jaw Deformity in Triploid Atlantic Salmon (*Salmo salar* L.).** *Plos One* 11

Lower jaw deformity (LJD) is a skeletal anomaly affecting farmed triploid Atlantic salmon (*Salmo salar* L.) which leads to considerable economic losses for industry and has animal welfare implications. The present study employed transcriptome analysis in parallel with real-time qPCR techniques to characterise for the first time the LJD condition in triploid Atlantic salmon juveniles using two independent sample sets: experimentally-sourced salmon (60 g) and commercially produced salmon (100 g). A total of eleven genes, some detected/identified through the transcriptome analysis (*fbn2*, *gal* and *gphb5*) and others previously determined to be related to skeletal physiology (*alp*, *bmp4*, *col1a1*, *col2a1*, *fgf23*, *igf1*, *mmp13*, *ocn*), were tested in the two independent sample sets. *Gphb5*, a recently discovered hormone, was significantly ( $P < 0.05$ ) down-regulated in LJD affected fish in both sample sets, suggesting a possible hormonal involvement. In-situ hybridization detected *gphb5* expression in oral epithelium, teeth and skin of the lower jaw. *Col2a1* showed the same consistent significant ( $P < 0.05$ ) down-regulation in LJD suggesting a possible cartilaginous impairment as a distinctive feature of the condition. Significant ( $P < 0.05$ ) differential expression of other genes found in either one or the other sample set highlighted the possible effect of stage of development or condition progression on transcription and showed that anomalous bone development, likely driven by cartilage impairment, is more evident at larger fish sizes. The present study improved our understanding of LJD suggesting that a cartilage impairment likely underlies the condition and *col2a1* may be a marker. In addition, the involvement of *gphb5* urges further investigation of a hormonal role in LJD and skeletal physiology in general.

Ghosh B, Nguyen TD, Crosbie PBB, Nowak BF, Bridle AR (2016) **Oral vaccination of first-feeding Atlantic salmon, *Salmo salar* L., confers greater protection against yersiniosis than immersion vaccination.** *Vaccine* 34:599-608

*Yersinia ruckeri* is a ubiquitous pathogen of finfish capable of causing major mortalities in

farmed fish stocks. It can be transmitted vertically from parent to progeny as well as horizontally in the water column from both clinically infected fish and asymptomatic carriers, and is consequently capable of infecting fish at early stages of development. Immunisation strategies that can protect small fry are therefore critical for the effective management of fish health, as is the ability to detect covertly infected fish. In this study, first feeding Atlantic salmon fry (<0.5 g) were immunised either by oral administration of a microencapsulated *Y. ruckeri* vaccine formulation (0.38 g initial weight), or via immersion in bacterin suspension (0.26 g), with and without a booster immersion vaccination at 1 g size. Protection in groups receiving only immersion immunisation did not differ significantly from untreated controls when challenged with *Y. ruckeri* at approximately 5 g size, while orally immunised fish were significantly better protected than untreated controls ( $F = 4.38$ ,  $df = 4,10$ ,  $P = 0.026$ ), with RPS varying between 29.4% (ORAL) and 51% (ORAL + DIP). A quantitative real-time PCR assay was used to successfully detect covertly infected fish among challenge survivors, indicating more than 50% of surviving fish in each group were infected with no significant differences between immunised fish and untreated controls

Johnson-Mackinnon J, Oldham T, Nowak B (2016) **Amoebic gill disease: a growing threat.** *Microbiology Australia*:140-142

The risk of disease outbreaks is predicted to increase due to climate change. For farmed fish an example is amoebic gill disease (AGD). While initially reported only in farmed salmonids in Washington State, USA, and Tasmania, Australia, it has now become an issue for Atlantic salmon farming worldwide and affects a range of other farmed marine fish species. Local high temperature anomalies and a lack of rainfall have been associated with the outbreaks of AGD. This worldwide presence is at least partly due to the cosmopolitan nature of the parasite and its low host-specificity. The disease can be treated using freshwater or hydrogen peroxide baths, but the treatments increase the cost of salmon production. Management of AGD contributes 20% to production costs of Atlantic salmon in Tasmania.

Morrison RN, Young ND, Knowles G, Cornish MC, Carson J (2016) **Isolation of Tasmanian Rickettsia-like organism (RLO) from farmed salmonids: identification of multiple serotypes and confirmation of pathogenicity.** *Dis Aquat Org* 122:85-103

Atlantic salmon *Salmo salar* L. farmed in south-east Tasmania, Australia, are susceptible to infection by the Tasmanian Rickettsia-like organism (TRLO), a Gram-negative bacterium. Here, we report the first isolation of TRLO from south-east Tasmania in pure culture and show that the bacterium is culturable on both specialised enriched agar and in cell culture using the CHSE-214 cell line. In vitro cultured TRLO was used to reproducibly elicit disease in Atlantic salmon parr held in fresh water. In inoculated fish, TRLO was observed intracytoplasmically in peripheral blood leucocytes, suggesting that these cells are responsible for haematogenous dispersal of the bacterium within the host. Fish with experimentally induced disease presented with gross and histopathological changes similar to TRLO-infected fish at commercial marine farms. TRLO was also isolated in culture from farmed Atlantic salmon in the Tamar River and Macquarie Harbour production areas in Tasmania, both of which have no history of TRLO-associated disease. These TRLO isolates appear to be serologically distinct from each other as well as from isolates obtained from south-east Tasmania, linking each serotype to a specific geographical location within Tasmania. Despite the lack of clinical evidence of TRLO-linked disease in fish grown in the Tamar River and Macquarie Harbour, experimental infection trials demonstrably showed the pathogenic potential of these TRLO serovars. Together, these data provide evidence that

TRLO is a fastidious, facultative intracellular bacterium and confirm TRLO as a pathogen of Atlantic salmon, causing a disease designated Tasmanian salmonid rickettsiosis.

Nuez-Ortin WG, Carter CG, Nichols PD, Wilson R (2016) **Sequential protein extraction as an efficient method for improved proteome coverage in larvae of Atlantic salmon (*Salmo salar*)**. *Proteomics* 16:2043-2047

Understanding diet- and environmentally induced physiological changes in fish larvae is a major goal for the aquaculture industry. Proteomic analysis of whole fish larvae comprising multiple tissues offers considerable potential but is challenging due to the very large dynamic range of protein abundance. To extend the coverage of the larval phase of the Atlantic salmon (*Salmo salar*) proteome, we applied a two-step sequential extraction (SE) method, based on differential protein solubility, using a nondenaturing buffer containing 150 mM NaCl followed by a denaturing buffer containing 7 M urea and 2 M thiourea. Extracts prepared using SE and one-step direct extraction were characterized via label-free shotgun proteomics using nanoLC-MS/MS (LTQ-Orbitrap). SE partitioned the proteins into two fractions of approximately equal amounts, but with very distinct protein composition, leading to identification of approximate to 40% more proteins than direct extraction. This fractionation strategy enabled the most detailed characterization of the salmon larval proteome to date and provides a platform for greater understanding of physiological changes in whole fish larvae. The MS data are available via the ProteomeXchange Consortium PRIDE partner repository, dataset PXD003366.

Nuez-Ortin WG, Carter CG, Wilson R, Cooke I, Nichols PD (2016) **Preliminary Validation of a High Docosahexaenoic Acid (DHA) and -Linolenic Acid (ALA) Dietary Oil Blend: Tissue Fatty Acid Composition and Liver Proteome Response in Atlantic Salmon (*Salmo salar*) Smolts**. *Plos One* 11

Marine oils are important to human nutrition as the major source of docosahexaenoic acid (DHA), a key omega-3 long-chain ( $\geq$  C-20) polyunsaturated fatty acid (n-3 LC-PUFA) that is low or lacking in terrestrial plant or animal oils. The inclusion of fish oil as main source of n-3 LC-PUFA in aquafeeds is mostly limited by the increasing price and decreasing availability. Fish oil replacement with cheaper terrestrial plant and animal oils has considerably reduced the content of n-3 LC-PUFA in flesh of farmed Atlantic salmon. Novel DHA-enriched oils with high alpha-linolenic acid (ALA) content will be available from transgenic oilseeds plants in the near future as an alternative for dietary fish oil replacement in aquafeeds. As a preliminary validation, we formulated an oil blend (TOFX) with high DHA and ALA content using tuna oil (TO) high in DHA and the flaxseed oil (FX) high in ALA, and assessed its ability to achieve fish oil-like n-3 LC-PUFA tissue composition in Atlantic salmon smolts. We applied proteomics as an exploratory approach to understand the effects of nutritional changes on the fish liver. Comparisons were made between fish fed a fish oil-based diet (FO) and a commercial-like oil blend diet (fish oil + poultry oil, FOPO) over 89 days. Growth and feed efficiency ratio were lower on the TOFX diet. Fish muscle concentration of n-3 LC-PUFA was significantly higher for TOFX than for FOPO fish, but not higher than for FO fish, while retention efficiency of n-3 LC-PUFA was promoted by TOFX relative to FO. Proteomics analysis revealed an oxidative stress response indicative of the main adaptive physiological mechanism in TOFX fish. While specific dietary fatty acid concentrations and balances and antioxidant supplementation may need further attention, the use of an oil with a high content of DHA and ALA can enhance tissue deposition of n-3 LC-PUFA in relation to a commercially used oil blend.

Oldham T, Rodger H, Nowak BF (2016) **Incidence and distribution of amoebic gill disease (AGD) - An epidemiological review.** *Aquaculture* 457:35-42

Amoebic gill disease (AGD), first documented thirty years ago in sea-caged salmonids, is an ever increasing global concern in finfish aquaculture. The result of gill infection by *Neoparamoeba perurans*, clinical AGD has been observed in fourteen countries distributed across six continents and in fifteen species of finfish. The greatest impacts of AGD have been on farmed Atlantic salmon during the seawater grow-out phase. When left untreated AGD has resulted in up to 80% mortality, and even mild infections reduce production performance and fish welfare. This review summarizes and analyses three decades of AGD research and outbreaks, with focus on the causal triad of pathogen, host and environment.

Pennacchi Y, Adams MB, Nowak BF, Bridle AR (2016) **Immune gene expression in the gills of Atlantic salmon (*Salmo salar* L.) following experimental reinfection with *Neoparamoeba perurans*.** *Aquaculture* 464:410-419

Amoebic gill disease (AGD) is caused by *Neoparamoeba perurans* and represents a significant threat to Atlantic salmon marine farming in several countries worldwide. Sequential natural reinfection with *N. perurans* after treatment occurs after the first AGD outbreak during the grow-out phase of Atlantic salmon culture. Little is known about the immune response of Atlantic salmon following reinfection with *N. perurans*. In a previous single exposure study, using gill biopsies with various severity of AGD-lesion, we reported that the immune signalling in AGD-affected gills was highly dependent on the ratio of normal to hyperplastic gill tissues. Here, following experimental reinfection we investigated the transcriptional immune response in the gills of AGD-affected Atlantic salmon. Furthermore, we reported the inflammatory and immune response following a single exposure to *N. perurans* during late infection and compared it to the reinfected fish. Fish groups were selected based on the gross gill scores carried out during the trial. Two gill biopsies were collected from each AGD-affected individual, one with no lesion and one partly including AGD-lesion. Furthermore, gill biopsies were collected from uninfected controls. Pro-inflammatory and immune-related genes were characterised at the mRNA level by using a quantitative RT-PCR. Targeted immune genes included IL-1 $\beta$ , TCR- $\alpha$  chain, CD8, CD4, MHC-II $\alpha$ , MHC-I, IgM and IgT. Histopathology and image analysis were used to assess the severity and to verify the reliability of the gross gill score as AGD severity assessment method to select fish groups for gene expression studies. Overall the expression at the mRNA level of the immune and pro-inflammatory genes analysed showed little change in AGD-affected gills of experimentally reinfected fish and of fish exposed once to *N. perurans*. Statement of relevance: This research further our understanding of the mechanisms underlying AGD in Atlantic salmon and provides the bases to the potential development of immune prophylactic treatments or other practical solutions.

2015

Bridle AR, Davenport DL, Crosbie PBB, Polinski M, Nowak BF (2015) ***Neoparamoeba perurans* loses virulence during clonal culture.** *Int J Parasitol* 45:575-578

Amoebic Gill Disease affects farmed salmonids and is caused by *Neoparamoeba perurans*. Clonal cultures of this amoeba have been used for challenge experiments, however the effect of long-term culture on virulence has not been investigated. Here we show, using in vitro and in vivo methods, that a clone of *N. perurans* which was virulent 70 days after clonal culture lost virulence after 3 years in clonal culture. We propose that this is related either to

the lack of attachment to the gills or the absence of an extracellular product, as shown by the lack of cytopathic effect on Chinook salmon embryo cells. The avirulent clonal culture of *N. perurans* allowed us to propose two potential virulence mechanisms/factors involved in Amoebic Gill Disease and is an invaluable tool for host-pathogen studies of Amoebic Gill Disease.

Ghosh B, Nowak BF, Bridle AR (2015) **Alginate Microencapsulation for Oral Immunisation of Finfish: Release Characteristics, Ex Vivo Intestinal Uptake and In Vivo Administration in Atlantic Salmon, *Salmo salar* L.** *Mar Biotechnol* 17:841-853

This study examined the feasibility of alginate microcapsules manufactured using a low-impact technology and reagents to protect orally delivered immunogens for use as immune prophylactics for fish. Physical characteristics and protein release kinetics of the microcapsules were examined at different pH and temperature levels using a microencapsulated model protein, bovine serum albumin (BSA). Impact of the microencapsulation process on contents was determined by analysing change in bioactivity of micro encapsulated lysozyme. Feasibility of the method for oral immune prophylaxis of finfish was assessed using FITC-labelled microcapsules. These were applied to distal intestinal explants of Atlantic salmon (*Salmo salar*) to investigate uptake ex vivo. Systemic distribution of microcapsules was investigated by oral administration of FITC-labelled microcapsules to Atlantic salmon fry by incorporating into feed. The microcapsules produced were structurally robust and retained surface integrity, with a modal size distribution of 250–750 nm and a tendency to aggregate. Entrapment efficiency of micro encapsulation was 51.2 % for BSA and 43.2 % in the case of lysozyme. Microcapsules demonstrated controlled release of protein, which increased with increasing pH or temperature, and the process had no significant negative effect on bioactivity of lysozyme. Uptake of fluorescent-labelled microcapsules was clearly demonstrated by intestinal explants over a 24-h period. Evidence of microcapsules was found in the intestine, spleen, kidney and liver of fry following oral administration. Amenability of the microcapsules to intestinal uptake and distribution reinforced the strong potential for use of this microencapsulation method in oral immune prophylaxis of finfish using sensitive immunogenic substances.

Mardones, J. I. , Dorantes-Aranda, J. J. , Nichols, P.D. , Hallegraeff, G.M. (2015) **Fish gill damage by the dinoflagellate *Alexandrium catenella* from Chilean fjords: Synergistic action of ROS and PUFA**, *Harmful Algae* 49:40–49

Outbreaks of the dinoflagellate *Alexandrium catenella* have caused significant economic losses to the salmon industry in the south of Chile. However, the precise ichthyotoxic mechanism by this paralytic shellfish toxin (PST) - producing dinoflagellate remains poorly understood. The rainbow trout cell line RTgill-W1 assay was used to investigate fish gill damage by multiple *A. catenella* strains under different environmental conditions, and potency of whole cells, lysed cells and culture medium were compared. Cytotoxic potency was highly variable among strains and strongly correlated to dinoflagellate cell-abundance. Lysed cells produced more gill damage than intact cells and supernatant, reducing gill cell viability down to 20% of controls at 4000 cells mL<sup>-1</sup>. However, gill cells exposed to pure PST fractions (C1&C2, STX, GTX 1&4) exhibited only very limited loss of viability (<30%), even at cell concentrations equivalent to exceeding those detected in southern Chilean fjords. *A. catenella* lytic compounds rapidly (1-4 days) degraded in the light, but were pH (7-9) and temperature stable (17-85 degrees C), thus arguing against the involvement of proteinaceous compounds. Superoxide production by Chilean dinoflagellate strains was as

high as by the raphidophyte *Chattonella marina* (max. 8.67 +/- 0.14 pmol O<sub>2</sub>(-) cell<sup>(-1)</sup> h<sup>(-1)</sup>), variable among strains and enhanced by cell disruption and environmental stress (low nutrients, low salinity, low or high pH). Fatty acid profiles included high concentrations of the long-chain (>= C-20) polyunsaturated fatty acids (PUFA) - docosahexaenoic acid (DHA, 22:6 omega 3; 16-20% of total fatty acids) in addition to several other PUFA, which were highly cytotoxic against gill cells as a purified fraction (LC50 1.30 µg mL<sup>(-1)</sup>). In addition, potency was enhanced 9-fold in combination with superoxide anion (LC50 0.15 µg mL<sup>(-1)</sup>). In conclusion, the present study demonstrates that fish gill damage during *A. catenella* fish-kill events in Chilean fjords cannot be explained by PST toxins, but can to a large extent be accounted for by the synergistic interaction between ROS (superoxide anion) and DHA, and potentially other PUFA, notably under conditions that promote cell lysis.

Valdenegro-Vega VA, Cook M, Crosbie P, Bridle AR, Nowak BF (2015) **Vaccination with recombinant protein (r22C03), a putative attachment factor of *Neoparamoeba perurans*, against AGD in Atlantic salmon (*Salmo salar*) and implications of a co-infection with *Yersinia ruckeri*.** *Fish Shellfish Immunol* 44:592-602

Amoebic gill disease (AGD) affects salmonids during the marine grow-out phase in the Tasmanian industry and in other major salmonid producing countries. During the period post-transfer to seawater, the bacterial condition yersiniosis can also cause high levels of mortality in Atlantic salmon grown in Tasmania, in addition to the hatchery outbreaks. The recombinant protein r22C03, a mannose-binding protein-like (MBP-like) similar to attachment factors of other amoebae, was tested as a vaccine candidate against AGD in a large scale challenge trial. Fish were immunised with r22C03 combined with FCA via intraperitoneal (i.p.) injection, and given a booster five weeks later by either i.p. injection (RP group) or by a dip-immersion (mRP). Fish were then challenged twice with *Neoparamoeba perurans*: the initial challenge 16 weeks after primary immunisation was terminated due to presence of ulcerative lesions in the skin of salmon; the second challenge was carried out after five weeks of treatment with oxytetracycline. These skin lesions might have been associated with a concurrent infection with *Yersinia ruckeri*, which was detected by real-time qPCR in serum of a large proportion of moribund and survivor fish after the AGD challenge. Before and during the *N. perurans* infection, levels of antibodies against r22C03 were measured by ELISA in serum, skin mucus and supernatant from skin and gill explants. For the second challenge, the average size of AGD lesions was recorded from histology sections and survival curves were obtained. Before AGD challenge, r22C03 induced antibody responses in serum and explants with both vaccination strategies. At the end of the challenge, levels of antibodies were lower than before challenge irrespective of treatment. Both vaccinated groups presented increased serum antibody responses, while only mRP presented antibody responses in skin mucus, and no significant antibody responses were measured in the explants. Antibodies did not confer protection to *N. perurans* infection, as no difference was observed in the survival curves of the vaccinated and control groups, and there was no effect on the gill lesion size. The concurrent yersiniosis infection probably represented more closely infection patterns observed in commercial settings. However, it could have interfered with the survival results and with the ability of the fish to respond to the amoebae infection.

Valdenegro-Vega VA, Polinski M, Bridle A, Crosbie P, Leef M, Nowak BF (2015) **Effects of single and repeated infections with *Neoparamoeba perurans* on antibody levels and immune gene expression in Atlantic salmon (*Salmo salar*).** *Fish Shellfish Immunol*

42:522-529

Amoebic gill disease (AGD) is the main health problem for the salmon industry in Tasmania, Australia and is now reported in most salmon producing countries. Antibody and gene expression responses to the pathogen, *Neoparamoeba perurans*, have been studied independently following primary exposure; however, the effects of sequential reinfection, which can often occur during net-pen culture of salmon, remain unclear. The association between the transcription of immune globulin (Ig) and their systemic and mucosal antibody levels in regards to AGD is unknown. Herein, we assessed the antibody responses as well as Ig transcription in the gills of Atlantic salmon infected only once and also sequentially with *N. perurans*. After four successive AGD challenges, no significant differences in plasma or skin mucus levels of IgM were observed between AGD-naïve and challenged fish. However, IgM gene expression in gill lesions of AGD-affected fish increased up to 31 d after infection, while no changes in IgT, TCR and CD8 transcription were observed. Changes at IgM transcription level did not match the lack of antibody response in mucus, which is possibly explained by weak correlations existing between protein and mRNA abundances in cells and tissues. In the second experiment, which investigated Ig responses to AGD at the transcriptional as well as antibody production level in salmon after a single infection, the levels of serum or skin mucus IgM antibody were not affected and no changes in the IgM or IgT transcription were induced.

Wright DW, Nowak B, Oppedal F, Bridle A, Dempster T (2015) **Depth distribution of the amoebic gill disease agent, *Neoparamoeba perurans*, in salmon sea-cages.**

*Aquaculture Environment Interactions* 7:67-74

Identifying where and when parasites occur in farming environments is vital to understand transmission dynamics and develop preventative measures that reduce host-parasite encounters. A major parasite concern for Atlantic salmon farming is *Neoparamoeba perurans*, a marine amoeba that causes the potentially fatal amoebic gill disease (AGD), for which few control options exist. We explored whether free-living *N. perurans* abundance differs among depths in commercial Atlantic salmon *Salmo salar* sea-cages. Water samples collected from the surface to 10 m depth at multiple cage sites and times, and subsequently subjected to qPCR analysis, revealed that *N. perurans* abundance was influenced by depth at the time of year when amoeba numbers were highest, with more amoebae in surface waters. No distinct depth patterns were observed when amoebae were in low abundance. Across all times, temperature and salinity were largely homogeneous throughout cage depths. Possible factors explaining the presence of amoebae at the surface are discussed. Our results suggest that excluding caged salmon from upper cage depths where *N. perurans* is more abundant could be an effective management strategy to reduce the speed at which initial infections occur and delay the development of AGD outbreaks.

Zainathan SC, Carlile G, Carson J, McColl KA, Crane MS, Williams LM, Hoad J, Moody NJG, Aiken HM, Browning GF, Nowak BF (2015) **Development and application of molecular methods (PCR) for detection of Tasmanian Atlantic salmon reovirus.** *J Fish Dis* 38:739-754

Molecular (PCR) diagnostic tests for the detection and identification of aquareovirus in general, and Tasmanian Atlantic salmon reovirus (TSRV) specifically, were developed, and their diagnostic sensitivity and specificity were determined and compared with virus isolation in cell culture. Intra laboratory and interlaboratory comparison of PCR (conventional hemi-nested RT-PCR & RT-qPCR) and virus isolation in cell culture using finfish cell lines, CHSE-

214 and EPC, was carried out for the detection and identification of TSRV using field samples of farmed Atlantic salmon *Salmo salar*, L. from various aquaculture sites around Tasmania. The interlaboratory comparison of diagnostic methods was carried out between two laboratories, AAHL-CSIRO and DPIPWE-Tasmania. A total of 144 fish from nine sites (12-33 fish per site) were sampled from two regions of Tasmania (Tamar River estuary in the north and Huon River estuary in the south-east) during late spring to early summer of 2009, and the data were analysed using different statistical approaches. The prevalence of TSRV ranged from 6% to 22% in both regions. All the diagnostic methods (data from both laboratories) had high specificity, while the estimated sensitivity varied between tests with RT-qPCR being the most sensitive (95.2%) method followed by virus isolation and then conventional hemi-nested RT-PCR.

2014

Bridle A, Nowak B (2014) **Vaccination against yersiniosis**. In: Gudding R, Lillehaug A, Evensen O (eds) *Fish Vaccination*. John Wiley & Sons, Chichester, UK

Yersiniosis is a disease caused by *Yersinia ruckeri*, which mostly affects salmonids during their hatchery stage. Yersiniosis is now endemic across all the major salmonid farming areas of the world. This chapter discusses the occurrence, significance, etiology, and pathogenesis of yersiniosis. It then describes the different vaccination procedures and the effects and side-effects of vaccination against yersiniosis. Licensed in 1976, a bacterin produced from formalin-killed *Y. ruckeri* was the first vaccine against enteric red mouth (ERM) and the first commercialized fish vaccine. Licensing of fish vaccines is regulated by laws and regulations outlined in the European Pharmacopoeia monographs and guidance documents.

Crosbie PBB, Bridle AR, Nowak BF (2014) **Culture and cryopreservation of *Neoparamoeba perurans* (AGD)**. FRDC Project No 2012/048 Report: Atlantic Salmon Aquaculture Subprogram. AMC, Launceston, Tasmania

This project has shown that the culture of *N. perurans* can be improved by incubating culture plates at 10°C. At lower temperatures the growth of contaminating organisms can be slowed which allows longer periods between sub-culturing. Cryopreservation techniques have been attempted however viability of cells post thaw has not been demonstrated. The toxicity of some cryoprotectant compounds used on *N. perurans* has been assessed and concentrations not detrimental to the amoebae were identified. The results from the infectivity trials where clonal cultures of *N. perurans* were assessed for their ability to cause AGD were the most intriguing. Although the cultures were shown to cause AGD there is some evidence of a loss of virulence of the clones in culture over 2.5 years. This observation requires verification but if true could open up opportunities to do comparative studies between virulent and non-virulent *N. perurans*.

dos Santos CCN, Adams MB, Leef MJ, Nowak BF (2014) **Changes in the interbranchial lymphoid tissue of Atlantic salmon (*Salmo salar*) affected by amoebic gill disease**. *Fish Shellfish Immunol* 41:600-607

The interbranchial lymphoid tissue (ILT) was recently described in the gills of salmonids. This study examined changes in the ILT during a parasitic infection in marine environment, using amoebic gill disease (AGD) as a model. Atlantic salmon (*Salmo salar*) experimentally infected with *Neoparamoeba perurans* were sampled at 0, 3, 7, 14 and 28 days post challenge. Transversal sections of three areas of the gills (dorsal, medial and ventral) were



histologically assessed for morphological and cellular changes. AGD induced morphological changes and a cellular response in the ILT of affected fish. These changes included a significant increase in the ILT surface area in fish 28 days after AGD challenge, compared to control fish at the same time point. The length of the ILT increased significantly 28 days post exposure in the dorsal area of the gill arch in the fish affected by AGD. The lymphocyte density of the ILT increased after AGD challenge, peaking at 7 days post exposure; however, by 28 days post exposure, a reduction of lymphocyte density to values close to pre-infection levels was observed. PCNA immunostaining revealed that epithelial hyperplasia was the most likely factor contributing to the ILT enlargement in the affected fish.

Nowak B, Valdenegro-Vega V, Crosbie P, Bridle A (2014) **Immunity to Amoeba.**

*Developmental and Comparative Immunology* 43:257-267

Amoebic infections in fish are most likely underestimated and sometimes overlooked due to the challenges associated with their diagnosis. Amoebic diseases reported in fish affect either gills or internal organs or may be systemic. Host response ranges from hyperplastic response in gill infections to inflammation (including granuloma formation) in internal organs. This review focuses on the immune response of Atlantic salmon to *Neoparamoeba perurans*, the causative agent of Amoebic Gill Disease (AGD)

Pennacchi Y, Leef MJ, Crosbie PBB, Nowak BF, Bridle AR (2014) **Evidence of immune and inflammatory processes in the gills of AGD-affected Atlantic salmon, *Salmo salar* L.** *Fish Shellfish Immunol* 36:563-570

Amoebic gill disease (AGD) is a disease caused by the ectoparasite *Neoparamoeba perurans* which affects several cultured marine fish worldwide. The characterisation of pro-inflammatory and immune related genes at the mRNA level in AGD-affected Atlantic salmon gills was performed at 10 days post-inoculation using 2D quantitative RT-PCR, a method of mapping transcriptional responses in tissues. The genes of interest were IL-1b, TNF-a, TCR-a chain, CD8, CD4, MHC-IIa, MHC-I, IgM and IgT. A significant increase in expression of the mRNA of all the genes was observed in the gills of AGD-affected fish. Contrary to previous studies, our data suggest that the parasite, *N. perurans*, elicits a classical inflammatory response in the gills of AGD-affected fish and indicates that the mRNA expression of immune genes within gill lesions misrepresents the cellular immune response in the gills during AGD.

Polinski M, Bridle A, Neumann L, Nowak B (2014) **Preliminary evidence of transcriptional immunomodulation by praziquantel in bluefin tuna and Atlantic salmon in vitro cultures.** *Fish Shellfish Immunol* 38:42-46

Praziquantel (PZQ), long-used in veterinary and human medicine for the treatment of helminth parasites, is known to enhance humoral and cellular immune responsiveness in mammals but has unknown direct immunomodulatory capabilities in fish. In the present study, we examined the ability of PZQ to induce gene transcriptional changes in immune-competent primary tissue/organ cultures of two highly important yet evolutionarily discrete fish species e Southern bluefin tuna *Thunnus maccoyii* and Atlantic salmon *Salmo salar*. These cultures consisted of mixed blood cell population for both species, as well as intestinal explants from bluefin. Although expression profiles varied between species and tissue/organ type, PZQ induced both T-cell receptor (more than twofold) and IL-8 transcriptional expression (more than fourfold). Additionally, increased expression of other inflammatory cytokines including IL-1b was detected in blood cell cultures from both species, and a general pattern of heightened antiviral signalling was observed. Specifically, elevated

transcription of Type I (IFN $\alpha$ ) and Type II (IFN $\gamma$ ) interferon in Atlantic salmon blood cultures along with elevated expression of MHC class I in blood cultures of both species. These findings provide preliminary evidence for direct immunomodulation by PZQ in fish and insight into its potential capacity as an immune stimulant/adjuvant in the rapidly expanding aquaculture industry.

Valdenegro-Vega VA, Crosbie P, Bridle A, Leef M, Wilson R, Nowak BF (2014)

**Differentially expressed proteins in gill and skin mucus of Atlantic Salmon (*Salmo salar*) affected by amoebic gill disease.** *Fish Shellfish Immunol* 40:69-77

The external surfaces of fish, such as gill and skin, are covered by mucus, which forms a thin interface between the organism and water. Amoebic gill disease (AGD) is a parasitic condition caused by *Neoparamoeba perurans* that affects salmonids worldwide. This disease induces excessive mucus production in the gills. The host immune response to AGD is not fully understood, and research tools such as genomics and proteomics could be useful in providing further insight. Gill and skin mucus samples were obtained from Atlantic salmon (*Salmo salar*) which were infected with *N. perurans* on four successive occasions. NanoLC tandem mass spectrometry (MS/MS) was used to identify proteins in gill and skin mucus of Atlantic salmon affected by AGD. A total of 186 and 322 non-redundant proteins were identified in gill and skin mucus respectively, based on stringent filtration criteria, and statistics demonstrated that 52 gill and 42 skin mucus proteins were differentially expressed in mucus samples from AGD-affected fish. By generating protein-protein interaction networks, some of these proteins formed part of cell to cell signalling and inflammation pathways, such as C-reactive protein, apolipoprotein 1, granulins, cathepsin, angiogenin-1. In addition to proteins that were entirely novel in the context in the host response to *N. perurans*, our results have confirmed the presence of protein markers in mucus that have been previously predicted on the basis of modified mRNA expression, such as anterior gradient-2 protein, annexin A-1 and complement C3 factor. This first proteomic analysis of AGD-affected salmon provides new information on the effect of AGD on protein composition of gill and skin mucus. Future research should focus on better understanding of the role these components play in the response against infection with *N. perurans*.

Valdenegro-Vega VA, Crosbie PBB, Cook MT, Vincent BN, Nowak BF (2014)

**Administration of recombinant attachment protein (r22C03) of *Neoparamoeba perurans* induces humoral immune response against the parasite in Atlantic salmon (*Salmo salar*).** *Fish Shellfish Immunol* 38:294-302

This study investigated the use of a recombinant protein of *Neoparamoeba perurans*, the causative agent of Amoebic gill disease (AGD), as an immunogen to generate systemic and mucosal antibody responses against the parasite. Genes encoding *N. perurans* homologs of mannose-binding protein (MBP) from *Acanthamoeba* spp. have been identified. From these, a *Neoparamoeba* MBP like EST has been identified and produced as a recombinant fusion protein. Attachment of *N. perurans* to the gill might be reduced by antibody-mediated interference of this protein, but this is dependent on the presence and level of functional antibodies in the mucus. Fish were immunized with the protein via i.p. injection with Freund's complete adjuvant (FCA); and serum and skin mucus samples were collected before and after immunization. Antibodies (IgM) present in samples were characterized via Western blot and their levels measured with an ELISA. The immunization was able to induce a systemic IgM response 8 weeks after primary exposure and a mucosal response 4 weeks post initial immunization, which were specific to the recombinant protein but not to antigens obtained

from crude amoebic preparations. However, adherence of the antibodies to the parasite was observed using immunocytochemistry, and both, serum and skin mucus IgM, were able to bind the surface of formalin-fixed *N. perurans*. This finding may contribute to further research into the development of a vaccine for AGD.

2013

Andrews M, Cobcroft JM, Battaglione SC, Valdenegro V, Martin MB, Nowak BF (2013) **Parasitic crustaceans infecting cultured striped trumpeter *Latris lineata*. *Aquaculture* 416:280-288**

Cultured striped trumpeter *Latris lineata* was held in sea cage systems or a land-based facility in south-eastern Tasmania. Visual checks of metazoan ectoparasites were conducted on six cohorts (T1 to T6) in the land-based facility from 2006 to 2007, and three cohorts (C1 to C3) held in cages from 2007 to 2008. Three parasite species were recorded; a cymothoid *Ceratothoa banksii*; a chondracanthid *Chondracanthus goldsmidi*; and a caligid *Caligus nuenonae*. All three parasite species were present on the striped trumpeter in the sea cages with *C. nuenonae* and *C. goldsmidi* found in very low prevalence on all cohorts. There was no significant effect of cohort or season on the parasites' prevalence or intensity. Cohort C1 had the highest numbers of *C. nuenonae* with prevalence of 2.5% (intensity  $1.0 \pm 0.0$  parasites fish<sup>-1</sup>), whilst cohort C3 had the highest prevalence of *C. goldsmidi* (3.3%, intensity  $1.0 \pm 0.0$ ). The isopod *C. banksii* was recorded in increasing prevalence in cohorts C1 and C2 during 2008, cohort C1 had a prevalence ranging from 9.8% (intensity  $1.0 \pm 0.0$ ) to 17.5% (intensity  $1 \pm 0.0$ ) whereas prevalence in cohort C2 ranged from 27.7% (intensity  $1.21 \pm 0.1$ ) to 67.2% (intensity  $1.8 \pm 0.1$ ). The two copepod species were recorded on the fish held in the land-based facility. *C. nuenonae* was found on fish from two cohorts at a prevalence of 22.3% (intensity  $1.4 \pm 0.1$  parasites) in cohort T1 and 4.3% ( $1.0 \pm 0.0$ ) in cohort T2. In contrast, *C. goldsmidi* was present during all parasite checks of cohorts T2 to T6 with the percentage of infected fish ranging from 27.2% (intensity  $1.3 \pm 0.1$ ) in cohort T2 to 100% (intensity  $32.8 \pm 1.9$ ) in cohort T4. Treatments against *C. goldsmidi* were conducted on cohorts T2 to T6 including manual removal of adult parasites and Neguvon baths. There was no apparent reduction in the parasite prevalence within season during follow-up surveys after ~6 months. An eleven month re-infection experiment was conducted with *C. goldsmidi*; striped trumpeter from which parasites were removed showed significantly lower prevalence ( $F = 161.8$ ,  $df 1,20$ ,  $P < 0.001$ ) than those fish from which parasites were not removed. The study suggests that effective control of parasitic crustaceans is likely to be an important factor in the successful culture of sea-caged striped trumpeter.

Cadoret K, Bridle AR, Leef MJ, Nowak BF (2013) **Evaluation of fixation methods for demonstration of *Neoparamoeba perurans* infection in Atlantic salmon, *Salmo salar* L., gills. *J Fish Dis* 36:831-839**

Formaldehyde-based fixatives are generally employed in histopathology despite some significant disadvantages associated with their usage. Formaldehyde fixes tissue by covalently cross-linking proteins, a process known to mask epitopes which in turn can reduce the intensity of immunohistochemical stains widely used in disease diagnostics. Additionally, formaldehyde fixation greatly limits the ability to recover DNA and mRNA from fixed specimens to the detriment of further downstream molecular analyses. Amoebic gill disease (AGD) has been reliably diagnosed from histological examination of gills although complementary methods such as in situ hybridization (ISH) and polymerase chain reaction

(PCR) are required to confirm the presence of *Neoparamoeba perurans*, the causative agent of AGD. As molecular techniques are becoming more prevalent for pathogen identification, there is a need to adapt specimen collection and preservation so that both histology and molecular biology can be used to diagnose the same sample. This study used a general approach to evaluate five different fixatives for Atlantic salmon, *Salmo salar* L., gills. Neutral-buffered formalin and seawater Davidson's, formaldehyde-based fixatives commonly used in fish histopathology, were compared to formalin-free commercial fixatives PAXgene((R)), HistoChoiceMB\* and RNA later. Each fixative was assessed by a suite of analyses used to demonstrate AGD including routine histochemical stains, immunohistochemical stains, ISH and DNA extraction followed by PCR. All five fixatives were suitable for histological examination of Atlantic salmon gills, with seawater Davidson's providing the best quality histopathology results. Of the fixatives evaluated seawater Davidson's and PAXgene((R)) were shown to be the most compatible with molecular biology techniques. They both provided good DNA recovery, quantity and integrity, from fixed and embedded specimens. The capacity to preserve tissue and cellular morphology in addition to allowing molecular analyses of the same specimens makes seawater Davidson's and PAXgene((R)) appear to be the best fixation methods for diagnosis and research on AGD in Atlantic salmon gills.

Leef MJ, Nowak BF (2013) **Atlantic Salmon Aquaculture Subprogram: The effects of AGD on gill function - use of a perfused gill model.** FRDC 2011/069 Report. Fisheries Research & Development Corporation, Launceston, Tasmania

This project has successfully adapted an isolated perfused gill model specifically to Atlantic salmon and has provided in vitro results of gill function and branchial vascular resistance in AGD-affected animals. This model represents a novel in vitro method for AGD research

Nowak B, Cadoret K, Feist SW, Bean TP (2013) **Laser-capture dissection and immunohistochemistry reveals chloride and mucous-cell specific gene expression in gills of seawater acclimated Atlantic salmon *Salmo salar*.** *J Fish Biol* 83:1459-1467

Laser-capture microdissection and immunohistochemistry were used to show that gene and protein expression varied in different cell types in the gills of Atlantic salmon *Salmo salar*, with chloride cells found to express high levels of sodium potassium ATPase and mucous cells expressing elevated levels of anterior gradient protein. It is therefore important that studies of gene expression in gill tissue take account of the proportion of the various cell types present.

Nowak BF (2013) **Immune gene expression in Atlantic salmon during amoebic gill disease - A review.** *Fish Shellfish Immunol* 34:1669-1669

The interbranchial lymphoid tissue (ILT) is a tissue recently described in the gills of salmonids. It is located at the base of the gill filaments in the interbranchial septum, and is an intraepithelial agglomeration of T cells, surrounded by a continuous superficial and basal layer of cytokeratin positive cells. The ILT was reported to be an important site for immune surveillance and a future target for vaccine approaches. Amoebic gill disease (AGD) is an infection caused by a *Neoparamoeba perurans* and is a significant threat for several fish species cultured in the marine environment worldwide. The gills of fish infected with AGD exhibit epithelial hyperplasia, increased mucus production and fish show respiratory distress. Mortality occurs if AGD is left untreated. At the moment, freshwater bathing is the main treatment for AGD. However, new techniques such as vaccines and dietary immunostimulants have been investigated. This study aimed to examine the cellular response in the ILT to a parasitic infection. Fish experimentally infected with *N. perurans*

were sampled at 0, 3, 7, 14 and 28 days post infection. Transversal sections of three areas of the gills (dorsal, middle and ventral) were cut and histologically analyzed. Preliminary results show that the area of the ILT was significantly larger in fish with AGD 28 days post infection than in control fish at the same time point ( $p < 0.001$ ). The cell density of the ILT was found to be significantly lower in infected fish than in control fish ( $p = 0.032$ ) 28 days post infection. Furthermore, the height of the ILT was significantly higher in the dorsal site of the gill arch 28 days post infection in infected fish than in control fish ( $p < 0.001$ ). These preliminary results indicate changes in the morphology of the interbranchial lymphoid tissue during AGD. This study contributes to our understanding of salmon mucosal immunity.

Stride MC, Polkinghorne A, Miller TL, Nowak BF (2013) **Molecular Characterization of "Candidatus Similichlamydia latridicola" gen. nov., sp nov (Chlamydiales: "Candidatus Parilichlamydiaceae"), a Novel Chlamydia-Like Epitheliocystis Agent in the Striped Trumpeter, *Latris lineata* (Forster).** *Appl Environ Microbiol* 79:4914-4920

Histological analysis of gill samples taken from individuals of *Latris lineata* reared in aquaculture in Tasmania, Australia, and those sampled from the wild revealed the presence of epitheliocystis-like basophilic inclusions. Subsequent morphological, in situ hybridization, and molecular analyses were performed to confirm the presence of this disease and discovered a Chlamydia-like organism associated with this condition, and the criteria set by Fredericks and Relman's postulates were used to establish disease causation. Three distinct 16S rRNA genotypes were sequenced from 16 fish, and phylogenetic analyses of the nearly full length 16S rRNA sequences generated for this bacterial agent indicated that they were nearly identical novel members of the order Chlamydiales. This new taxon formed a well-supported clade with "Candidatus Parilichlamydia carangidicola" from the yellowtail kingfish (*Seriola lalandi*). On the basis of sequence divergence over the 16S rRNA region relative to all other members of the order Chlamydiales, a new genus and species are proposed here for the Chlamydia-like bacterium from *L. lineata*, i.e., "Candidatus Similichlamydia latridicola" gen. nov., sp. nov.

Valdenegro-Vega VA, Crosbie P, Cook M, Nowak BF (2013) **Use of a recombinant protein from the amoebae *Neoparamoeba perurans* as a vaccine candidate against AGD in Atlantic salmon.** *Fish Shellfish Immunol* 34:1681-1681

Amoebic gill disease (AGD) is the main disease affecting salmonids in Tasmania, Australia. The aetiological agent is *Neoparamoeba perurans* and the clinical presentation produces severe mortalities if not treated. The current treatment (fresh water bathing) decreases the number of amoebae on the gills, but since it has to be repeated several times it is costly for the industry and stressful on the fish. The development of a vaccine remains a high priority for the local industry. Previous work identified a Mannose binding-like protein (MBL) in *N. perurans*, similar to attachment factors of other amoebae, suggesting that by interfering with this MBL and blocking attachment of *N. perurans*, the severity of AGD could be reduced. Fish were immunized with this vaccine candidate using two different delivery strategies. Both systemic and mucosal antibody responses were measured using ELISA for a period of 12 weeks. Additionally, the production of antibodies by local cells in mucosal tissues was also measured using tissue explants obtained from gills and skin. Both vaccination regimes induced an increase in the production of antibodies against the antigen at the mucosal level during 12 weeks. Following this period, fish were challenged with the parasite for 3 weeks and the survival curves were recorded for vaccinated and unvaccinated fish. Additionally, gene expression of IgM and the mucosal immunoglobulin IgT were measured in mucosal

tissues before and after challenge for both vaccinated and not immunized fish and correlated with the ELISA results. This study provided insight into the physiological effects and survival outcomes of a novel vaccine candidate against *N. perurans* and could represent an initial step into the development of an efficient vaccine against AGD.

Valdenegro-Vega VA, Crosbie P, Vincent B, Cain KD, Nowak BF (2013) **Effect of immunization route on mucosal and systemic immune response in Atlantic salmon (*Salmo salar*)**. *Vet Immunol Immunopathol* 151:113-123

This study aimed to assess systemic and mucosal immune responses of Atlantic salmon (*Salmo salar*) exposed to two protein-hapten antigens – dinitrophenol (DNP) and fluorescein isothiocyanate (FITC) each conjugated with keyhole limpet haemocyanin (KLH) – administered using different delivery strategies. Fish were exposed to the antigens through different routes, and were given a booster 4 weeks post initial exposure. Both systemic and mucosal antibody responses were measured for a period of 12 weeks using an enzyme linked immunosorbent assay (ELISA). Only fish exposed to both antigens via intraperitoneal (IP) injection showed increased systemic antibody response starting 6 weeks post immunization. No treatment was able to produce a mucosal antibody response; however there was an increase in antibody levels in the tissue supernatant from skin explants obtained 12 weeks post immunization from fish injected with FITC. Western blots probed with serum and culture supernatant from skin explants showed a specific response against the antigens. In conclusion, IP injection of hapten-antigen in Atlantic salmon was the best delivery route for inducing an antibody response against these antigens in this species. Even though IP injection did not induce an increase in antibody levels in the skin mucus, there was an increased systemic antibody response and an apparent increase of antibody production in mucosal tissues as demonstrated by the increased level of specific antibody levels in supernatants from the tissue explants.

Zainathan SC, Carson J, Crane MS, Nowak BF (2013) **Laboratory evaluation of sample collection methods (organs vs swabs) for Tasmanian salmon reovirus detection in farmed Atlantic salmon, *Salmo salar* L.** *J Fish Dis* 36:427-436

The use of swabs relative to organs as a sample collection method for the detection of Tasmanian salmon reovirus (TSRV) in farmed Tasmanian Atlantic salmon, *Salmo salar* L., was evaluated by RT-qPCR. Evaluation of individual and pooled sample collection (organs vs swabs) was carried out to determine the sensitivity of the collection methods and the effect of pooling of samples for the detection of TSRV. Detection of TSRV in individual samples was as sensitive when organs were sampled compared to swabs, and in pooled samples, organs demonstrated a sensitivity of one 10-fold dilution higher than sampling of pooled swabs. Storage of swabs at 4 degrees C for t=24h demonstrated results similar to those at t=0. Advantages of using swabs as a preferred sample collection method for the detection of TSRV compared to organ samples are evident from these experimental trials.

2012

Adams MB, Crosbie PBB, Nowak BF (2012) **Preliminary success using hydrogen peroxide to treat Atlantic salmon, *Salmo salar* L., affected with experimentally induced amoebic gill disease (AGD)**. *J Fish Dis* 35:839-848

Currently, the only effective and commercially used treatment for amoebic gill disease (AGD) in farmed Tasmanian Atlantic salmon is freshwater bathing. Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), commonly used throughout the aquaculture industry for a range of topical skin and gill

infections, was trialled in vitro and in vivo to ascertain its potential as an alternative treatment against AGD. Under in vitro conditions, trophozoites of *Neoparamoeba perurans* were exposed to three concentrations of H<sub>2</sub>O<sub>2</sub> in sea water (500, 1000 and 1500 mg L<sup>-1</sup>) over four durations (10, 20, 30 and 60 min) each at two temperatures (12 and 18 degrees C). Trophozoite viability was assessed immediately post-exposure and after 24 h. A concentration/duration combination of 1000 mg L<sup>-1</sup> for >10 min demonstrated potent amoebicidal activity. Subsequently, Atlantic salmon mildly affected with experimentally induced AGD were treated with H<sub>2</sub>O<sub>2</sub> at 12 and 18 degrees C for 15 min at 1250 mg L<sup>-1</sup> and their re-infection rate was compared to freshwater-treated fish over 21 days. Significant differences in the percentage of filaments affected with hyperplastic lesions (in association with amoebae) and plasma osmolality were noted between treatment groups immediately post-bath. However, the results were largely equivocal in terms of disease resolution over a 3-week period following treatment. These data suggest that H<sub>2</sub>O<sub>2</sub> treatment in sea water successfully ameliorated a clinically light case of AGD under laboratory conditions.

**Bridle AR, Koop BF, Nowak BF (2012) Identification of Surrogates of Protection against Yersiniosis in Immersion Vaccinated Atlantic Salmon. *Plos One* 7**

Simple cost-effective bacterins are the earliest and most successfully used commercial vaccines in fish. In particular, those prepared from *Yersinia ruckeri* have proven effective at controlling Enteric Red Mouth Disease (ERM) and yersiniosis in rainbow trout and Atlantic salmon, respectively. However, the emergence of outbreaks of ERM caused by atypical biotypes of *Y. ruckeri* and reports of vaccine failure resulting in mass mortality of hatchery Atlantic salmon has reinvigorated interest in vaccines against fish bacterial diseases. Therefore the objective of this study was to identify surrogates of protection against yersiniosis using cDNA microarray to characterise the response of host genes in the gills of unvaccinated and vaccinated Atlantic salmon challenged with *Y. ruckeri*. Differentially expressed genes were identified using two-way ANOVA and restricted to those with >2.5-fold change at P<0.05. Using cDNA microarray we identified the expression of 6 genes in response to infection and 4 genes associated with the protective host response to yersiniosis. Analysis by real-time PCR confirmed that three immunologically relevant genes, namely a cathelicidin (47-fold) and a C-type lectin (19-fold) increased in response to yersiniosis. Including collagenase (17-fold increase), an important tissue remodelling and repair enzyme, these genes represent 3 of 6 non-protective and/or pathological responses to yersiniosis. Genes associated with the protective host response included an immunoglobulin gene and a selenoprotein that showed significant fold changes (15-fold increases each), highlighting the importance of antibody-mediated protection against yersiniosis. These findings provide much needed knowledge of the host-pathogen interaction in response to bacterial infection and immunisation in fish. Significantly, we identified a transcriptional biosignature consisting of predominantly immune-relevant genes (14 up and 3 down-regulated) in the gills of Atlantic salmon after immersion vaccination and before bacterial challenge. This biosignature may be used as a surrogate of protection and therefore as a predictor of vaccine success against yersiniosis.

**Codabaccus BM, Carter CG, Bridle AR, Nichols PD (2012) The "n-3 LC-PUFA sparing effect" of modified dietary n-3 LC-PUFA content and DHA to EPA ratio in Atlantic salmon smolt. *Aquaculture* 356:135-140**

n-3 Long-chain (>= C-20) polyunsaturated fatty acids (LC-PUFA) are used extensively by fish via beta-oxidation when in dietary surplus. Therefore it is of interest to optimize n-3 LC-

PUFA deposition in fish via a reduction in beta-oxidation which may be induced by manipulation of dietary fatty acids. This study tested whether Atlantic salmon smolt fed a diet with a higher docosahexaenoic acid (DHA): eicosapentaenoic acid (EPA) ratio and a lower content of n-3 LC-PUFA to that of fish oil (FO) based diets would enhance deposition of n-3 LC-PUFA in fish tissues. Comparisons were made between fish fed: a FO diet, a blend of 50% rapeseed and 50% tuna oil diet (model oil, MO 1), a blend of 50% rapeseed, 25% tuna and 25% FO diet (MO 2), and a blend of 50% FO and 50% chicken fat diet (FO/CF). The dietary DHA: EPA ratio was in the order MO 1>MO 2>FO/CF similar to FO. Dietary n-3 LC-PUFA content was approximately 2-fold lower in fish fed the MO 1, MO 2 and FO/CF diets compared to the FO diet. There were comparable amounts of n-3 LC-PUFA in the muscle of FO, MO 1 and FO/CF fed fish. Our findings indicate that the right balance in both absolute and relative amounts of EPA and DHA can promote n-3 LC-PUFA retention.

Codabaccus MB, Bridle AR, Nichols PD, Carter CG (2012) **Restoration of Fillet n-3 Long-Chain Polyunsaturated Fatty Acid Is Improved by a Modified Fish Oil Finishing Diet Strategy for Atlantic Salmon (*Salmo salar* L.) Smolts Fed Palm Fatty Acid Distillate.** *J Agric Food Chem* 60:458-466

Reducing the lipid content in fish prior to feeding a fish oil finishing diet (FOFD) has the potential to improve n-3 long-chain ( $\geq C(20)$ ) polyunsaturated fatty acid (LC-PUFA) restoration. This study had two main objectives: (1) determine whether feeding Atlantic salmon smolt a 75% palm fatty acid distillate diet (75PFAD) improves the apparent digestibility (AD) of saturated fatty acids (SPA) and (2) examine whether a food deprivation period after growth on 75PFAD leads to higher n-3 LC-PUFA restoration in the fillet when applying a FOFD. The AD of SPA was higher for 75PFAD compared to that of a fish oil (FO) diet. The relative level (as % total fatty acids (FA)) of n-3 LC-PUFA was higher in unfed fish compared to that in continuously fed fish after 21 and 28 day FOFD periods, respectively. Our results suggest that a food deprivation period prior to feeding a FOFD improves the efficiency of n-3 LC-PUFA restoration in the fillet of Atlantic salmon smolt.

Crosbie PBB, Bridle AR, Cadoret K, Nowak BF (2012) **In vitro cultured *Neoparamoeba perurans* causes amoebic gill disease in Atlantic salmon and fulfils Koch's postulates.** *Int J Parasitol* 42:511-515

Amoebic gill disease (AGD) in marine farmed Atlantic salmon is of growing concern worldwide and remains a significant health issue for salmon growers in Australia. Until now the aetiological agent, *Neoparamoeba perurans*, has not been amenable to in vitro culture and therefore Koch's postulates could not be fulfilled. The inability to culture the amoeba has been a limiting factor in the progression of research into AGD and required the maintenance of an on-going laboratory-based infection to supply infective material. Culture methods using malt yeast agar with sea water overlaid and subculturing every 3-4 days have resulted in the establishment of a clonal culture of *N. perurans*, designated clone 4. Identity of the amoeba was confirmed by PCR. After 70 days in culture clone 4 infected Atlantic salmon, causing AGD, and was re-isolated from the infected fish. Diagnosis was confirmed by histology and the infectious agent identified by PCR and in situ hybridisation using oligonucleotide primers and probes previously developed and specific to *N. perurans*. This study has fulfilled Koch's postulates for *N. perurans* as a causative agent of AGD and illustrates its free-living and parasitic nature.

Crosbie PBB, Bridle AR, Nowak BF (2012) **Extension funding application - AGD Vaccine Phase III.** FRDC Project No 2008/218: FRDC Salmon Subprogram. University of Tasmania



and Fisheries Research & Development Corporation, Launceston, Tasmania

We have fulfilled Koch's postulates for *Neoparamoeba perurans* and showed that this species of amoeba causes Amoebic Gill Disease in Atlantic salmon by infecting fish with *N. perurans* after 20 weeks in culture. This was only possible when we developed successful in vitro culture methods for this species. This now allows further research into the biology of the amoeba. We determined generation time for cultured *N. perurans* to be 26.66 h. We have investigated the role of saccharide-inhibitable lectins. The results from the experiments looking at the ability of monosaccharides to inhibit amoebae attachment to the gills of salmon indicated that when amoebae were exposed to galactose at concentrations of 50 and 5 mM there was significantly less pathology seen after 72 h compared to the other galactose concentrations and the positive control.

Flood MJ, Purser GJ, Carter CG (2012) **The effects of changing feeding frequency simultaneously with seawater transfer in Atlantic salmon *Salmo salar* L. smolt.** *Aquacult Int* 20:29-40

The effects on group feed intake and growth performance of changing feeding frequency simultaneously with seawater transfer of Atlantic salmon *Salmo salar* were investigated. Two feeding regimes of one feed per day (1F) and eight feeds per day (8F) were compared for groups of Atlantic salmon in freshwater. Following seawater transfer groups were either fed on their pre-transfer regimes or swapped to the other regime, resulting in four treatments (n = 3). Regardless of the pre-transfer feeding regime, 1F groups had significantly (P < 0.05) lower feed intake immediately following transfer than 8F groups. However, groups that underwent a change in feeding frequency did not have significantly lower feed intake immediately following transfer than those kept on the pre-transfer feeding regime. During the freshwater phase, overall mean feed intake of 8F groups was significantly greater than 1F groups, whilst there was no significant difference in mean feed intake for any of the treatments during the seawater phase. Growth was better in groups fed 8F in freshwater than those fed 1F in freshwater regardless of post-transfer feeding regime. There were no significant differences in growth depensation throughout the experiment, suggesting that there were no overall differences in hierarchy strength amongst treatments. The main finding of this experiment was that a single meal per day immediately following seawater transfer results in initially significantly lower feed intake than the higher feeding frequency regardless of pre-transfer feeding regime, consequently multiple daily feeds is the recommended feeding regime following seawater transfer.

Morrison RN, Young ND, Nowak BF (2012) **Description of an Atlantic salmon (*Salmo salar* L.) type II interleukin-1 receptor cDNA and analysis of interleukin-1 receptor expression in amoebic gill disease-affected fish.** *Fish Shellfish Immunol* 32:1185-1190

Previously, we showed that IL-1b transcription is induced in the gills of amoebic gill disease (AGD) affected fish in an AGD lesion-restricted fashion. However, in this environment, there is very little evidence of inflammation on histopathological or transcriptional levels and we hypothesised that aberrant signalling may occur. As a first step in investigating this issue, we cloned and sequenced the Atlantic salmon IL-1 receptor type II (IL-1RII) mRNA, and then examined the expression of both the IL-1RI (IL-1 receptor-like protein) and II during *Neoparamoeba perurans* infection. In gill lesions from AGD affected fish, a step-wise temporal increase in the relative expression of IL-1b coincided with a significant reduction in IL-1RI, whereas the IL-1RII mRNA remained unchanged. Down-regulation of IL-1RI could explain the paucity of inflammation in affected tissue, although simultaneous up-regulation of

IL-1b-inducible transcripts indicated that this is not due to a complete blockage of the IL-1RI pathway. Rather, it appears that IL-1RI transcription is reduced and this rate limits the effects of chronic IL-1b overexpression.

Nowak B (2012) *Neoparamoeba perurans*. In: Woo PTK, Buchmann K (eds) **Fish Parasites: Pathobiology and Protection**. CAB International, London, United Kingdom

*Neoparamoeba perurans* Young, Crosbie, Adams, Nowak et Morrison 2007 is a marine amoeba (Amoebozoa, Dactylopodida) which colonizes fish gills resulting in outbreaks of amoebic gill disease (AGD) in fish farmed in marine. The transmission is horizontal. Either cohabitation with infected fish or exposure to amoebae isolated from the gills of fish affected by AGD resulted in successful experimental infections. As few as 10 amoebae/L of water cause amoebic gill disease in naïve Atlantic salmon. There is a positive correlation between the number of amoebae in the water and the severity of the lesions. While our knowledge of *N. perurans* and AGD has significantly increased during the last 10 years there are still many unanswered questions about the pathogen and the disease. As the disease is increasingly affecting fish farmed in marine environment and is one of the more significant emerging diseases in mariculture, further research is necessary to improve our ability to manage AGD.

2011

Adams MB (2011) **Effect of AQUI-S® anaesthesia during freshwater treatment on AGD re-development**. AQUI-S New Zealand

Barnes R, King H, Carter CG (2011) **Hypoxia tolerance and oxygen regulation in Atlantic salmon, *Salmo salar* from a Tasmanian population**. *Aquaculture* 318:397-401

For aquatic ectotherms, increasing water temperatures cause an exponential increase in metabolic rate and decreasing oxygen solubility. Fish species that regulate their metabolic rate to low dissolved oxygen concentrations are understood to be hypoxia tolerant whereas salmonid fish are considered to be classic metabolic conformers and their metabolic rate is dependent on the environmental oxygen concentration. This study examined Atlantic salmon, *Salmo solar*, undergoing a progressive hypoxia at optimal temperatures and at temperatures nearing the upper thermal tolerance limit for the species to determine if metabolic regulation occurred. Oxygen consumption was measured on individual Atlantic salmon (150.7 +/- 40.8 g) in 66-L static respirometers; oxygen measurements were taken every 5 min until the fish lost equilibrium. Metabolic regulation was observed at all temperatures and occurred in 67, 50 and 50% of the fish at 14, 18 and 22 degrees C, respectively. The plateau metabolic rate ( $VO_{2PL}$ ) was 293.4 +/- 24.5 mg.kg.h(-1) at 22 degrees C which was significantly higher than in the 14 and 18 degrees C treatments (191.1 +/- 24.5 and 203.9 +/- 12.6 mg.kg.h(-1), respectively). This difference was also reflected in the critical oxygen threshold ( $P_{crit}$ ) where the value for the 22 degrees C treatment (4.59 +/- 0.32 mg.L(-1)) was significantly higher than those of the 14 and 18 degrees C treatments (3.46 +/- 0.14 and 3.39 +/- 0.26 mg.L(-1) respectively). These results indicate that some fish from the Tasmanian population of Atlantic salmon have the ability to regulate metabolic rate to low oxygen concentrations and therefore show a relatively high degree of hypoxia tolerance.

Barnes R, King H, Carter CG (2011) **Oxygen regulation in Tasmanian Atlantic salmon. Project No 2010/203 Project Report:** Atlantic Salmon Subprogram. Institute for Marine and Antarctic Studies, Launceston, Tasmania

This project primarily addresses physiological "robustness" in Atlantic salmon. Critical issues for the Tasmanian salmon industry include understanding the physiological response of salmon to hypoxia due to decreased dissolved oxygen (DO) and increases in other dissolved metabolic wastes. The Tasmanian salmon industry has a selective breeding program and robustness/resilience is one of the main characteristics the industry is interested in developing further. Meanwhile, the Industry is investing heavily in order to manage the DO environment experienced by its fish. For example, SALTAS spends more than \$500,000 annually (i.e., 10% of its operational budget) on oxygen to help combat the high temperature/low DO issues.

This research was primarily designed to gather baseline data on a wide range of parameters relating to decreasing dissolved oxygen. The current project has confirmed that oxygen regulation does occur in the Tasmanian population of Atlantic salmon. A number of key findings regarding oxygen regulation have resulted from this project and include differences among pedigreed families, differences between the metabolic rates of oxygen regulators and conformers, differences between male and female fish and that oxygen regulation occurs under both static and flow conditions.

Bridle A, Nosworthy E, Polinski M, Nowak B (2011) **Evidence of an Antimicrobial-Immunomodulatory Role of Atlantic Salmon Cathelicidins during Infection with *Yersinia ruckeri*.** *Plos One* 6

Cathelicidins are a family of antimicrobial peptides that act as effector molecules of the innate immune system with broad spectrum antimicrobial properties. These evolutionary conserved cationic host-defence peptides are integral components of the immune response of fish, which are generally believed to rely heavily on innate immune defences to invading pathogens. In this study we showed that Atlantic salmon cathelicidin 1 and 2 (asCATH1 and asCATH2) stimulated peripheral blood leukocytes increasing the transcription of the chemokine interleukin-8. Further, functional differences were identified between the two cathelicidins. In the presence of serum, asCATH1 displayed greatly diminished host haemolytic activity, while the constitutively expressed asCATH2 had no haemolytic activity with or without serum. These findings support our hypothesis that fish cathelicidins exert their primary antimicrobial action at the site of pathogen invasion such as epithelial surfaces. Further, we hypothesise that like their mammalian counterparts in the presence of serum they act as mediators of the innate and adaptive immune response via the release of cytokines thus indirectly protecting against a variety of pathogens. We highlight the importance of this immunomodulatory role from the involvement of asCATHs during an infection with the fish pathogen *Yersinia ruckeri*. While we were able to demonstrate in vitro that asCATH1 and 2, possessed direct microbicidal activity against the fish pathogen, *Vibrio anguillarum*, and a common gram negative bacterium, *Escherichia coli*, little or no bactericidal activity was found against *Y. ruckeri*. The contribution of either asCATH in the immune response or as a potential virulence factor during yersiniosis is highlighted from the increased expression of asCATH1 and 2 mRNA during an in vivo challenge with *Y. ruckeri*. We propose that Atlantic salmon cathelicidins participate in the interplay between the innate and adaptive immune systems via the release of cytokines enabling a more effective response to invading pathogens.

Carter CG, Hauler RC (2011) **Effect of high digestible protein to digestible energy ratio on lysine utilisation by Atlantic salmon, *Salmo salar* L., parr.** *Aquaculture* 311:209-214

This study investigated the efficiency of lysine utilisation for liveweight gain (LG), protein gain (PG) and lysine gain (LysG) in Atlantic salmon (*Salmo solar* L) parr fed a diet with a high digestible protein (DP) to digestible energy (DE) ratio. Twelve diets containing 10.15 to 20.79 g dietary digestible lysine (DDLys) kg<sup>-1</sup> at a constant 25.0 g DP MJ DE<sup>-1</sup> (25.0 DP DE<sup>-1</sup>) were fed at a fixed ration for 50 days. With increasing DDLys there was a significant (P<0.001) linear increase in LG. Whole-body crude protein and lysine content increased with increasing wet weight (W), with weight exponents b (W(b)) of 1.58 and 2.47, respectively. There were significant linear relationships between digestible lysine intake (DLysI) and LG, PG and LysG. The relationship between DLysI and LysG predicted a maintenance lysine requirement of 7.7 mg kg<sup>-0.75</sup> d<sup>-1</sup> and an efficiency of 77% for lysine utilisation for lysine gain above maintenance lysine intake. To further examine the effect of the DP DE ratio on lysine utilisation comparison was made to a similar study that used a lower DP DE ratio of 19.8 DP DE<sup>-1</sup>. Comparison between linear relationships for LG, PG and LysG from the two experiments showed that there were no significant differences between the efficiency of lysine utilisation (slopes) for LG, PG or LysG, nor were there significant differences between the elevations for PG and LysG. This study demonstrated that at two fixed but different protein and energy intakes the dietary DP DE ratio did not influence the efficiency of lysine utilisation for lysine gain over a range of dietary lysine and digestible lysine intakes.

Carter CG, Sajjadi M (2011) **Low fishmeal diets for Atlantic salmon, *Salmo salar* L., using soy protein concentrate treated with graded levels of phytase.** *Aquacult Int* 19:431-444

The experiment aimed at determining the efficient use of phytase (Phy) in Atlantic salmon diets that had low (4.5%) fishmeal and contained 60% soy protein concentrate (SPC). Phytase was either included at 250, 500, 1,000 or 4,000 U Phy kg<sup>-1</sup> diet or the SPC was pre-treated prior to making diets using 250, 500 or 1,000 U Phy kg<sup>-1</sup> SPC. Fish were fed the experimental diets for 12 weeks, and there were no differences in survival among treatments nor were there differences in growth performance between the phytase-pre-treated SPC diets. Feed intake and weight gain were significantly lower for diets supplemented below 1,000 U Phy kg<sup>-1</sup> compared to all other diets. Apparent digestibility (AD) of phosphorus was significantly lower without the use of phytase (45.43 +/- A 2.06%) than for all other treatments. AD phosphorus increased from 55.70 +/- A 1.81% at the lowest phytase supplementation (250 U Phy kg<sup>-1</sup>) to 80.87 +/- A 2.12% at the highest (4,000 U Phy kg<sup>-1</sup>). There was no difference in AD phosphorus between the diet with the highest supplementation (4,000 U Phy kg<sup>-1</sup>) and the pre-treated diets. There were no differences in whole-body dry material, crude protein or total lipid, whereas bone ash was significantly lower for diets supplemented below 1,000 U Phy kg<sup>-1</sup>. Ash and phosphorus in the whole body and bone increased with increasing added phytase. At and above an inclusion of 1,000 U Phy kg<sup>-1</sup>, bone ash (51.26 +/- A 0.12% bone weight) and bone phosphorus (11.21 +/- A 0.04% bone weight) reached concentrations that were no different to the pre-treated diets. In conclusion, phytase improved Atlantic salmon's growth performance fed low fishmeal diets containing SPC, and at least 1,000 U Phy kg<sup>-1</sup> diet was required to have the same effect as pre-treatment of SPC with 250 U Phy kg<sup>-1</sup> SPC.

Codabaccus BM, Bridle AR, Nichols PD, Carter CG (2011) **An extended feeding history with a stearidonic acid enriched diet from parr to smolt increases n-3 long-chain**

**polyunsaturated fatty acids biosynthesis in white muscle and liver of Atlantic salmon (*Salmo salar* L.).** *Aquaculture* 322:65-73

Vegetable oils (VO) are globally accepted alternatives for fish oil (FO) in aquafeeds. The lack of n-3 long-chain ( $\geq$  C-20) polyunsaturated fatty acids (n-3 LC-PUFA) in VO is a major constraint. Echium oil (EO), rich in stearidonic acid (SDA), has the potential to increase endogenous n-3 LC-PUFA biosynthesis. We tested whether feeding Atlantic salmon an EO-based diet in both freshwater and seawater would increase n-3 LC-PUFA levels by comparing the fatty acid (FA) profiles in liver and white muscle to fish fed FO and rapeseed oil (RO)-based diets. The gene expression of n-3 LC-PUFA biosynthetic enzymes was measured to demonstrate the underlying mechanism of n-3 LC-PUFA biosynthesis. After prolonged feeding with EO diet from freshwater to seawater phases, EO fish had higher n-3 LC-PUFA levels in both liver and white muscle compared to RO fish. However, FO fish had the highest n-3 LC-PUFA levels in examined tissues. Delta 6 Desaturase gene expression in liver and white muscle was up-regulated in RO fish only, liver Delta 5 desaturase gene expression was reduced in seawater and liver FA elongase gene expression was regulated by an interaction between dietary oil and environment. This study showed that feeding Atlantic salmon from parr to smolt using an SDA enriched diet increases n-3 LC-PUFA biosynthesis in liver and white muscle through increased supply of the n-3 LC-PUFA precursor SDA. The down regulation of Delta 5 desaturase gene expression in the liver of seawater fish may explain environmental differences in n-3 LC-PUFA biosynthesis.

Codabaccus MB, Bridle AR, Nichols PD, Carter CG (2011) **Effect of feeding Atlantic salmon (*Salmo salar* L.) a diet enriched with stearidonic acid from parr to smolt on growth and n-3 long-chain PUFA biosynthesis.** *Brit J Nutr* 105:1772-1782

Vegetable oils (VO) have become the predominant substitute for fish oil (FO) in aquafeeds; however, the resultant lower content of n-3 long-chain ( $\geq$  C20) PUFA (n-3 LC-PUFA) in fish has put their use under scrutiny. The need to investigate new oil sources exists. The present study tested the hypothesis that in Atlantic salmon (*Salmo salar* L.), a high intake of stearidonic acid (SDA) from Echium oil (EO) would result in increased n-3 LC-PUFA biosynthesis due to a lower requirement for Delta 6 desaturase. Comparisons were made with fish fed on diets containing rapeseed oil (RO) and FO in freshwater for 112d followed by 96d in seawater. EO fish had higher whole-carcass SDA and eicosatetraenoic acid (ETA) in freshwater and prolonged feeding on the EO diet in seawater resulted in higher SDA, ETA, EPA and docosapentaenoic acid (DPA) compared with RO fish. Fatty acid mass balance of freshwater fish indicated higher biosynthesis of ETA and EPA in EO fish compared with fish fed on the other diets and a twofold increase in n-3 LC-PUFA synthesis compared with RO fish. In seawater, n-3 biosynthetic activity was low, with higher biosynthesis of ETA in EO fish and appearance of all desaturated and elongated products along the n-3 pathway. SDA-enriched VO are more suitable substitutes than conventional VO from a human consumer perspective due to the resulting higher SDA content, higher total n-3 and improved n-3: n-6 ratio obtained in fish, although both VO were not as effective as FO in maintaining EPA and DHA content in Atlantic salmon.

Costa AA, Leef MJ, Bridle AR, Carson J, Nowak BF (2011) **Effect of vaccination against yersiniosis on the relative percent survival, bactericidal and lysozyme response of Atlantic salmon, *Salmo salar*.** *Aquaculture* 315:201-206

The bacterium *Yersinia ruckeri* serovar O1b causes yersiniosis in Atlantic salmon, *Salmo salar*, in the southern hemisphere. Despite vaccination this disease has resulted in

significant hatchery losses in the Tasmanian Atlantic salmon aquaculture industry. A poor response to vaccination in juveniles, 1–5 g, has led to the investigation of the suitability of the current formalin killed whole-cell vaccine Yersinivac-B. In this study trypsin was added to the Yersinivac-B to expose the bacteria's protective O-antigen to make the vaccine more immunogenic. At six weeks post vaccination, the effect of Yersinivac-B and the novel trypsinated Yersinivac-B vaccine on body mucus lysozyme and mucus and serum bactericidal activity of fish was determined over a 48 h period following challenge with *Y. ruckeri*. Body and gill mucus lysozyme and mucus and serum bactericidal activity was also determined in surviving fish at 10 weeks post *Y. ruckeri* challenge. Following the challenge period of 14 days the trypsinated Yersinivac-B fish demonstrated a significantly higher percent survival compared to the Yersinivac-B and control unvaccinated fish. Body mucus lysozyme concentration was also significantly elevated at 8 h post challenge in the trypsinated Yersinivac-B fish compared to controls. This variable however appears unlikely to play a significant role in protection as positive bactericidal activity was not found in the mucus of any fish following challenge. Bactericidal activity was not observed in the serum or mucus of any challenge survivors. At 8 h post challenge the trypsinated Yersinivac-B fish demonstrated the highest serum bactericidal activity. However, the unvaccinated control fish also displayed positive serum bactericidal activity despite being unlikely to have been previously exposed to *Y. ruckeri*. A significantly higher gill mucus lysozyme concentration in control survivors compared to vaccinated fish suggests that this response may be important in the protection of unvaccinated fish against yersiniosis. This research has highlighted the potential use of trypsin to increase the efficacy of Yersinivac-B. It has also contributed to better understanding of the role of humoral immune responses during a *Y. ruckeri* challenge.

Nowak BF, Hayward CJ, Gonzalez L, Bott NJ, Lester RIG (2011) **Sea lice infections of salmonids farmed in Australia.** *Aquaculture* 320:171-177

Sea lice cause significant issues in the mariculture of salmonids. However, there have been no reports about sea lice from salmonid farming in Australia. Here, we investigated the presence of sea lice on salmonids in Australian mariculture. *Caligus longirostris* was present on Atlantic salmon and rainbow trout (archival samples only) farmed in Tasmania. *C. longirostris* was found on salmon only from two cages, in which fish had not been bathed in fresh water (as is routinely practiced in Australia to treat Amoebic Gill Disease) for several months. A prevalence of 1.5% was recorded in one cage with Atlantic salmon of average weight 5.51 kg, which were graded for a selective breeding program and as a result not treated with fresh water for 236 days. A prevalence of 1.9% was found in an experimental cage in which the salmon (average weight 5.62 kg) were not bathed for 294 days. A total of five individuals of *C. longirostris* were found during the field survey—four females and one male. All females were non-ovigerous. In addition, archival samples of caligids collected from rainbow trout farmed in Western Australia were identified as *C. chistos*. As both of these species of caligids have been reported from wild marine fish in Australia, their presence on farmed salmonids is an example of host-switching. Bayesian Inference analyses of mitochondrial cytochrome c oxidase 1 gene (*cox1*) and partial 28S ribosomal DNA showed that *C. longirostris* formed highly supported clades with *C. elongatus* and *C. gurnadi*.

Pinkiewicz TH, Purser GJ, Williams RN (2011) **A computer vision system to analyse the swimming behaviour of farmed fish in commercial aquaculture facilities: A case study using cage-held Atlantic salmon.** *Aquacult Eng* 45:20-27

Knowledge of fish behaviour plays an important role in aquaculture farm management. Video systems are the most common and cost-effective way of observing behaviours in commercial aquaculture operations. However long term observation is not feasible due to a limited ability to analyse footage manually. This paper describes preliminary findings obtained via computer vision software that was developed to automatically analyse fish movement and behaviours in aquaculture sea cages. Results show that the system is capable of detecting fish shapes in video recordings and from these shapes quantifying changes in swimming speed and direction continuously throughout the day. Also variations between days were detected and these may have been associated with the daily shift in the tidal cycle. The system has the potential to act as an alarm to farm operators, informing them about unusual fish behaviours on a continuous, real-time basis. It also has potential to assist in the evaluation of fish welfare.

Safari O, Farhangi M, Carter CG, Yakhchali B, Shawrang P (2011) **Effect of sieve size on chemical composition and functional properties of canola meal (*Brassica napus*) protein fractions as fishmeal replacement.** *Afr J Biotechnol* 10:11764-11771

Canola meal (CM) use in animal nutrition is limited due to the inclusion of various antinutritional factors (ANF). This study aimed to evaluate the effect of sieving on nutrient and ANF contents of CM. Five sieves with mesh size (diameter) of 16, 18, 20, 30 and 40 were used in this regard. With an increase in the mesh size, the recovery percentage significantly decreased (73.7 vs. 18.3), while crude protein content (370.7 vs. 378.1 g/kg), gross cost (0.47 vs. 1.91 US\$/kg) and protein unit cost (1.27 vs. 1.66 US\$/kg) were significantly increased ( $P < 0.05$ ). In the next stage, only the sieved canola meal obtained through mesh size 16, 18 and 20 was selected for further investigation. The amounts of crude protein, crude fat and total NSP of the processed products were not significantly differed. However, the amounts of acid detergent fiber (218.2 g/kg vs. 206.7 g/kg) and neutral detergent fiber (330.8 g/kg vs. 319.1 g/kg) significantly decreased by increasing mesh size. Glucosinolates (20.67 vs. 21.82  $\mu\text{mol/g DM}$ ) and phytate (40.6 g/kg vs. 62.0 g/kg) had significant increment ( $P < 0.05$ ) on the other hand. Considering the weighted averages of different measured variables from PCA, the mesh size 16 was selected for use in the production of a canola protein concentrate.

2010

Bridle AR, Crosbie PBB, Cadoret K, Nowak BF (2010) **Rapid detection and quantification of *Neoparamoeba perurans* in the marine environment.** *Aquaculture* 309:56-61

The protozoan parasite *Neoparamoeba perurans* is the causative agent of amoebic gill disease (AGD) and an emerging threat to the aquaculture of marine finfish species worldwide. Despite several years of research and continuing efforts the culture of *N. perurans* remains elusive. As a result current detection methods rely on molecular techniques namely, polymerase chain reaction (PCR) and in situ hybridization (ISH). In this study, a total DNA extraction technique combined with a highly sensitive real-time PCR assay using primers specific for *N. perurans* was developed and validated. Using this method we were able to detect a single 18S rRNA gene copy and readily detected *N. perurans* with the lowest detection limit for *N. perurans* cells spiked in sea water being one cell (100% detection rate). The genome of *N. perurans* contains multiple copies of the 18S rRNA gene, and an estimate of 2880 copies per cell was derived from real-time PCR

calibration curves for cell suspensions and plasmid DNA. The developed method was applied to seawater samples collected from both an experimental AGD infection tank and a variety of environmental sites including those used to culture Atlantic salmon (*Salmo salar* L.) in Tasmania, Australia. Detectable populations were highly abundant from sites in and closely surrounding cage culture of Atlantic salmon. Furthermore, the method when applied to gill swabs from an on-farm gill pathology assessment demonstrated that non-destructive semi-quantitative analysis of amoebae loads from these fish was possible. Not only does this study provide evidence that *N. perurans* is a free-living amoeba but the quantitative nature of this novel assay clearly demonstrates the impact of marine cage aquaculture on the prevalence of this fish pathogen and is a step towards establishing the distribution of *N. perurans* in the marine environment and its relationship with AGD outbreaks.

Carter CG, Katersky RS, Barnes J, Hauler RC, Bridle AR (2010) **Redefining nutrient requirements of fish in sub-optimum environments**. In: Crovetto GM (ed) Energy and Protein Metabolism and Nutrition, Book 127. EAAP

Provided protein and energy requirement modelling for Atlantic salmon held under sub-optimum conditions of elevated temperature and low dissolved oxygen.

Crosbie PBB, Bridle AR, Leef MJ, Nowak BF (2010) **Effects of different batches of *Neoparamoeba perurans* and fish stocking densities on the severity of amoebic gill disease in experimental infection of Atlantic salmon, *Salmo salar* L.** *Aquacult Res* 41:e505-e516

Currently, there are two methods of inducing laboratory-based amoebic gill disease (AGD) in Atlantic salmon, *Salmo salar* L.: cohabitation with infected fish or exposure to a suspension of amoebae. Amoebic gill disease cannot be induced with cultured amoebae; therefore, the only source of the infective organism is salmon with the disease. For experimental purposes and to maintain pathogen supply, salmon are kept in an infection tank and amoebae are isolated from salmon once the disease establishes. In this way, discrete batches of amoebae are collected periodically. This study investigated the infective ability of different batches of amoebae. Furthermore, the effect of stocking density of salmon on the progression of AGD was also examined. The infective ability of different batches of amoebae isolated periodically from AGD-affected salmon varied in terms of quantifiable pathology. Salmon stocking density had a significant impact on survival after amoebae challenge, with morbidity beginning 23 days post challenge in tanks stocked at 5.0 kg m<sup>-3</sup> and 29 days for those stocked at 1.7 kg m<sup>-3</sup>. For uniform initiation of AGD in multiple tanks, amoebae batches should be equally divided and added to tanks until the required concentration is reached and to maintain a standard biomass between replicate tanks and treatments.

Nowak BF, Bryan J, Jones SRM (2010) **Do salmon lice, *Lepeophtheirus salmonis*, have a role in the epidemiology of amoebic gill disease caused by *Neoparamoeba perurans*?** *J Fish Dis* 33:683-687

Amoebic gill disease (AGD) is an acute to chronic proliferative condition of farmed Atlantic salmon caused by *Neoparamoeba perurans* (see Young, Crosbie, Adams, Nowak & Morrison 2007; Young, Dykova, Snekvik, Nowak & Morrison 2008a). AGD and *N. perurans* have been reported in most salmon-producing countries except Canada. In western North America, AGD occurs in Washington State but not in the adjacent British Columbia (B.C.). Despite the ubiquitous distribution of the disease and its causative agent, reservoir populations of the amoeba and the mechanism(s) of transmission to and among farmed fish have not been elucidated. The purpose of this study was to conduct a preliminary survey for



reservoirs of *N. perurans* at or adjacent to salmon populations suspected to be affected with AGD.

Taylor RS, Crosbie PB, Cook MT (2010) **Amoebic gill disease resistance is not related to the systemic antibody response of Atlantic salmon, *Salmo salar* L.** *J Fish Dis* 33:1-14  
Amoebic gill disease (AGD) is a proliferative gill tissue response caused by *Neoparamoeba perurans* and is the main disease affecting Australian marine farmed Atlantic salmon. We have previously proposed that macroscopic gill health ('gill score') trajectories and challenge survival provide evidence of a change in the nature of resistance to AGD. In order to examine whether the apparent development of resistance was because of an adaptive response, serum was sequentially sampled from the same individuals over the first three rounds of natural AGD infection and from survivors of a subsequent non-intervention AGD survival challenge. The systemic immune reaction to 'wildtype' *Neoparamoeba* sp. was characterized by Western blot analysis and differentiated to putative carbohydrate or peptide epitopes by periodate oxidation reactions. The proportion of seropositive fish increased from 46% to 77% with each AGD round. Antibody response to carbohydrate epitope(s) was immunodominant, occurring in 43-64% of samples. Antibodies that bound peptide epitope were identified in 16% of the challenge survivors. A 1:50 (single-dilution) enzyme-linked immunosorbent assay confirmed a measurable immune titre in 13% of the survivors. There was no evidence that antibodies recognizing wildtype *Neoparamoeba* provided significant protection against AGD.

van Gelderen R, Carson J, Gudkovs N, Nowak B (2010) **Physical characterisation of *Tenacibaculum maritimum* for vaccine development.** *J Appl Microbiol* 109:1668-1676  
*Tenacibaculum maritimum* is a well known fish pathogen worldwide, affecting many fish species including Atlantic salmon in Tasmania, Australia. The aim of this study was to characterise and understand the similarities and differences between the isolates in order to select isolates for later pathogenicity and vaccination trials. Several physical characterisation tests were carried out: whole cell protein profiles, lipopolysaccharide profiles (LPS), extracellular product profiles (ECP), indirect immunofluorescent antibody test (IFAT) and hydrophobicity. The Tasmanian strains of *T. maritimum* appear relatively homogeneous physically, but antigenically different. All isolates were hydrophobic and produce a variety of ECP profiles. There were two isolates that stand out in all assays (89/4747 and 01/0356-7) and showed great variation from the other isolates. All isolates have been confirmed as *T. maritimum*. Based on the tests carried out three isolates were chosen for in vivo trials: 89/4747, 89/4762, 00/3280. This is the first study to characterise *T. maritimum* isolates from Tasmanian waters. The opportunity to develop vaccines for the Tasmanian salmonid aquaculture industry is enhanced by a greater understanding of the physical characteristics of pathogens.

van Gelderen R, Carson J, Nowak B (2010) **Experimentally induced marine flexibacteriosis in Atlantic salmon smolts *Salmo salar*. I. Pathogenicity.** *Dis Aquat Org* 91:121-128

*Tenacibaculum maritimum* causes marine flexibacteriosis in many cultured fish species, including Atlantic salmon *Salmo salar* in Tasmania, Australia. Several aspects of the pathogenicity of this bacterium were investigated in naive Atlantic salmon smolts using different isolates, growth conditions and doses to produce a model of infection. We found that *T. maritimum* is pathogenic to Atlantic salmon using either marine Shieh's or marine Ordal's culture medium. The use of aeration in broth culture produced a dose effect in

challenge due to a 'clumping' of the bacteria during culture. The virulence of a strain appears to be connected with this 'clumping', the more adherent the cells, the more pathogenic the strain. Differences in virulence between 3 strains was apparent, with 1 of the strains (89/4747) being non-pathogenic and unable to produce disease in the host. The 2 other strains (89/4762, 00/3280) were highly virulent, resulting in 100% mortalities within 3 d. A reproducible model of infection has been established in the present study using strain 89/4762. Results from the present study provide a better insight into the nature of the disease.

Villavedra M, To J, Lemke S, Birch D, Crosbie P, Adams M, Broady K, Nowak B, Raison RL, Wallach M (2010) **Characterisation of an immunodominant, high molecular weight glycoprotein on the surface of infectious *Neoparamoeba* spp., causative agent of amoebic gill disease (AGD) in Atlantic salmon.** *Fish Shellfish Immunol* 29:946-955

Amoebic gill disease can be experimentally induced by the exposure of salmonids to *Neoparamoeba* spp. freshly isolated from infected fish, while cultured amoebae are non-infective. Results from our previous work suggested that one key difference between infectious and non-infectious *Neoparamoeba* were the highly glycosylated molecules in the glycocalyx. To characterise these surface glycans or glycoproteins we used a monoclonal antibody (mAb 44C12) specific to a surface molecule unique to infective parasites. This mAb recognised a carbohydrate epitope on a high molecular weight antigen (HMWA) that make up 15-19% of the total protein in a soluble extract of infectious parasites. The HMWA consisted of at least four glycoprotein subunits of molecular weight (MW) greater than 150 kDa that form disulfide-linked complexes of MW greater than 600 kDa. Chemical deglycosylation yielded at least four protein bands of approximate MW 46, 34, 28 and 18 kDa. While a similar HMWA complex was present in non-infective parasites, the glycoprotein subunits were of lower MW and exhibited differences in glycosylation. The four glycoproteins subunits recognised by mAb 44C12 were resistant to degradation by PNGase F, PNGase A, O-glycosidase plus beta-1, 4-galactosidase, beta-N-acetylglucosaminidase and neuraminidase. The major monosaccharides in the HMWA from infectious parasites were rhamnose, fucose, galactose, and mannose while sialic acids were absent. The carbohydrate portion constituted more than 90% of the total weight of the HMWA from infectious *Neoparamoeba* spp. Preliminary results indicate that immunisation of salmon with HMWA does not lead to protection against challenge infection; rather it may even have an immunosuppressive effect

2009

Adams MB, Gross KA, Nowak BF (2009) **Branchial mechanical injury does not accelerate the progression of experimentally induced amoebic gill disease (AGD) in Atlantic salmon *Salmo salar* L.** *Aquaculture* 290:28-36

It remains unclear whether the pathological severity of AGD can be exacerbated by injury to the branchial epithelium; a circumstance that may arise in situ due to contact with harmful water-borne agents. Here, results from an experimental laboratory infection are given, testing the assertion that branchial injury would accelerate the pathological development of AGD. The lamellar epithelium of the left and right, first and second, anterior hemibranchs of Atlantic salmon were damaged by physical abrasion and subsequently exposed to *Neoparamoeba perurans*. Control groups of non-damaged/infected and damaged/non-infected fish were synchronously maintained for 32 days. Further undamaged fish were

selected for mechanical damage at day 16, following the onset of AGD. Gills were collected from each group and processed for routine histology at 2, 4, 8, 16, 24 & 32 days post-exposure to *Neoparamoeba* spp. Mechanical injury initially resulted in oedema, telangiectasis, haemorrhaging and leucocytic infiltration. During subsequent recovery, the lamellae were fused with undifferentiated cells, mucous cells and leucocytes. The degree of lamellar fusion (due to mechanical injury) had dissipated substantially by the conclusion of the trial. Trophozoites of *N. perurans* were largely undetected upon mechanically injured portions of the gills for the duration of the experiment. Concurrently, there was no significant difference between injured and control fish in terms of AGD development over time, neither was there a significant difference within fish when comparing damaged and undamaged hemibranchs. Together, these data suggest mechanical injury does not present an enhanced opportunity for attachment and/or colonization of the gill epithelium in Atlantic salmon during experimental infection with *N. perurans*.

Carter CG, Irwin K, Glencross B (2009) **Evaluation of the variability in the apparent digestible value of *Lupinus angustifolius* and *L. luteus* ingredients to Atlantic salmon, *Salmo salar*.** In: Glencross BD (ed) *Aquaculture Feed Grants Program Final report* to Grains and Fisheries Research and Development Corporation. Department of Fisheries, Western Australia, North Beach, Western Australia

The apparent digestibility of nutrients from kernel meals made from two narrow-leafed lupin (*Lupinus angustifolius*) and two yellow lupin (*L. luteus*) varieties were compared. Two additional ingredients, a *L. luteus* protein concentrate and a soybean reference, were also included. The ingredients were added to a basal fish meal mash at 30% and the diets extruded. Each diet was fed to three groups, one in each of three time-blocks, of Atlantic salmon (500 g) kept in 2000 l of seawater at 15°C. After 8 days the salmon were stripped of faeces and apparent digestibility calculated. There was no significant ( $P > 0.2$ ) difference between ingredient apparent digestibility for crude lipid. The reference diet showed a low crude protein digestibility in one time period, when these data were removed there were significant differences between ingredient apparent digestibility for crude protein. Soybean and *L. angustifolius* cv. Myallie (MKM) had significantly lower AD for crude protein than *L. luteus* cv. Wodjil kernel meal (LKM) and protein concentrate (LPC). A *L. angustifolius* cv. Belara kernel meal (BKM) was not significantly different to any of the other ingredients. A second experiment aimed to determine the apparent digestibility of several varieties of *Lupinus angustifolius* kernel meals fed to seawater Atlantic salmon (*Salmo salar* L.). Faecal samples were stripped after 10 days on 5 experimental feeds containing 70% of a reference diet (REF) and 30% of either *L. angustifolius* (cv. Gungarru) kernel meal (GKM), *L. angustifolius* (cv. Mandelup) kernel meal (MaKM), *L. angustifolius* (cv. Myallie) kernel meal (MKM), *L. angustifolius* (cv. Tanjil) kernel meal (TKM), or *L. angustifolius* (cv. 2173M) kernel meal (2173KM). The apparent digestibility for crude protein was significantly higher for GKM and MKM than for MAKM. Gross energy, crude lipid and phosphorus digestibility were not different between ingredients. Ingredient crude protein digestibility was broadly similar to other similar studies.

Carter CG, Irwin K, Glencross B (2009) **Gastrointestinal evacuation rate in seawater Atlantic salmon (*Salmo salar*) fed diets containing fish meal, soybean meal, lupin kernel meals and lupin protein concentrates.** In: Glencross B (ed) *Aquaculture Feed Grants Program Final report* to Grains and Fisheries Research and Development Corporation. Department of Fisheries, Western Australia, North Beach, Western Australia

The experiment aimed to assess the effect of plant proteins on the gastrointestinal evacuation rate (GIER) of Atlantic salmon (*Salmo salar* L.) smolts ( $144.2 \pm 5.8$  g) held at 15°C. Ingredients tested were *L. luteus* protein concentrate (LPC), *L. luteus* (cv Wodjil) kernel meal (LKM), *L. angustifolius* (cv. Belara) kernel meal (BKM), *L. angustifolius* (cv. Myallie) kernel meal (MKM), soybean meal (SBM). A reference mash that included an inert marker (0.1%) was formulated and 5 experimental diets made to include 30% of each plant protein ingredient. Two sets of each diet containing either 0.1% Yttrium oxide or 0.1% Ytterbium oxide as inert markers were made. Calculation of the GIER was based on the replacement of one marker with the second marker in faeces collected after the markers were changed in the diets being fed. A model described by marker (%) =  $(a - d) / ((1 + (T/c) - b) + d)$  was used to derive values for the slope (parameter b) of an S-shaped curve and the time taken for the second marker to replace half the first marker (parameter c). In relation to the time taken for 50% replacement (parameter c), the ingredients were divided into two groups: the kernel meals (LKM, BKM, MKM) had values of 8.5 to 8.8 h compared with 10.2 to 11.4 h for the other ingredients (LPC, REF, SBM). Groupings in the slope value (parameter b) were less obvious although the kernel meals had the lowest values and LPC had the highest value, almost twice that of the lowest, MKM. The higher slope values indicated more of the gut contents tended to be evacuated together whereas the lower slope values indicated a more gradual evacuation. Apparent digestibility for dietary nitrogen was positively correlated with slope ( $r = 0.829$ ;  $P < 0.01$ ;  $n = 6$ ). Overall, the analysis suggested that more of the lupin kernel meals were evacuated sooner but in a more gradual manner. In comparison, the other meals, particularly LPC, remained in the gastrointestinal tract longer but were then evacuated more rapidly in a consolidated mass.

Carter CG, Ward LR, Glencross B (2009) **Biological value to Atlantic salmon of lupin kernel meal compared with soybean at different inclusions and water temperatures.** In: Glencross B (ed) *Aquaculture Feed Grants Program Final report* to Grains and Fisheries Research and Development Corporation. Department of Fisheries, Western Australia, North Beach, Western Australia

The experiment aimed to compare the biological value of a lupin kernel meal (*L. angustifolius* cv Coromup) with fish meal and with soybean at two temperatures and two inclusion levels. Inclusion levels of lupin and soybean were 15 and 25% at 14°C and 15% at 18°C. Diets were formulated to be isonitrogenous and isoenergetic on a gross compositional basis and to have a marginal crude protein content (40%). Inclusion of 15% reflected maximum industry inclusion rates for lupin whereas 25% inclusion reflected a higher level in order to investigate whether performance changed at the higher level. The temperature of 14°C reflected an optimum summer temperature and was compared with an elevated summer temperature of 18°C, but one at which salmon would still be fed commercially. Following initial analysis a two-way ANOVA compared the effects of diet and temperature using a data set restricted to the 15% inclusion. There was no interaction between temperature and diet for any performance parameter analysed and the key results were: for change in weight both diet ( $P = 0.009$ ) and temperature ( $P = 0.001$ ) were significant factors, LM15 and 14°C showed significantly higher change in weight; weight specific feed intake was also significantly higher for LM15 but it was higher at 18°C. This meant growth efficiency was not different between diets but was lower at 18°C. Thus, in terms of growth performance LM15 appeared to be the better diet at both temperatures. However, exposure to plant meals as well as to high temperature, in addition to ingredient effects, contributed to moderate / severe morphological changes observed in the intestinal mucosa.

van Gelderen R, Carson J, Nowak B (2009) **Effect of extracellular products of *Tenacibaculum maritimum* in Atlantic salmon, *Salmo salar* L.** *J Fish Dis* 32:727-731

*Tenacibaculum maritimum* (formerly *Flexibacter maritimus*) is a well-known pathogen in a number of cultured fish species worldwide (Wakabayashi, Hikida & Masumura 1986; Alsina & Blanch 1993; Chen, Henry-Ford & Groff 1995; Handlinger, Soltani & Percival 1997; Ostland, LaTrace, Morrison & Ferguson 1999). It is a marine bacterium that causes necrotic lesions on the body, head, fins and gills, with erosive lesions on the external surface as the prominent clinical sign (Carson, McCosh & Schmidtke 1992). In Australia, the main species affected are Atlantic salmon, *Salmo salar* L., and rainbow trout, *Oncorhynchus mykiss* (Walbaum), in sea-cage culture in Tasmania (Handlinger et al. 1997). Experimental investigation into the pathogenesis of *T. maritimum* showed that challenge at higher doses (c.  $1 \cdot 10^8$  cells mL<sup>-1</sup>) had an acute lethal effect on Atlantic salmon (van Gelderen 2007). Mortalities occurred within days and the clinical sign was the disintegration of the epithelium. In addition, a lack of an inflammatory response is characteristic of early flexibacteriosis lesions. Handlinger et al. (1997) suggested that this was the result of powerful exotoxins that prevent a host response. These findings pointed to a possible role of toxins in the pathogenicity of *T. maritimum* in Atlantic salmon. Effects of *T. maritimum* toxins have been explored in red and black sea bream, *Pagrus major* (Temminck and Schlegel), and *Acanthopagrus schlegeli* (Bleeker) (Baxa, Kawai & Kusuda 1988). In both fish species, extracellular products (ECP) showed insignificant *in vitro* activity; however, this did not correspond with the toxic effects observed *in vivo* with ECP recording the lowest LD50. Baxa et al. (1988) did indicate that the pathogenicity of *T. maritimum* in black and red sea bream may be ascribed in part to ECP. The current study investigated ECP toxicity *in vivo* to observe direct effects rather than *in vitro* activity of different toxins. Further, this study provides the first observations of *T. maritimum* ECP toxicity in Atlantic salmon.

van Gelderen R, Carson J, Nowak B (2009) **Experimental vaccination of Atlantic salmon (*Salmo salar* L.) against marine flexibacteriosis.** *Aquaculture* 288:7-13

*Tenacibaculum maritimum* infections can cause losses in mariculture, however there is no commercially available vaccine in Australia. A vaccination trial was undertaken using 4 groups: Control, IP Control, Vaccine, and Vaccine + Adjuvant. Within the challenge period of 27 days, significant protection was demonstrated in fish injected with the Vaccine + Adjuvant compared to all other groups. RPS values were calculated at 79.6% and 78.0%. Fish vaccinated without adjuvant did show lower mortalities than unvaccinated fish, however, it was not significantly different from both unvaccinated groups. Low RPS values at 27.7% and 22.0% indicate that the vaccine without adjuvant could not provide sufficient protection from a moderate challenge of *T. maritimum*. Side effects of the oil based adjuvant, Freund's incomplete adjuvant (FIA) were noted. All fish injected with the Vaccine + Adjuvant had black/brown pigment associated with the external surface of the fundic region of the stomach. Histological examination revealed this material to be black/brown pigmentation most likely caused by melanin. An inflammatory response was noted around this material with the formations of granulomas and cysts.

Vincent BN, Adams MB, Nowak BF, Morrison RN (2009) **Cell-surface carbohydrate antigen(s) of wild-type *Neoparamoeba* spp. are immunodominant in sea-cage cultured Atlantic salmon (*Salmo salar* L.) affected by amoebic gill disease (AGD).** *Aquaculture* 288:153-158

A proportion of Atlantic salmon experimentally affected by amoebic gill disease (AGD) develop a serum antibody response to wild-type *Neoparamoeba* spp.. These antibodies bind cell-surface epitope(s) and in most cases the epitope(s) are sensitive to sodium periodate oxidation. In this study, blood was obtained from triploid and diploid sea-farmed Atlantic salmon after 8, 10 and 13 months of sea-cage culture. An additional group of Atlantic salmon broodstock was sampled at 15 months after transfer to sea. Anti-*Neoparamoeba* spp. (anti-NP) antibodies that bound cell-surface carbohydrate antigens of wild-type *Neoparamoeba* spp. were detected in several of these samples. In all cases, the presence of plasma anti-NP antibodies does not appear to be associated with the level of AGD-like gross gill pathology. These results provide further evidence for the development of an antibody response in AGD-affected Atlantic salmon and that carbohydrate epitopes of wild-type *Neoparamoeba* spp. are immunodominant.

2008

Adams MB, Villavedra M, Nowak BF (2008) **An opportunistic detection of amoebic gill disease in blue warehou, *Seriolella brama* Gunther, collected from an Atlantic salmon, *Salmo salar* L., production cage in south eastern Tasmania.** *J Fish Dis* 31:713-717

This communication describes the histopathological and immunohistochemical identification of *Neoparamoeba* sp. eliciting early signs of disease within the gills of blue warehou, *Seriolella brama* Günther, an endemic species that migrates throughout the southern temperate waters of Australia and New Zealand (Knuckey & Sivakumaran 2001).

Carter C, Katersky RS, Barnes JC, Bridle AR, Hauler RC (2008) **Assessment of fish growth performance under limiting environmental conditions:** Aquaculture nutrition subprogram. FRDC 2004/237 Report. Tasmanian Aquaculture and Fisheries Institute, Launceston, Tasmania

This research has advanced our understanding of how fish growth is influenced by nutrition, by environment and by the interaction between nutritional and environmental factors. When the research started the majority of nutrition research considered the performance of feeds under optimum environmental conditions. In stark contrast the Australian aquaculture industry is increasingly facing the proposition of growing fish under sub-optimum conditions. Atlantic salmon are grown at elevated summer temperatures whereas barramundi and several sub-tropical species are affected by low winter temperatures. In addition, aquafeed companies lacked critical information about the optimum balance of protein and energy required for feed formulations at elevated temperatures. The research addressed a significant need for fundamental and applied information about nutrition of fish under limiting environment conditions. Integration of molecular techniques into the research program enhanced the value of the research considerably.

Embar-Gopinath S, Bowman J, Carson J, Crosbie P, Nowak B (2008) **A culture-dependent 16S rRNA gene-based approach to identify gill bacteria associated with amoebic gill disease in Atlantic salmon.** *Bull Eur Assoc Fish Pathol* 28:27-34

The culturable gill bacterial populations associated with amoebic gill disease (AGD) in Atlantic salmon (*Salmo salar*) were identified using biochemical tests, cluster analysis and 16S rRNA gene-based approaches. The gills of fish with clinical signs of AGD were dominated by isolates that had biochemical profiles similar to the representative strains

identified as *Winogradskyella* spp. and *Staphylococcus* spp. Such strains could not be cultured from the AGD-negative samples. This study discusses the possibility of association of culturable salmonid gill bacteria in AGD.

Haugarvoll E, Bjerkas I, Nowak BF, Hordvik I, Koppang EO (2008) **Identification and characterization of a novel intraepithelial lymphoid tissue in the gills of Atlantic salmon.** *J Anat* 213:202-209

In addition to being the respiratory organ in fish, the gills form a barrier against the external milieu. Innate and adaptive immune system components have been detected in the gills, but lymphoid cell accumulations similar to that seen in the mammalian mucosa have not been described. The present investigations revealed cell accumulations on the caudal edge of interbranchial septum at the base of the gill filaments in the Atlantic salmon. Cytokeratin immunohistochemical staining and identification of a basal membrane and desmosome cell junctions by electron microscopy showed that the cell accumulation was located intraepithelially. Major histocompatibility complex (MHC) class II(+) cells were detected by immunohistochemistry, and laser capture micro-dissection and subsequent RT-PCR analysis revealed expression of T-cell receptor transcripts in the investigated tissue, suggesting the presence of T cells. The intraepithelial tissue reported here may be a suitable location for immune surveillance of gill infections, as well as a target site for new vaccine approaches and investigations of epithelial immunity. This is the first description of a lymphocyte cell aggregation within a teleostian gill epithelium network, illustrating a phylogenetically early form of leukocyte accumulations in a respiratory organ.

Miller MR, Bridle AR, Nichols PD, Carter CG (2008) **Increased Elongase and Desaturase Gene Expression with Stearidonic Acid Enriched Diet Does Not Enhance Long-Chain (n-3) Content of Seawater Atlantic Salmon (*Salmo salar* L.).** *J Nutr* 138:2179-2185

Atlantic salmon (*Salmo salar* L.) can produce (n-3) long-chain (LC)-PUFA when fed biosynthetic precursors. This has potential for developing sustainable aquafeeds. Echium oil (EO) is rich in stearidonic acid [SDA, 18:4(n-13)] and bypasses the initial Delta 6 desaturase (FAD6) step in the (n-3) LC-PUFA biosynthetic pathway. EO was fed to seawater Atlantic salmon for 12 wk, and compared with fish fed a diet containing canola oil (CO), a source of alpha-linolenic acid [ALA; 18:3(n-3)] or fish oil (FO) that provides (n-3) LC-PUFA. Fatty acid (FA) composition of liver, white muscle, and whole fish was measured to show whether dietary precursors were endogenously biosynthesized to LC-PUFA. Gene expression of liver FA elongase and FAD5 was upregulated in EO fish compared with FO fish. Furthermore, dietary precursors affected the FA concentrations of direct biosynthetic products in all tissues. The increased gene expression in the EO fish was reflected by an increased FA concentration of eicosapentaenoic acid [20:5(n-3)] in the liver compared with the CO fish. However, the high concentrations of (n-3) LC-PUFA found in seawater Atlantic salmon fed diets rich in FO were not attained via biosynthesis from precursors (ALA or SDA) in diets.

Miller MR, Nichols PD, Carter CG (2008) **The digestibility and accumulation of dietary phytosterols in Atlantic salmon (*Salmo salar* L.) smolt fed diets with replacement plant oils.** *Lipids* 43:549-557

Phytosterols occur in high concentration in canola (*Brassica napus* L.) and other vegetable oils such as from the borage plant Echium (*Echium plantagineum* L.). We investigated if Atlantic salmon (*Salmo salar*) digest and accumulate dietary phytosterols in significant amounts in muscle and liver. Phytosterols are lipid soluble, lower cholesterol and reduce the risk of coronary heart disease in humans. We aimed to determine if fatty fish, such as

salmon, can be used as a delivery source of this functional food component. Three diets containing canola oil (CO), Echium oil (EO) and fish oil (FO) were fed to Atlantic salmon smolt over 9 weeks. The digestibility of natural abundances of phytosterols by Atlantic salmon was poor compared to cholesterol. However, phytosterols accumulated in liver and muscle of fish. Significantly increased concentrations of 24-methylenecholesterol, campesterol, beta-sitosterol and total phytosterol occurred in livers of EO fed fish compared to FO fed fish. Campesterol concentrations increased in CO fed fish compared to the FO fed fish. We demonstrated that natural abundances of dietary phytosterols are digested by and accumulated in liver and white muscle of Atlantic salmon smolt. However, phytosterol levels in salmon muscle will not be a major source of phytosterols in human diets and would not be expected to significantly effect human cardiovascular health.

Miller MR, Nichols PD, Carter CG (2008) **n-3 Oil sources for use in aquaculture - alternatives to the unsustainable harvest of wild fish.** *Nutr Res Rev* 21:85-96

The present review examines renewable sources of oils with n-3 long-chain ( $\geq$  C(20)) PUFA (n-3 LC-PUFA) as alternatives to oil from wild-caught fish in aquafeeds. Due to the increased demand for and price of wild-caught marine sources of n-3 LC-PUFA-rich oil, their effective and Sustainable replacement in aquafeeds is an industry priority, especially because dietary n-3 LC-PUFA from eating fish are known to have health benefits in human beings. The benefits and challenges involved in changing dietary oil in aquaculture are highlighted and four major potential Sources of n-3 LC-PUFA for aquafeeds, other than fish oil, are compared. These sources of oil, which contain n-3 LC-PUFA, specifically EPA (20 : 5n-3) and DHA (22 : 6n-3) or precursors to these key essential fatty acids, are: (1) other marine Sources of oils (2) vegetable oils that contain biosynthetic precursors, such as stearidonic acid, which may be used by fish to produce n-3 LC-PUFA; (3) single-cell oil sources of n-3 LC-PUFA; (4) vegetable oils derived from oil-seed crops that have undergone genetic modification to contain n-3 LC-PUFA. The review focuses on Atlantic salmon (*Salmo salar* L.), because it is the main intensively cultured finfish species and it both uses and stores large amounts Of Oil, in particular n-3 LC-PUFA, in the flesh.

Morrison RN, Nowak BF (2008) **Immunohistochemical detection of anterior gradient-2 in the gills of amoebic gill disease-affected Atlantic salmon, *Salmo salar* L.** *J Fish Dis* 31:699-705

The aim of this study was to develop an immunological reagent that binds asAG-2, and then use this reagent to detect cells expressing asAG-2 in AGD-affected Atlantic salmon. Our results suggest that as AG-2 mRNA upregulation in the gills of AGD-affected fish is closely linked with an increase in the abundance of mucous cells and that as AG-2 may play a role in the recruitment of these cells. Whether this occurs by metaplasia (differentiation) or hyperplasia (division) is unknown but the anti-asAG-2 antiserum will be a useful tool in addressing this issue

Nowak B, Young N, Morrison R, Attard M, Vincent B, Crosbie P (2008) **Establishment of challenge for AGD.** FRDC Project No 2004/215, Aquafin CRC Project 341 (3) Report: Atlantic Salmon Aquaculture Subprogram. Tasmanian Aquaculture and Fisheries Institute, Launceston, Tasmania

The main objectives of this project were to provide essential service for AGD research. During this project we standardised existing AGD challenge protocol and developed a new in vivo gill attachment challenge assay. Both challenge protocols have been successfully applied in AGD research. Research on virulent amoebae resulted in a description of a new



species, which consequently has been shown to be involved in all AGD cases worldwide. This discovery led to the development of new diagnostic tests, which are now available for confirmation of AGD infections and further research. In conclusion, this project has not only provided essential support for all AGD research by supplying amoebae and salmon and running AGD challenges for the experimental vaccines, but also increased our knowledge and understanding of AGD.

Sajjadi M, Carter CG (2008) **Effect of feeding rate on nutrient digestibility in Atlantic salmon, *Salmo salar* L.** *Iran J Fish Sci* 7:241-256

A digestibility trial was conducted to examine the effect of feeding rate on dry matter, gross energy, crude protein and phosphorus digestibility in Atlantic salmon (*Salmo salar*). Duplicate groups of fish were fed 0.25, 0.5, 0.75, 1.0, 1.25 and 1.9% BW/day. The faeces were collected by Guelph-type collectors for five successive days. Dry matter, protein and phosphorus digestibilities were all significantly ( $P < 0.05$ ) affected by feeding rate. Dry matter digestibility was significantly lower in fish fed 0.25% BW day in comparison with fish fed 0.5, 0.75 and 1% BW day. Protein digestibility was significantly lower in fish fed 0.25% BW/day in comparison with 1.25% BW day. Phosphorus digestibility was significantly lower in fish fed 0.25% BW/day in comparison with all other treatments except for 1% BW day. There were no significant differences for energy digestibility between fish fed with different amount of feed. The main effect was reduced digestibility at the lowest level of intake with no obvious relationship between feeding rate and digestibility above this amount. This was explained by a relatively higher loss of endogenous faecal nitrogen and phosphorus at sub-maintenance feeding.

Vincent BN, Nowak BF, Morrison RN (2008) **Detection of serum anti-NeoParamoeba spp. antibodies in amoebic gill disease-affected Atlantic salmon *Salmo salar*.** *J Fish Biol* 73:429-435

Amoebic gill disease (AGD)-affected Atlantic salmon *Salmo salar* sometimes developed a serum-antibody response to wild-type NeoParamoeba spp. Five of 103 AGD-affected *S. salar* sampled possessed detectable antibodies that bound wild-type NeoParamoeba spp. western blotting revealed two distinctly different binding profiles. (C) 2008 The Authors Journal compilation (C) 2008 The Fisheries Society of the British Isles.

Wilkinson R, Longland R, Woolcott H, Carter C, Porter M (2008) **Environmental control of growth and early maturation in salmonids.** FRDC Project No 2005/201, Aquafin CRC Project 1A6(2). Tasmanian Aquaculture and Fisheries Institute, Launceston, Tasmania  
At present the culture of Atlantic salmon within Australia produces approximately 26,000 t of fish per annum and is a direct employer of over 1100 workers with the majority of farmed fish sold nationally and only 12% exported. Environmental conditions, such as increased temperatures and high light intensities, found within Tasmania, provide exceptional growing conditions for Atlantic salmon with growth rates far exceeding other salmon producing nations. However, the life history strategy of Atlantic salmon has evolved to ensure maximum opportunity for reproduction which means that both male and female fish are able to initiate maturation and reproduce at several times during their life cycle if environmental and nutritional factors are favourable (Thorpe et al., 1990). Consequently salmon farmed within Tasmania have a far greater rate of early maturation resulting in a percentage of the stock being unsaleable due to poor flesh quality. Previous work has resulted in the development of artificial lighting strategies to alter the timing and/or completely inhibit these reproductive events on farms and has been successful in providing harvestable fish year-

round. The current study seeks to build on the previous work to ultimately gain a greater understanding of the physiological mechanisms responsible for maturation in salmonids.

Wynne JW, O'Sullivan MG, Cook MT, Stone G, Nowak BF, Lovell DR, Elliott NG (2008) **Transcriptome analyses of amoebic gill disease-affected Atlantic salmon (*Salmo salar*) tissues reveal localized host gene suppression.** *Mar Biotechnol* 10:388-403

The transcriptome response of Atlantic salmon (*Salmo salar*) displaying advanced stages of amoebic gill disease (AGD) was investigated. Naive smolt were challenged with AGD for 19 days, at which time all fish were euthanized and their severity of infection quantified through histopathological scoring. Gene expression profiles were compared between heavily infected and naive individuals using a 17 K Atlantic salmon cDNA microarray with real-time quantitative RT-PCR (qPCR) verification. Expression profiles were examined in the gill, anterior kidney, and liver. Twenty-seven transcripts were significantly differentially expressed within the gill; 20 of these transcripts were down-regulated in the AGD-affected individuals compared with naive individuals. In contrast, only nine transcripts were significantly differentially expressed within the anterior kidney and five within the liver. Again the majority of these transcripts were down-regulated within the diseased individuals. A down-regulation of transcripts involved in apoptosis (procathepsin L, cathepsin H precursor, and cystatin B) was observed in AGD-affected Atlantic salmon. Four transcripts encoding genes with antioxidant properties also were down-regulated in AGD-affected gill tissue according to qPCR analysis. The most up-regulated transcript within the gill was an unknown expressed sequence tag (EST) whose expression was 218-fold (+/- SE 66) higher within the AGD affected gill tissue. Our results suggest that Atlantic salmon experiencing advanced stages of AGD demonstrate general down-regulation of gene expression, which is most pronounced within the gill. We propose that this general gene suppression is parasite-mediated, thus allowing the parasite to withstand or ameliorate the host response.

Young ND, Cooper GA, Nowak BF, Koop BF, Morrison RN (2008) **Coordinated down-regulation of the antigen processing machinery in the gills of amoebic gill disease-affected Atlantic salmon (*Salmo salar* L.).** *Mol Immunol* 45:2581-2597

Several important cultured marine fish are highly susceptible to an ectoparasitic condition known as amoebic gill disease (AGD). In AGD-affected fish, modulation of IL-1 beta, p53 and p53-regulated transcripts is restricted to the (multi)focal AGD-associated gill lesions. To determine whether this lesion-restricted modulation of transcripts occurs on a transcriptome-wide scale and to identify mechanisms that underpin the susceptibility of fish to AGD, we compared the transcriptome of AGD lesions with "nonrill" tissue from AGD-affected and healthy individuals. Global gene expression profiling using a 16 K salmonid microarray, revealed a total of 176 significantly regulated annotated features and of those, the modulation of 99 (56%) was lesion-restricted. Annotated transcripts were classified according to functional gene ontology. Within the immune response category, transcripts were almost universally down-regulated. In AGD-affected tissue, significant, coordinated down-regulation of the major histocompatibility complex class I (MHC I) pathway-related genes occurred during the later stages of infection and appeared to be mediated by down-regulation of interferon-regulatory factor (IRF)-1, independent of interferon-alpha, interferon-gamma and IRF-2 expression. Within this micro-environment, suppression of the MHC I and possibly the MHC II pathways may inhibit the development of acquired immunity and could explain the unusually high susceptibility of Atlantic salmon to AGD. (C) 2008 Elsevier Ltd. All rights reserved.

Young ND, Dykova I, Snekvik K, Nowak BF, Morrison RN (2008) ***Neoparamoeba perurans* is a cosmopolitan aetiological agent of amoebic gill disease.** *Dis Aquat Org* 78:217-223  
Previously we described a new member of the *Neoparamoeba* genus, *N. perurans*, and showed that it is an agent of amoebic gill disease (AGD) of Atlantic salmon *Salmo salar* cultured in southeast Tasmania, Australia. Given the broad distribution of cases of AGD, we were interested in extending our studies to epizootics in farmed fish from other sites around the world. Oligonucleotide probes that hybridise with the 18S rRNA of *N. perurans*, *N. branchiphila* or *N. pemaquidensis* were used to examine archival samples of AGD in Tasmania as well as samples obtained from 4 host fish species cultured across 6 countries. In archival samples, *N. perurans* was the only detectable amoeba, confirming that it has been the predominant aetiological agent of AGD in Tasmania since epizootics were first reported. *N. perurans* was also the exclusive agent of AGD in 4 host species across 6 countries. Together, these data show that *N. perurans* is a cosmopolitan agent of AGD and, therefore, of significance to the global mariculture industry.

2007

Crosbie PBB, Adams MB, Attard MG, Nowak BF (2007) **Development of an in vivo assay to assess attachment of *Neoparamoeba* sp (an amphizoic gymnamoeba) to the gills of Atlantic salmon, *Salmo salar* L.** *J Fish Dis* 30:187-190

The aim of this study was to develop an assay to measure *Neoparamoeba* sp. attachment to Atlantic salmon gills over a short period of time and use it to assess the impact of antibiotics on the challenge inoculum and initiation of AGD in the laboratory. The assay could also be applied to testing the potential of any treatments or prophylactic measures that may affect *Neoparamoeba* sp. attachment to salmon gills and subsequent progression to AGD.

Hauler RC, Carter CG, Edwards SJ (2007) **Feeding regime does not influence lysine utilisation by Atlantic salmon, *Salmo salar* L., parr.** *Aquaculture* 273:545-555

This study investigated whether the efficiency of lysine utilisation for liveweight gain, protein gain and lysine gain was affected by feeding regime in Atlantic salmon (*Salmo salar* L.) parr. Twelve diets containing from 10.15 to 20.79 g dietary digestible lysine kg<sup>-1</sup> were hand-fed either to satiation or to a controlled fixed ration. The controlled ration was set to equal the feed intake of the basal (lowest) lysine diet so that any growth above that of the group fed the basal diet at the controlled ration was due entirely to the additional dietary lysine. For both feeding regimes, with increasing dietary lysine there were significant linear increases in liveweight gain ( $P < 0.001$ ) and in protein ( $P < 0.001$ ) and lysine ( $P < 0.01$ ) concentration of liveweight gain. Increasing dietary lysine resulted in a significant ( $P < 0.001$ ) linear increase in feed intake at satiation. Efficiency of lysine utilisation for liveweight gain above maintenance lysine intake was significantly ( $P < 0.005$ ) higher at satiation than for the controlled ration: 47.7 compared to 34.9 mg liveweight gain per mg digestible lysine intake, respectively. However, feeding regime had no significant effect on the efficiency of lysine utilisation for protein or lysine gain. This study demonstrated that feed intake does not influence the efficiency of lysine utilisation for protein or lysine gain in Atlantic salmon parr. The change in weight-specific lysine composition suggests a robust physiological mechanism maintaining the efficiency of lysine utilisation for lysine gain in Atlantic salmon parr.

Jones MA, Powell MD, Becker JA, Carter CG (2007) **Effect of an acute necrotic bacterial gill infection and feed deprivation on the metabolic rate of Atlantic salmon *Salmo salar*.** *Dis Aquat Org* 78:29-36

In this study, experiments were conducted to examine the effect of an acute necrotic bacterial gill infection on the metabolic rate (M-O<sub>2</sub>) of Atlantic salmon *Salmo salar*. Fed and unfed Atlantic salmon smolts were exposed to a high concentration (5 x 10<sup>12</sup> CFU ml<sup>-1</sup>) of the bacteria *Tenacibaculum maritimum*, their routine and maximum metabolic rates (M-O<sub>2</sub>rou and M-O<sub>2</sub>max', respectively) were measured, and relative metabolic scope determined. A significant decrease in metabolic scope was found for both fed and unfed infected groups. Fed infected fish had a mean +/- standard error of the mean (SEM) decrease of 2.21 +/- 0.93  $\mu\text{M O}_2 \text{ g}^{-1} \text{ h}^{-1}$ , whilst unfed fish a mean SEM decrease of 3.16 +/- 1.29  $\mu\text{M O}_2 \text{ g}^{-1} \text{ h}^{-1}$ . The decrease in metabolic scope was a result of significantly increased M-O<sub>2</sub>rou of both fed and unfed infected salmon. Fed infected fish had a mean SEM increase in M-O<sub>2</sub>rou of 1.86 +/- 0.66  $\mu\text{M O}_2 \text{ g}^{-1} \text{ h}^{-1}$ , whilst unfed infected fish had a mean SEM increase of 2.16 +/- 0.32  $\mu\text{M O}_2 \text{ g}^{-1} \text{ h}^{-1}$ . Interestingly, all groups maintained M-O<sub>2</sub>max regardless of infection status. Increases in M-O<sub>2</sub>rou corresponded to a significant increase in blood plasma osmolality. A decrease in metabolic scope has implications for how individuals allocate energy; fish with smaller metabolic scope will have less energy to allocate to functions such as growth, reproduction and immune response; which may adversely affect the efficiency of fish growth.

Leef MJ, Harris JO, Powell MD (2007) **Metabolic effects of amoebic gill disease (AGD) and chloramine-T exposure in seawater-acclimated Atlantic salmon *Salmo salar***. *Dis Aquat Org* 78:37-44

Our aim was to determine possible metabolic effects amoebic gill disease (AGD) on Atlantic salmon *Salmo salar*. Standard (R-S) and routine (R-ROU) metabolic rates were evaluated by continually measuring oxygen consumption in 2 independent tanks of fish (18.69 +/- 1.01 kg m<sup>-3</sup>, mean +/- SE). Active metabolic rate (R-ACT) and metabolic scope (R-ACT - R-S) were assessed using a chasing protocol and determined at 3 time periods: (1) pre-infection, (2) 3 d post-infection, and (3) 2 d post-treatment. On Day 3 of the study, the fish were infected with amoebae isolated from the gills of AGD-affected salmon (2300 cells l<sup>-1</sup>). No significant elevations in R-ACT or metabolic scope were detected 3 d post-infection and 2 d post-treatment; however, significant elevations in R-S and R-ROU were detected 3 d post-infection and 2 d post-treatment. Assessment of R-ROU data, especially for the light period, also indicated a rise in oxygen consumption rate over the course of the experiment. Treatment of AGD-affected Atlantic salmon with chloramine-T (CL-T) appeared to briefly mitigate the rise in R-S, as there was a 30% drop (though non-significant) in R-S following treatment. Despite this, R-S continued the upward trend 1 d following treatment. These results suggest that over the course of AGD development, R-S in Atlantic salmon increases. Therefore, considering the physical conditions which constrain R-ACT, we expect that metabolic scope would become compromised in fish more heavily affected with AGD. Treatment with CL-T shows promise for mitigating the respiratory effects of AGD and potentially minimising the loss of metabolic scope.

Leef MJ, Harris JO, Powell MD (2007) **The respiratory effects of chloramine-T exposure in seawater acclimated and amoebic gill disease-affected Atlantic salmon *Salmo salar* L.** *Aquaculture* 266:77-86

The aim of the present study was to examine the respiratory effects of chloramine-T, a proposed novel chemotherapeutic treatment for seawater-acclimated Atlantic salmon *Salmo salar* (L.) affected by amoebic gill disease (AGD). Following a surgical recovery period of 20-24 h, fish, both healthy (N=21) and AGD-affected (N=13) were exposed to either a 1-hour

pulse of seawater containing chloramine-T at a therapeutic concentration of 10 mg L<sup>-1</sup> (experimental), or 100 ml of sterile seawater (sham-treated controls). Arterial blood samples were repeatedly taken from a dorsal aortic catheter prior to exposure (0 h), immediately following exposure (1 h) and then at 3, 6, 12 and 24 h and various respiratory parameters measured. Results showed that there were no significant effects relating to chloramine-T exposure regardless of disease status. Additional examination of the pH-bicarbonate diagrams confirmed that there was minimal acid-base disturbance in fish exposed to chloramine-T regardless of disease status. Significant changes seen within the examined haematological parameters of both healthy and AGD-affected fish appeared to be related to the repeated withdrawal of blood for analysis of respiratory parameters. Overall these results suggest that the use of chloramine-T in full-strength seawater at the therapeutic concentration of 10 mg L<sup>-1</sup> for 1 h had no significant respiratory effect in healthy or AGD-affected Atlantic salmon. Additionally, these results help to highlight the potential beneficial use of chloramine-T as a commercial treatment for gill diseased in marine Atlantic salmon.

Leef MJ, Hill JV, Harris JO, Powell MD (2007) **Increased systemic vascular resistance in Atlantic salmon, *Salmo salar* L., affected with amoebic gill disease.** *J Fish Dis* 30:601-613

Previous investigations into the pathophysiology of amoebic gill disease (AGD) have suggested that there are probable cardiovascular effects associated with this disease. In the present study Atlantic salmon, *Salmo salar* L., were experimentally infected by cohabitation with diseased individuals. Two commonly used vasodilators, sodium nitroprusside (SNP) and captopril, the angiotensin-converting enzyme (ACE) inhibitor, were used as tools to investigate possible vasoconstriction and/or renin-angiotensin system (RAS) dysfunction in AGD-affected animals. Within the SNP trial, results showed that AGD-affected fish exhibited lowered cardiac output (Q), lowered cardiac stroke volume (V-S) and a significantly elevated systemic vascular resistance (R-S) compared with non-affected naive counterparts. These effects were totally abolished following SNP administration (40 µg kg<sup>-1</sup>), however significant cardiovascular effects associated with SNP were not observed. Within the captopril trial, where AGD-affected fish were more diseased compared with the SNP trial, a significant hypertension was observed in AGD-affected fish. Captopril administration (10(-4) mol L<sup>-1</sup> at 1 mL kg<sup>-1</sup>) resulted in a significant drop in dorsal aortic pressure (P-DA) for both AGD-affected and naive control fish. In terms of peak individual responses, captopril administration effectively lowered P-DA in both AGD-affected and naive control groups equally. The drop in P-DA following SNP administration however was significantly greater in AGD-affected fish potentially suggesting disease-related vasoconstriction. The lack of significant cardiovascular effects directly associated with both SNP and captopril administrations possibly relate to the 6 h recovery period following surgical procedures. However, while variable, these results do suggest that there are significant cardiovascular effects including vasoconstriction and hypertension associated with AGD.

Miller MR, Nichols PD, Carter CG (2007) **Replacement of dietary fish oil for Atlantic salmon parr (*Salmo salar* L.) with a stearidonic acid containing oil has no effect on omega-3 long-chain polyunsaturated fatty acid concentrations.** *Comp Biochem Phys B* 146:197-206

The worldwide increase in aquaculture production and the concurrent decrease of wild fish stocks has made the replacement of fish oil in aquafeeds an industry priority. Oil from a plant source *Echium plantagineum* L., Boraginaceae, has high levels of stearidonic acid (SDA,

18:4 omega 3, 14%) a biosynthetic precursor of omega-3 long-chain ( $\geq$  C-20) polyunsaturated fatty acids (omega 3 LC-PUFA). Atlantic salmon (*Salmo salar* L.) parr were fed a control fish oil diet (FO) or one of 3 experimental diets with 100% canola oil (CO) 100% SDA oil (SO), and a 1:1 mix of CO and SDA oil (MX) for 42 days. There were no differences in the growth or feed efficiency between the four diets. However, there were significant differences in the fatty acid (FA) profiles of the red and white muscle tissues. Significantly higher amounts of SDA, eicosapentaenoic acid (20:5 omega 3, EPA), docosahexaenoic acid (22:6 omega 3, DHA) and total omega 3 FA occurred in both red and white muscle tissues of fish fed SO and FO compared with those fed CO. Feeding SO diet resulted in W LC-PUFA amounts in the white and red muscle being comparable to the FO diet. This study shows that absolute concentration ( $\mu$ g/g) of EPA, DHA and total omega 3 have been maintained over 6 weeks for Atlantic salmon fed 14% SDA oil. The balance between increased biosynthesis and retention of W LC-PUFA to maintain the concentrations observed in the SO fed fish remains to be conclusively determined, and further studies are needed to ascertain this. (c) 2006 Elsevier Inc. All rights reserved.

Miller MR, Nichols PD, Carter CG (2007) **Replacement of fish oil with thraustochytrid *Schizochytrium* sp L oil in Atlantic salmon parr (*Salmo salar* L) diets.** *Comp Biochem Phys A* 148:382-392

Replacing fish oil with that from a docosahexaenoic acid (22:6 omega 3, DHA) rich single cell micro-organism, thraustochytrid *Schizochytrium* sp. L, in diets for Atlantic salmon (*Salmo salar*) was investigated. Four experimental diets containing 100% thraustochytrid oil (TO), 100% palm oil (PO) and a 4:1 palm and thraustochytrid oil mixture (MX) were compared to a fish oil (FO) diet over 9 weeks. A saltwater transfer challenge occurred at the end of the trial for 14 days to test the diet treatments on the ability of salmon to smolt. There were no significant differences in the feed consumption of the diets or the digestibility of the omega 3 or omega 6 PUFA, indicating no differences in the digestibility of fatty acids between diets. No significant differences were noted between the growth of fish on the four diet treatments. Significant differences were noted in the fatty acid profiles of the fish muscle tissues between all diets. Fish on the TO diet had a significantly greater percentage of DHA in muscle tissue compared with fish on all other diets. Blood osmolarity, which is inversely related to the ability of salmon to smolt, from the TO and FO fed fish was significantly lower than that of fish on the PO diet. This study showed that thraustochytrid oil can be used to replace fish oil in Atlantic salmon diets without detriment to the growth of parr. Including thraustochytrid oil in fish diets significantly increases the amount of DHA in Atlantic salmon muscle and therefore is a candidate for use in oil blends for salmon diets. Thraustochytrid oil provides a renewable source of essential fatty acids, in particular DHA, for aquafeeds.

Morrison RN, Zou J, Secombes CJ, Scapigliati G, Adams MB, Nowak BF (2007) **Molecular cloning and expression analysis of tumour necrosis factor-alpha in amoebic gill disease (AGD)-affected Atlantic salmon (*Salmo salar* L.).** *Fish Shellfish Immunol* 23:1015-1031

Tumour necrosis factor-alpha (TNF-alpha) is a key mediator of inflammation during amoebiasis of humans and mice. Atlantic salmon (*Salmo salar* L.) are also susceptible to infection by amoebae (*Neoparamoeba* spp.), inflicting a condition known as amoebic gill disease (AGD). Here, the role of TNF-alpha in AGD-pathogenesis was examined. Two Atlantic salmon TNF-alpha transcripts designated TNF-alpha 1 and TNF-alpha 2 together with their respective genes were cloned and sequenced. TNF-alpha 1 is 1379 bp and

consists of a 738 bp open reading frame (ORF) translating into a predicted protein of 246 amino acids. TNF-alpha 2 is 1412 bp containing an ORF and translated protein the same length, as TNF-alpha 1. An anti-rainbow trout TNF-alpha polyclonal antibody that bound recombinant Atlantic salmon TNF-alpha 1 and TNF-alpha 2 was used to detect constitutive and inducible expression of TNF-alpha in various tissues. The anti-TNF-alpha antibody bound to a TNF-like protein approximate to 60 kDa that was constitutively expressed in a number of tissues in healthy Atlantic salmon. However, this protein was not detected in lysates from mitogen-stimulated head kidney leucocytes, despite up-regulation of TNF-alpha mRNAs under the same conditions. During the early onset of AGD in Atlantic salmon, there were no demonstrable differences in the gill tissue expression of TNF-alpha 1, TNF-alpha 2 nor the interleukin-1 beta (IL-1 beta), inducible nitric oxide synthase (NOS) and interferon gamma (IFN-gamma) mRNAs compared to tissue from healthy fish. In Atlantic salmon with advanced AGD, IL-1 beta but not TNF-alpha 1 or TNF-alpha 2 mRNAs was up-regulated and was lesion-restricted. Given that *Neoparamoeba* spp. modulated both TNF-alpha 2 and IL-1 beta in head kidney leucocytes in vitro, it appears that rather than being recalcitrant to *Neoparamoeba* spp.-mediated TNF-alpha expression, either the parasite can influence the cytokine response during infection, there is ineffective signalling for TNF-alpha expression, or there are too few cells at the site of infection with the capacity to produce TNF-alpha. These data support our previous observation that IL-1 beta mRNA expression is up-regulated in AGD-affected tissue and that TNF-alpha is not intrinsic in AGD-pathogenesis.

Nowak B, Adams M, Vincent B, O'Brien D, Mitchell D (2007) **Effects of husbandry on Amoebic Gill Disease**. FRDC Project No 2004/214, Aquafin CRC Project 343: Atlantic Salmon Aquaculture Subprogram. Tasmanian Aquaculture and Fisheries Institute, Launceston, Tasmania

Before this project our knowledge of the effects of husbandry on Amoebic Gill Disease (AGD) was limited. This project allowed on-farm assessment of effects of husbandry procedures and stock characteristics on AGD severity. Furthermore, we investigated the potential to re-use fresh water for more than one bath. Preliminary results were promising. Further trials, including some on a larger scale should be undertaken to confirm that re-use of freshwater bath has a commercial potential.

Out of season smolt subjected to artificial lighting regimes and transferred to estuarine sites, where a marked halocline is present, required earlier bathing than fish from cages where no artificial lighting was used. However the advantage of fish subjected to artificial lighting not maturing would outweigh the disadvantage of the need for an earlier bath. This is because maturing fish were more affected by AGD than non-maturing fish. Neither supplemental oxygenation nor high-energy diet affected AGD. There was no statistically significant difference between males and females with regard to AGD. There was no evidence that ploidy had an effect on AGD, however the trial was compromised by the priorities of commercial farm management. There was no significant effect of gill damage on the severity of Amoebic Gill Disease. In conclusion, this project has not only increased our understanding of the effects of husbandry on AGD but also has enhanced our ability to investigate AGD in the future. This will ultimately lead to direct benefits for the salmon industry.

Nowak B, Bridle A, Gross K, Vincent B, Young N, Morrison R (2007) **Use of immunomodulation to improve fish performance in Australian temperate water finfish culture**. FRDC Project No 2004/210, Aquafin CRC Project 342 (2): Atlantic Salmon Aquaculture Subprogram. Tasmanian Aquaculture and Fisheries Institute, Launceston,

## Tasmania

Before this project our knowledge of immune response in Amoebic Gill Disease (AGD) was fundamentally limited and more information was required to assess the potential for immunomodulators in the management of AGD. We confirmed that injection of bacterial DNA motif (CpG oligonucleotides) six days before AGD challenge can offer significant protection to Atlantic salmon (relative percent survival up to 52.5%). However, there was no effect if the fish were challenged immediately post injection with bacterial DNA. This suggests that while there is a potential benefit from the use of immunostimulants, their application is limited because their efficacy is directly linked to the timing of an outbreak, which can be unpredictable in the field. While fish which survived an initial AGD episode show increased resistance to subsequent AGD infection, in contrast to some diseases this effect cannot be simply explained by the presence of antibodies. The duration of exposure (or number of exposures) appears to be important for the development of serum antibodies. Mucus antibodies could not be detected in Atlantic salmon that survived AGD challenge. Microarray experiments and further gene expression studies suggested that there is a loss of cell-cycle control in AGD lesions. Furthermore, immune pathways are affected since the down-stream effect(s) of the initial inflammatory signals were not detectable. It is possible that this significantly contributes to the extremely high rate of mortality in unmitigated AGD epizootics.

While we have achieved our objectives and answered many of the original questions, new issues have emerged from our research. These include a lack of understanding of the mechanisms of inhibition of inflammatory and immune pathways, significance of antibody response (if any) in AGD, and the potential for vaccine antigen discovery through the use of anti-peptide antibody. The presence and role of a more localised antibody response in the gill mucus or epithelium (currently undetectable) warrants further investigation. In conclusion, we now have a better understanding of AGD pathogenesis and the reasons why the host immune response is ineffective in this disease. In particular, we have shown that immune pathways are inhibited in Atlantic salmon affected by AGD.

### Nowak BF (2007) **Parasitic diseases in marine cage culture - An example of experimental evolution of parasites?** *Int J Parasitol* 37:581-588

Rapid development of fish culture in marine cages has been associated with an emergence of parasitic diseases. There is a general trend to an increase in infections with ectoparasites with direct life cycles and a reduced diversity of parasites in aquaculture. Some mariculture creates conditions that are similar to serial passage experiments, which are used to study adaptation during experimental evolution of pathogens. In particular, increased density of fish, repeated introduction of naive hosts, homogenous host populations, fast growth and a potential decrease in genetic diversity are attributes of both aquaculture and serial passage experiments. Some free-living organisms, for example *NeoParamoeba* spp. and *Uronema* spp. parasitise fish in culture, but have not been reported from wild populations. Farming fish in marine cages can increase the risk of outbreaks of parasitic diseases, including those caused by opportunistic parasites. However, aquaculture has the potential to control parasitic diseases through selective breeding, vaccination and general fish health management.

Powell MD, Becker JA, Ransom J, Florent RL, Jones M (2007) **Commercial AGD and Salmon Health.** FRDC Project No 2004/213, Aquafin CRC Project 341(2): Atlantic Salmon Aquaculture Subprogram. Tasmanian Aquaculture and Fisheries Institute, Launceston,



## Tasmania

Prior to this project there had been investigations into some potential candidate amoebicides, with little success except for the possibility of oxidative disinfectants such as chloramine-T. This project has since tested a number of amoebicides using a progressive approach of in vitro toxicity, in vivo efficacy in the laboratory through to in vivo efficacy under field conditions in either semi-commercial or under full commercialised field trials.

Although the practical delivery of some of these as treatments of amoebic gill disease (AGD), such as chloramine-T bathing, appear not to be practicable, other avenues may have potential for further commercial development, such as the dietary inclusion of potential amoebicidal compounds, including bithionol and ionophore-based amoebicides. The project has explored the potential of bithionol, a registered amoebicidal drug, as an in-feed treatment, showing that AGD severity can be reduced by approximately 50%. Similarly, the project has examined the efficacy of an immunostimulant-based feed additive, Aquacite and Betabec which reduced mortality in Atlantic salmon with AGD but did not affect the intensity of infection.

This project has further characterised the effects of gill disease, in particular AGD, with respect to the metabolic cost of disease to the fish. This work has estimated that in excess of 17% of the ingested energy is likely to go to service the cost of AGD. This approach provides a useful tool to incorporate into bioeconomic models for assessing the efficacy of AGD treatments in the future.

Vincent BN, Adams MB, Crosbie PBB, Nowak BF, Morrison RN (2007) **Atlantic salmon (*Salmo salar* L.) exposed to cultured gill-derived *Neoparamoeba branchiphila* fail to develop amoebic gill disease (AGD).** *Bull Eur Assoc Fish Pathol* 27:112-115

Gill-derived *Neoparamoeba* spp. from Atlantic salmon cause amoebic gill disease (AGD) in naive recipients. Atlantic salmon were inoculated with clonal gill-derived *Neoparamoeba branchiphila* that had been cultured in the presence or absence of Atlantic salmon cutaneous mucus. *Neoparamoeba branchiphila* did not elicit AGD and the supplementation of cultures with cutaneous mucus did not influence virulence.

Wynne JW, Cook MT, Nowak B, Lovell D, O'Sullivan M, Stone G, Elliott NG (2007) **Associations between differential gene expression and increased AGD resistance in Atlantic salmon *Salmo salar*.** *Aquaculture* 272:S318-S318

Previous research suggests that resistance to amoebic gill disease (AGD) is to some extent genetically controlled. As a consequence of these findings, developing selective breeding programs to increase AGD resistance have become a major focus in Tasmania. In order to gain maximum efficiency from such breeding programs, genes which influence resistance must be identified. This study aimed to identify such genes by comparing differential gene expression between fish displaying high and low levels of AGD resistance. Briefly, a population of Atlantic salmon were experimentally challenged with AGD over a 19 day period. Following challenge all individuals were euthanised, organs dissected and the gills processed for routine histology. Next, the severity of AGD infection was determined by histopathological examination of the infected gill tissue. Differential gene expression was then assessed within the gill tissue using a 16K salmonid cDNA microarray with real time quantitative PCR validation. Preliminary results from this work will be presented and future research directions discussed.

Wynne JW, Cook MT, Nowak BF, Elliott NG (2007) **Major histocompatibility polymorphism associated with resistance towards amoebic gill disease in Atlantic salmon (*Salmo salar* L.)**. *Fish Shellfish Immunol* 22:707-717

The association between major histocompatibility (MH) polymorphism and the severity of infection by amoebic gill disease (AGD) was investigated across 30 full sibling families of Atlantic salmon. Individuals were challenged with AGD for 19 days and then their severity of infection scored by histopathological examination of the gills. Fish were then genotyped for the MH class I (Sasa-UBA) and MH class II alpha (Sasa-DAA) genes using polymorphic repeats embedded within the 30 untranslated regions of the Sasa-UBA and Sasa-DAA genes. High variation in the severity of infection was observed across the sample material, ranging from 0% to 85% gill filaments infected. In total, seven Sasa-DAA-3UTR and ten Sasa-UBA-3UTR marker alleles were identified across the 30 families. A significant association between the marker allele Sasa-DAA-3UTR 239 and a reduction in AGD severity was detected. There was also a significant association found between AGD severity and the presence of two Sasa-DAA-3UTR genotypes. While the associations between MH allele/genotypes and AGD severity reported herein may be statistically significant, the small sample sizes observed for some alleles and genotypes means these associations should be considered as suggestive and future research is required to verify their biological significance.

Wynne JW, Cook MT, Nowak BF, Elliott NG (2007) **Possible links between MH polymorphism and resistance to AGD in Atlantic salmon *Salmo salar***. *Aquaculture* 272:S318-S319

Amoebic gill disease (AGD) is the most significant health problem affecting the culture of Atlantic salmon in Australia. Research is underway to determine if AGD resistance is a heritable trait and therefore if there is potential to enhance resistance by selective breeding. In an attempt to accelerate the possible gains from future breeding programs we have undertaken a search for genes that may control or influence genetic resistance. Identifying such genes may allow the development of QTL for AGD resistance. The major histocompatibility (MH) class genes offer good candidates due to their important role in the immune system, high polymorphism and previous associations with resistance to other salmonids diseases. Within this study, Atlantic salmon were experimentally challenged with AGD over a 19 day period. Histopathological scoring of gill tissue was employed to determine the severity of AGD in all individuals. Fish were then genotyped for a MH class I microsatellite (UBA) and MH class II minisatellite (DAA) located within the 3' untranslated regions of their respective genes. This facilitated the examination of a link between the presence of specific MH alleles and the histopathological severity of AGD. Preliminary results from this analysis will be presented and their implications for a marker assisted selective breeding program discussed.

Young ND, Crosbie PBB, Adams MB, Nowak BF, Morrison RN (2007) ***Neoparamoeba perurans* n. sp., an agent of amoebic gill disease of Atlantic salmon (*Salmo salar*)**. *Int J Parasitol* 37:1469-1481

Amoebic gill disease (AGD) is a potentially fatal disease of some marine fish. Two amphizoic amoebae *Neoparamoeba pemaquidensis* and *Neoparamoeba branchiphila* have been cultured from AGD-affected fish, yet it is not known if one or both are aetiological agents. Here, we PCR amplified the 18S rRNA gene of non-cultured, gill-derived (NCGD) amoebae from AGD-affected Atlantic salmon (*Salmo salar*) using *N. pemaquidensis* and *N.*

branchiphila-specific oligonucleotides. Variability in PCR amplification led to comparisons of 18S rRNA and 28S rRNA gene sequences from NCGD and clonal cultured, gill-derived (CCGD) *N. pemaquidensis* and *N. branchiphila*. Phylogenetic analyses inferred from either 18S or 28S rRNA gene sequences unambiguously segregated a lineage consisting of NCGD amoebae from other members of the genus *Neoparamoeba*. Species-specific oligonucleotide probes that hybridise 18S rRNA were designed, validated and used to probe gill tissue from AGD-affected Atlantic salmon. The NCGD amoebae-specific probe bound AGD-associated amoebae while neither *N. pemaquidensis* nor *N. branchiphila* were associated with AGD-lesions. Together, these data indicate that NCGD amoebae are a new species, designated *Neoparamoeba perurans* n.sp. and this is the predominant aetiological agent of AGD of Atlantic salmon cultured in Tasmania, Australia.

2006

Attard MG, Crosbie PBB, Adams MB, Nowak BF (2006) **A preliminary study into effects of seawater acclimation on the progression of Amoebic Gill Disease (AGD) in out of season Atlantic salmon (*Salmo salar*, L.).** *Bull Eur Assoc Fish Pathol* 26:275-279

Three groups of out of season Atlantic salmon (*Salmo salar* L.) were challenged with *Neoparamoeba* spp. The first group was seawater acclimated for 7d and then maintained in full strength seawater for another 7d prior to challenge. The second group was acclimated in seawater for 7d only and the third group was moved directly to seawater from fresh water prior to challenge. 34 days post challenge survival was 19, 50 and 0% respectively.

Bridle AR, Morrison RN, Cunningham PMC, Nowak BF (2006) **Quantitation of immune response gene expression and cellular localisation of interleukin-1 beta mRNA in Atlantic salmon, *Salmo salar* L., affected by amoebic gill disease (AGD).** *Vet Immunol Immunopathol* 114:121-134

The characterisation of selected immune response genes during amoebic gill disease (AGD) in Atlantic salmon, *Salmo salar* L., was performed using semi-quantitative RT-PCR, quantitative real-time RT-PCR (qRT-PCR), and in situ hybridisation (ISH). The immune response genes of interest were interleukin-1 beta (IL-1 beta), inducible nitric oxide synthase (iNOS), serum amyloid A (SAA), and serum amyloid P-like pentraxin (SAP). Atlantic salmon were inoculated with the ectoparasite *Neoparamoeba* sp., the causative agent of AGD, and gill, liver and anterior kidney tissue sampled at 0, 7 and 14 d post-inoculation (p.i.). Semi-quantitative RT-PCR was performed on the tissue samples to identify up/down-regulated mRNA expression relative to uninfected control fish and normalised to the housekeeping gene, beta-actin. Interleukin-1 beta (IL-1 beta) was the only immune response gene of those investigated whose mRNA was differentially regulated in any of the tissues and was found to be up-regulated in the gills by semi-quantitative RT-PCR. Increased gill IL-1 beta mRNA expression was then accurately quantitated and confirmed using probe-based qRT-PCR. The cellular localisation of the IL-1 beta mRNA expression in the gills of uninfected and infected fish was then determined by ISH using an IL-1 beta-specific biotinylated cRNA probe. Expression of IL-1 beta mRNA was localised to filament and lamellar epithelium pavement cells in gills of uninfected and infected Atlantic salmon. These data implicate the involvement of IL-1 beta at the site of infection, the gills, of Atlantic salmon during AGD. This work supports previous studies that suggest IL-1 beta is important in the regulation of the fish immune response to parasitic infection but additionally shows the cellular localisation of fish IL-1 beta mRNA expression during infection.

Carson J, Higgins MJ, Wilson TK, Gudkovs N, Bryant TB (2006) **Vibriosis of aquatic animals: development of a national standard diagnostic technology.** FRDC Project No 2001/628: Aquatic Animal Health Subprogram. Tasmanian Aquaculture and Fisheries Institute, Launceston, Tasmania

Vibriosis is one of the most frequent causes of marine aquatic animal disease and affects a wide range of farmed species including finfish, crustacea and shellfish (Actis et al., 1999). In Australia, catastrophic losses are regularly observed in larvae and juveniles in hatcheries as well as in adult farmed animals. Vibriosis is a national problem that affects aquaculture from the temperate regions of the south through to tropical northern Australia. The purpose of this project is to develop a practical and reliable laboratory based test system for the identification of Australian Vibrionaceae.

Miller MR, Nichols PD, Barnes J, Davies NW, Peacock EJ, Carter CG (2006) **Regiospecificity profiles of storage and membrane lipids from the gill and muscle tissue of Atlantic salmon (*Salmo salar* L.) grown at elevated temperature.** *Lipids* 41:865-876

Regiospecific and traditional analysis, of both storage and membrane lipids, was performed on gill, white muscle, and red muscle samples taken from Atlantic salmon (*Salmo salar*) to gauge the effect of elevated water temperature. The fish, fed a commercial diet, were held at an elevated water temperature of 19 degrees C. Total n-3 PUFA, total PUFA, and n-3/n-6 and unsaturated/saturated fatty acid (UFA/SFA) ratios in the FA profile of the total lipid extract in the white muscle were fairly low compared with fish grown at 15 degrees C. Adaptation of structural and storage lipids at elevated temperatures was shown by a significant ( $P < 0.01$ ) reduction in PUFA especially in the percentage of EPA (6-8%). Further adaptation was indicated by the percentages of SFA, which were significantly ( $P < 0.05$ ) higher in gill (56%) and white muscle (58%) polar lipid fractions and coincided with lower percentages of n-3, n-6, and total PUFA. The regiospecific profiles indicated a high affinity of DHA to the sn-2 position in both the TAG (61-68%) and polar lipid (35-60%) fractions. The combination of detailed regiospecific and lipid analyses demonstrated adaptation of cell membrane structure in Atlantic salmon grown at an elevated water temperature.

Morrison RN, Cooper GA, Koop BF, Rise ML, Bridle AR, Adams MB, Nowak BF (2006) **Transcriptome profiling the gills of amoebic gill disease (AGD)-affected Atlantic salmon (*Salmo salar* L.): a role for tumor suppressor p53 in AGD pathogenesis?** *Physiol Genomics* 26:15-34

*NeoParamoeba* spp. are amphizoic amoebae with the capacity to colonize the gills of some marine fish, causing AGD. Here, the gill tissue transcriptome response of Atlantic salmon (*Salmo salar* L.) to AGD is described. Tanks housing Atlantic salmon were inoculated with *NeoParamoeba* spp. and fish sampled at time points up to 8 days post inoculation (pi.). Gill tissues were taken from AGD-affected fish, and a DNA microarray was used to compare global gene expression against tissues from AGD-unaffected fish. A total of 206 genes, representing 190 unique transcripts, were reproducibly identified as up- or downregulated in response to *NeoParamoeba* spp. infection. Informative transcripts having GO biological process identifiers were grouped according to function. Although a number of genes were placed into each category, no distinct patterns were observed. One Atlantic salmon cDNA that was upregulated in infected gill relative to noninfected gill at 114 and 189 h pi. showed significant identity with the *Xenopus*, mouse, and human anterior gradient-2 (AG-2) homologs. Two Atlantic salmon AG-2 mRNA transcripts, designated asAG-2/1 and asAG-

2/2, were cloned, sequenced, and shown to be predominantly expressed in the gill, intestine, and brain of a healthy fish. In AGD-affected fish, differential asAG-2 expression was confirmed in samples used for microarray analyses as well as in AGD-affected gill tissue taken from fish in an independent experiment. The asAG-2 up-regulation was restricted to AGD lesions relative to unaffected tissue from the same gill arch, while p53 tumor suppressor protein mRNA was concurrently downregulated in AGD lesions. Differential expression of p53-regulated transcripts, proliferating cell nuclear antigen and growth arrest and DNA damage-inducible gene-45 beta (GADD45 beta) in AGD lesions, suggests a role for p53 in AGD pathogenesis. Thus, AGD may represent a novel model for comparative analysis of p53 and p53-regulated pathways.

Morrison RN, Koppang EO, Hordvik I, Nowak BF (2006) **MHC class II+ cells in the gills of Atlantic salmon (*Salmo salar* L.) affected by amoebic gill disease.** *Vet Immunol Immunopathol* 109:297-303

Amoebic gill disease (AGD) is characterised by the association of *Neoparamoeba* sp. with hyperplastic gill tissue of affected fishes, however, the identity and role of host cells associated with AGD lesions are not known. Here, we investigated cells with an immunological role that were associated with AGD lesions by locating cellular MHC class II beta chain. A tank housing Atlantic salmon (*Salmo salar*) was inoculated with *Neoparamoeba* sp., and MHC class II beta chain expression in the gills was qualitatively assessed by immunohistochemistry. In AGD-naive control fish, MHC class II+ cells were detected basolateral to the interlamellar epithelium as well as upon the interlamellar and secondary epithelium. In the gills of AGD affected fish MHC class II+ cells were observed in both affected and unaffected tissue. Within AGD lesions, numerous MHC class II+ cells were present and these cells exhibited variable levels of expression suggesting that like mammals, MHC class II expression is highly regulated. The presence of MHC class II+ cells within gill lesions is indicative of immune cell trafficking and these cells could contribute in an antigen presentation capacity to the development of an antibody response in fish chronically affected by AGD.

Vincent BN, Morrison RN, Nowak BF (2006) **Amoebic gill disease (AGD)-affected Atlantic salmon, *Salmo salar* L., are resistant to subsequent AGD challenge.** *J Fish Dis* 29:549-559

There is inconsistent evidence of resistance of Atlantic salmon, *Salmo salar* L., to amoebic gill disease (AGD). Here, evidence is presented that demonstrates that Atlantic salmon exposed and subsequently challenged with AGD are more resistant than naive control fish. Seventy-three per cent of Atlantic salmon previously exposed to AGD survived to day 35 post-challenge compared with 26% exposed to *Neoparamoeba* sp. for the first time, yet the gill pathology of surviving naive control or previously exposed fish was not significantly different. Development of resistance to AGD is associated with anti-*Neoparamoeba* sp. antibodies that were detectable in serum of 50% of surviving Atlantic salmon previously exposed to AGD. However, anti-*Neoparamoeba* sp. antibodies were not detectable in cutaneous mucus of resistant fish. Increased resistance of Atlantic salmon after secondary *Neoparamoeba* sp. infection and detection of specific serum antibodies provides support for the development of a vaccine for AGD.

2005

Bridle AR, Carter CG, Morrison RN, Nowak BF (2005) **The effect of beta-glucan administration on macrophage respiratory burst activity and Atlantic salmon, *Salmo salar* L., challenged with amoebic gill disease - evidence of inherent resistance.** *J Fish Dis* 28:347-356

Previous studies have demonstrated that beta-glucans stimulate Atlantic salmon, *Salmo salar* L., head kidney macrophages both in vitro and in vivo and increase protection against various pathogens. Based on our previous work that showed potent immunostimulatory CpG motif-containing oligodeoxynucleotides increased resistance to amoebic gill disease (AGD), the present study investigated the immunostimulatory effects of three commercial beta-glucan-containing feeds and their ability to increase resistance to AGD. All three commercial beta-glucans were able to stimulate the respiratory burst activity of Atlantic salmon head kidney macrophages in vitro, albeit at different times and concentrations. However, dietary incorporation of the beta-glucans was unable to stimulate the in vivo respiratory burst activity of head kidney macrophages, or serum lysozyme production, and did not increase resistance against AGD. However, this trial showed for the first time that a small subpopulation of Atlantic salmon subjected to a severe AGD infection was able to resist becoming heavily infected and furthermore survive the challenge.

Crosbie PBB, Macleod C, Forbes S, Nowak BF (2005) **Distribution of *Neoparamoeba* sp in sediments around marine finfish farming sites in Tasmania.** *Dis Aquat Org* 67:61-66  
Marine sediment samples collected from various sites at 2 Atlantic salmon farms in Tasmania were analysed for the presence of *Neoparamoeba* sp., an amoeba associated with amoebic gill disease (AGD) in farmed Atlantic salmon. Environmental variables of the sediment layer at each site, including redox potential and sulphide concentration, were measured and the general biological condition assessed by video observation. Sediments and environmental data were collected on 4 occasions at each site over a 12 mo period. *Neoparamoeba* sp. was detected in populations of amoebae recovered by culture from all sites and in 50% of all sediment samples taken. There was evidence of a seasonal influence on the presence of the amoeba, but this was different at each farm. No *Neoparamoeba* sp. was recovered from any sites at Farm 1 during the winter of 2002 whereas at Farm 2 this was the case for the summer of 2003. There appeared to be no relationship between the presence of *Neoparamoeba* sp., salmon farming activities and environmental parameters.

Douglas-Helders GM, Saksida S, Nowak BF (2005) **Questionnaire-based risk assessment for amoebic gill disease (AGD) and evaluation of freshwater bathing efficacy of reared Atlantic salmon *Salmo salar*.** *Dis Aquat Org* 63:175-184

Factors causing amoebic gill disease (AGD), the main disease affecting the salmonid industry in Tasmania, are largely unknown. Managers of 57 sites, from 4 countries, in both the Southern and Northern Hemisphere were questioned regarding the 2000 smolt year-class Atlantic salmon. Questions included site characteristics, fish population characteristics, husbandry and management programs, through to freshwater bathing characteristics. Possible risk factors for AGD outbreaks that were identified in this study using farm managers' opinions were mixed sex stocks (Tasmania), downstream positioning from other salmonid farming sites (pooled), tidal sites (Tasmania), depth of site (pooled), rate of salinity change in halocline and depth of halocline (pooled, Tasmania), phyto-plankton blooms (pooled, Tasmania), antifouling paints (Tasmania, overseas) and automated feeding (pooled, overseas). The information from farm managers suggested that freshwater bathing is less effective with smaller bathing cages and lower levels of saturated oxygen in the bathing

water. Risk factors could not always be determined due to the complex interactions between the biological and chemical environment, host and pathogenic factors, coupled with low sample numbers.

Douglas-Helders M, Nowak B, Butler R (2005) **The effect of environmental factors on the distribution of *Neoparamoeba pemaquidensis* in Tasmania.** *J Fish Dis* 28:583-592

Aquaculture in Tasmania is mostly carried out in estuaries. These estuarine habitats show a great variety and form unique environments in which *Neoparamoeba pemaquidensis*, the amoebic gill disease (AGD)-causing protozoan, may or may not survive. Tasmania is divided into two zones, one where AGD is present and one where AGD is absent, but any ecological data to rationalize this distribution is lacking. In in vitro trials *N. pemaquidensis* strains were exposed to different concentrations of ammonium sulphate, copper sulphate, copper sulphate and tannin, and different *Neoparamoeba* densities, salinities and temperatures. A trial using field water samples investigated the survival of *N. pemaquidensis* in waters sourced from AGD-free and AGD-positive zones, and water analysis was performed to determine any differences. Significantly decreased protozoan survival was found with exposure to increasing copper sulphate concentrations from 10 to 100 000  $\mu\text{M}$  ( $P < 0.001$ ), salinity of 15 parts per thousand ( $P < 0.001$ ), low *Neoparamoeba* densities of 625 and 1250 cells  $\text{mL}^{-1}$  ( $P = 0.0005$ ), and water sourced from Macquarie Harbour ( $P < 0.001$ ). The water chemistry of this AGD-free zone showed significantly lower dissolved calcium and magnesium concentrations which may contribute to this area being AGD-free. Understanding of the ecology of *N. pemaquidensis* will enable better control and prevention strategies for Tasmanian salmon growers.

Embar-Gopinath S, Butler R, Nowak B (2005) **Influence of salmonid gill bacteria on development and severity of amoebic gill disease.** *Dis Aquat Org* 67:55-60

The relationship between salmonid gill bacteria and *Neoparamoeba* sp., the aetiological agent of amoebic gill disease (AGD) was determined in vivo. Fish were divided into 4 groups and were subjected to following experimental infections: Group 1, amoebae only; Group 2, *Staphylococcus* sp. and amoebae; Group 3, *Winogradskyella* sp. and amoebae; Group 4, no treatment (control). Fish (Groups 1, 2 and 3) were exposed to potassium permanganate to remove the natural gill microflora prior to either bacterial or amoebae exposure. AGD severity was quantified by histological analysis of gill sections to determine the percentage of lesioned filaments and the number of affected lamellae within each lesion. All amoebae infected groups developed AGD, with fish in Group 3 showing significantly more filaments with lesions than other groups. Typically lesion size averaged between 2 to 4 interlamellar units in all AGD infected groups. The results suggest that the ability of *Neoparamoeba* sp. to infect filaments and cause lesions might be enhanced in the presence of *Winogradskyella* sp. The possibility is proposed that the prevalence of more severe AGD is due to the occurrence of *Winogradskyella* sp. at high concentrations on the gills.

Embar-Gopinath S, Crosbie PBB, Nowak B (2005) **Use of potassium permanganate to disinfect Atlantic salmon (*Salmo salar*) gills.** *Bull Eur Assoc Fish Pathol* 25:291-296

This study determined the efficiency of potassium permanganate ( $\text{KMnO}_4$ ; 5 mg/l) in disinfecting Atlantic salmon gills and verified the effect of this disinfection process on subsequent development of amoebic gill disease (AGD).  $\text{KMnO}_4$  treated fish showed a significant reduction in their average bacterial colony forming units from the gills compared to untreated fish. However, there was no significant difference in AGD severity between  $\text{KMnO}_4$  treated and untreated groups.

Gross KA, Powell MD, Butler R, Morrison RN, Nowak BF (2005) **Changes in the innate immune response of Atlantic salmon, *Salmo salar* L., exposed to experimental infection with *Neoparamoeba* sp.** *J Fish Dis* 28:293-299

An experiment was conducted to determine the effect of *Neoparamoeba* sp. infection on the innate immune responses of Atlantic salmon. Atlantic salmon were experimentally infected with *Neoparamoeba* sp. and serially sampled 0, 1, 4, 6, 8 and 11 days post-exposure (dpe). Histological analysis of infected fish gill arches identified the presence of characteristic amoebic gill disease lesions as early as 1 dpe with a steady increase in the number of affected gill filaments over time. Immune parameters investigated were anterior kidney phagocyte function (respiratory burst, chemotaxis and phagocytosis) and total plasma protein and lysozyme. In comparison with non-exposed control fish basal respiratory burst responses were suppressed at 8 and 11 dpe, while phorbol myristate acetate-stimulated activity was significantly suppressed at 11 dpe. Variable differences in phagocytic activity and phagocytic rate following infection were identified. There was an increase in the chemotactic response of anterior kidney macrophages isolated from exposed fish relative to control fish at 8 dpe. Total protein and lysozyme levels were not affected by *Neoparamoeba* sp. exposure.

Leef MJ, Harris JO, Hill J, Powell MD (2005) **Cardiovascular responses of three salmonid species affected with amoebic gill disease (AGD).** *Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology* 175:523-532

The cardiovascular effects of amoebic gill disease (AGD) were investigated immediately following surgery in three salmonid species; Atlantic salmon (*Salmo salar* L.), brown trout (*Salmo trutta* L.) and rainbow trout (*Oncorhynchus mykiss* Walbaum). Fish, both naïve (control) and infected (AGD-affected) of each species, were fitted with dorsal aorta catheters and cardiac flow probes. Cardiac output and dorsal aortic pressures were then continuously measured over a 6-h period following surgery. Results showed that Atlantic salmon, brown trout and rainbow trout displayed similar dorsal aortic pressure, cardiac output, and systemic vascular resistance (mean dorsal aortic pressure divided by cardiac output) values. However, the only significant differences relating to disease status i.e. infected or control, were found in Atlantic salmon. Although no significant differences were seen in dorsal aortic pressure values, AGD-affected salmon displayed significantly elevated systemic vascular resistance at 4 and 6 h post surgery. Cardiac output was also approximately 35% lower in AGD-affected salmon compared to the nonaffected control counterparts. These results comparatively examine cardiac function in response to AGD across three salmonid species and highlight species-specific cardiovascular responses that occur in association with disease. It is suggested that the apparent cardiac dysfunction seen in AGD-affected Atlantic salmon could, under stressful conditions, become exacerbated. Cardiac failure is therefore suggested to be a possible physiological mechanism by which AGD causes or contributes to mortality in Atlantic salmon.

Leef MJ, Harris JO, Powell MD (2005) **Respiratory pathogenesis of amoebic gill disease (AGD) in experimentally infected Atlantic salmon *Salmo salar*.** *Dis Aquat Org* 66:205-213

The aim of this study was to investigate the respiratory responses of Atlantic salmon, *Salmo salar*, experimentally affected with amoebic gill disease (AGD). In Series I, arterial blood samples were taken over a 96 h period following amoebae addition to examine potential respiratory effects associated with initial exposure. No major significant treatment effects



were found between fish exposed to amoebae and control (non-exposed) fish. Arterial pH (pHa) was seen to be significantly elevated at 48 h in AGD fish relative to the 0 h time point. To investigate the long-term respiratory effects associated with infection, fish were similarly exposed to amoebae and sampled over a 16 d period. As for Series I, caudal blood pH was significantly elevated by Day 2 (48 h) compared to the pre (Day 0)-time point, suggesting that initial exposure to amoebae and/or amoebae attachment may have induced an initial respiratory alkalosis via increased ventilation frequency and/or amplitude. From Day 7 onwards, and coinciding with a significant increase in the percentage of affected gill filaments, blood pH decreased significantly, possibly indicating the onset of the characteristic respiratory acidosis that has previously been described for experimentally AGD affected Atlantic salmon. Although fish in this study showed up to 90% AGD-affected filaments, the corresponding respiratory results do not reflect a major acid–base disturbance. Therefore, the findings from the present study support the contention that, although AGD only affects the gill, AGD associated mortality in Atlantic salmon may not be primarily associated with respiratory failure.

Morrison RN, Crosbie PBB, Cook MT, Adams MB, Nowak BF (2005) **Cultured gill-derived *NeoParamoeba pemaquidensis* fails to elicit amoebic gill disease (AGD) in Atlantic salmon *Salmo salar*.** *Dis Aquat Org* 66:135-144

Amoebic gill disease (AGD) affects the culture of Atlantic salmon *Salmo salar* in the southeast of Tasmania. The disease is characterised by the presence of epizootic *NeoParamoeba* spp. in association with hyperplastic gill tissue. Gill-associated amoebae trophozoites were positively selected by plastic adherence for culture in seawater, where they proliferated using heat-killed *E. coli* as a nutrient source. One isolate of gill-harvested amoebae designated NP251002 was morphologically consistent to *N. pemaquidensis* under light, fluorescence and transmission electron microscopy. Rabbit anti-*N. pemaquidensis* antiserum bound to NP251002, and *N. pemaquidensis* small subunit (SSU) ribosomal DNA (18S rDNA) was detected in NP251002 genomic DNA preparations using PCR. A high degree of similarity in the alignment of the NP251002 18S rDNA PCR amplicon sequence with reference isolates of *N. pemaquidensis* suggested conspecificity. While short-term culture (72 h) of gill-harvested amoebae does not affect the capacity of amoebae to induce AGD, Atlantic salmon challenged with NP251002 after the trophozoites had been 34 and 98 d in culture exhibited neither gross nor histological evidence of AGD. It is not known if NP251002 were avirulent at the time of isolation, had down-regulated putative virulence factors or virulence was inhibited by the culture conditions. Therefore, the time in culture could be a limiting factor in maintaining virulence using the culture technique described here.

Morrison RN, Nowak BF (2005) **Bath treatment of Atlantic salmon (*Salmo salar*) with amoebae antigens fails to affect survival to subsequent amoebic gill disease (AGD) challenge.** *Bull Eur Assoc Fish Pathol* 25:155-160

There is no consistent evidence of resistance of Atlantic salmon (*Salmo salar*) to amoebic gill disease (AGD), despite either a prior history of AGD, passive immunisation or active immunisation. Here, fish were bathed in amoebae antigens from either an avirulent in vitro cultured strain or wild-type *NeoParamoeba pemaquidensis* and challenged with gill-derived amoebae 27 days post-treatment. Neither bath treatment enhanced survival compared to a placebo treated group of fish. Similarly treatment did not influence the proportion of AGD-affected gill filaments in fish surviving the AGD challenge. It is not known if the failure of the

treatments to elicit protection was mediated by a lack of an immune response or if an immune response was ineffective during the AGD challenge.

Powell MD, Attard M, Harris JO, Roberts SD, Leef MJ (2005) **Why fish die - the treatment and pathophysiology of AGD**. FRDC Project No 2001/205, Aquafin CRC Project 341. Tasmanian Aquaculture and Fisheries Institute, Launceston, Tasmania

Prior to this project, the study of amoebic gill disease patho-physiology was confined to using either fish infected by cohabitation or else clinically infected on fish farms under commercial conditions. This project developed a standardised process for acclimating Atlantic salmon smolts to seawater and infecting those salmon with *Neoparamoeba* sp. in the laboratory. This produced a reliable and repeatable challenge that could re-create AGD in the laboratory within 2-3 weeks. Although it developed more quickly than infections under field conditions, this condensed infection provides a tool with which to study the physiology of the disease and the efficacy of candidate treatments.

This project characterised the pathogenesis of AGD under laboratory conditions and supported suggestions that respiratory compromise was a minor cause of mortality in AGD affected salmon. Although the resting metabolic rate of the fish is increased with infection, probably reflecting an increased energetic cost of disease, the main cause of mortality appears to be due to acute cardiovascular compromise. Studies showed that increased vascular resistance (hypertension: high blood pressure) resulted in circulatory failure in AGD affected salmon. This same pathology was not however, seen in less susceptible salmonids such as rainbow trout. The effects of the hypertension could be partially reversed using drugs that lower blood pressure.

The role of mucus in AGD was closely studied. Fish affected by AGD showed a reduction in the viscosity of mucus, analogous to a “runny nose” which would slough off the offending *Neoparamoeba* sp. parasite from the gills. This was reflected in biochemical and histochemical changes in the composition of the mucus both during seawater acclimation and in response to infection in salmonids.

The project identified several potential improvements to bathing as a treatment for AGD. The use of softened water enhances mucus sloughing and is more effective at killing *Neoparamoeba* sp. Similarly, replacement of freshwater with a chloramine-T treatment in seawater may offer an alternative to freshwater bathing, especially as an emergency treatment or for farms that have limited access to freshwater. The use of artificially softened water is now at the point of commercial adoption by the aquaculture industry, and the use of chloramine-T in seawater is close to that point.

The use of in feed amoebocides or treatments that help to overcome the effects of AGD were also tested. *Neoparamoeba* sp. are resistant to many families of antiprotozoal drugs, although at least in vitro, *Neoparamoeba* sp. was sensitive to the drug bithionol. In addition, the use of mucolytic drugs to enhance mucus sloughing and reduced mucus viscosity showed promise in retarding the onset of AGD. Also, the use of nutritional supplements Aquacite and Betabec, maintained feed intake and growth in AGD affected fish under laboratory conditions.

In conclusion, this project has identified a primary cause of AGD related mortality and several potential treatment options. This has significantly increased our understanding of the pathophysiology of the disease as well as providing avenues for improvements in the commercial control of the disease.

Powell MD, Harris JO, Carson J, Hill JV (2005) **Effects of gill abrasion and experimental infection with *Tenacibaculum maritimum* on the respiratory physiology of Atlantic salmon *Salmo salar* affected by amoebic gill disease.** *Dis Aquat Org* 63:169-174

The effects of gill abrasion and experimental infection with *Tenacibaculum maritimum* were assessed in Atlantic salmon *Salmo salar* with underlying amoebic gill disease. The respiratory and acid-base parameters arterial oxygen tension (PaO<sub>2</sub>), arterial whole blood oxygen content (CaO<sub>2</sub>), arterial pH (pH(a)), haematocrit and haemoglobin concentrations were measured at intervals over a 48 h recovery period following surgical cannulation of the dorsal aorta. Mortality rates over the recovery period were variable, with gill abrasion and inoculation with *T. maritimum* causing the highest initial mortality rate and unabraded, uninoculated controls showing the lowest overall mortality rate. Fish with abraded gills tended to show reduced PaO<sub>2</sub> and lower CaO<sub>2</sub> compared with unabraded fish. Infection with *T. maritimum* had no effect on PaO<sub>2</sub> or CaO<sub>2</sub>. All fish showed an initial alkalosis at 24 h post-surgery/inoculation which was more pronounced in fish inoculated with *T. maritimum*. There were no significant effects of gill abrasion or infection upon the ratio of oxygen specifically bound to haemoglobin or mean cellular haemoglobin concentration. Histologically, 48 h following surgery, abraded gills showed multifocal hyperplastic lesions with pronounced branchial congestion and telangiectasis, and those inoculated with *T. maritimum* exhibited focal areas of branchial necrosis and erosion associated with filamentous bacterial mats. All fish examined showed signs of amoebic gill disease with multifocal hyperplastic and spongy lesions with parasome-containing amoeba associated with the gill epithelium. The results suggest that respiratory compromise occurred as a consequence of gill abrasion rather than infection with *T. maritimum*.

Villavedra M, McCarthy K, To J, Morrison R, Crosbie P, Broady K, Raison RL (2005) **Changes in antigenic profile during culture of *Neoparamoeba* sp., causative agent of amoebic gill disease in Atlantic salmon.** *Int J Parasitol* 35:1417-1423

Amoebic gill disease (AGD), the most serious infectious disease affecting farmed salmon in Tasmania, is caused by free-living marine amoeba *Neoparamoeba* sp. The parasites on the gills induce proliferation of epithelial cells initiating a hyperplastic response and reducing the surface area available for gaseous exchange. AGD can be induced in salmon by exposure to freshly isolated *Neoparamoeba* from AGD infected fish, however cultured *Neoparamoeba* are non-infective. We describe here antigenic differences between freshly isolated and in vitro cultured parasites, and within individual isolates of the parasite cultured under different conditions. Immunoblot analysis using polyclonal antisera. revealed differences in the antigen profiles of two cultured isolates of *Neoparamoeba* sp. when they were grown on agar versus in liquid medium. However, the antigen profiles of the two isolates were very similar when they were grown under the same culture conditions. Comparison of these antigen profiles with a preparation from parasites freshly isolated from infected gills revealed a very limited number of shared antigens. In addition monoclonal antibodies (mAbs) raised against surface antigens of cultured parasites were used in an indirect immunofluorescence assay to assess the expression of specific Surface antigens of *Neoparamoeba* sp. after various periods in culture. Significant changes in antigen expression of freshly isolated parasites were observed after 15 days of in vitro Culture. The use of mAb demonstrated progressive exposure/expression of individual antigens on the surface of the freshly isolated parasites during the period in culture.

Ward DA, Carter CG, Townsend AT (2005) **The use of yttrium oxide and the effect of faecal collection timing for determining the apparent digestibility of minerals and trace elements in Atlantic salmon (*Salmo salar*, L.) feeds.** *Aquacult Nutr* 11:49-59

The effectiveness of the external marker yttrium oxide (Y<sub>2</sub>O<sub>3</sub>) and sampling period for determining the apparent digestibility (AD) of minerals and trace elements within Atlantic salmon feeds were compared. Yttrium oxide was compared at inclusions of 0.01, 0.1, 1 and 10 g kg<sup>-1</sup> wet weight of the feeds. Samples were analysed for a range of mineral and trace elements via inductively coupled plasma optical emission spectroscopy after wet decomposition with concentrated nitric acid. Feed marked with 1 g kg<sup>-1</sup> provided the greatest recovery, and provided analytical values within an optimal range, and therefore, the effect of faecal sampling period on AD of mineral and trace element was then compared using the feed marked with 1 g kg<sup>-1</sup> Y<sub>2</sub>O<sub>3</sub>. Faeces were collected over three different time periods within a 24 h period: 4 x 6 h, 2 x 12 h and 1 x 24 h. Magnesium, manganese, phosphorus, iron and chromium displayed significant differences in AD relating to sampling period. An inclusion level of 1 g kg<sup>-1</sup> Y<sub>2</sub>O<sub>3</sub> and faecal sampling over at least an 18 h period after feeding proved to be the most effective method of determining AD of mineral and trace element in Atlantic salmon feed.

2004

Adams MB, Ellard K, Nowak BF (2004) **Gross pathology and its relationship with histopathology of amoebic gill disease (AGD) in farmed Atlantic salmon, *Salmo salar* L.** *J Fish Dis* 27:151-161

Gross pathological assessment of amoebic gill disease (AGD) is the only non-destructive, financially viable method for rapid and broad-scale disease management of farmed Atlantic salmon, *Salmo salar* L., in Tasmania. However, given the presumptive nature of this diagnosis, the technique has been considered questionable. This study investigated the degree of conformity between clinical signs and histological lesions observed in a commercial setting. Three groups of Atlantic salmon (n = 42, 100 and 100, respectively) were collected from various farm sites in southern Tasmania between December 2001 and April 2003. Micro-stereoscopic analysis showed that grossly affected tissue regions correspond to areas of hyperplastic lamellar fusion, generally in association with attached *Neoparamoeba* sp. Agreement between gross signs of AGD and histopathological diagnosis was compared. Kappa analysis indicated moderate to good agreement between methods (kappa = 0.52-0.74). Individual cases of disagreement were further scrutinized and several factors were found to influence the level of agreement between the two methods. Stage of disease development, lesions derived from other pathogens, assessor interpretation/experience, sampling methods, histological technique and/or experience were potential confounding factors. It was concluded that clinical diagnosis is acceptable as a farm-monitoring tool only. Removal of grossly affected tissue and subsequent histological examination is recommended to improve diagnostic accuracy.

Adams MB, Nowak BF (2004) **Experimental amoebic gill disease of Atlantic salmon, *Salmo salar* L.: further evidence for the primary pathogenic role of *Neoparamoeba* sp (Page, 1987).** *J Fish Dis* 27:105-113

Amoebic gill disease (AGD) has been attributed to infection by *Neoparamoeba* sp. The causal mechanisms for AGD lesion development and the primary pathogenic role of *Neoparamoeba* sp. require elucidation. Three groups of Atlantic salmon were exposed to

viable gill isolated amoebae, to sonicated amoebae, or to sea water containing viable amoebae without direct contact with gill epithelia. Fish were removed 8 days post-exposure and the gills assessed histologically for AGD. AGD occurred only when fish were exposed to viable trophozoites. Consequently, in an accompanying experiment, infection was evaluated histologically at 12, 24 and 48 h post-exposure in three groups of salmon, one group being mechanically injured 12 h prior to exposure. A progressive host response and significant increase ( $P < 0.001$ ) in the numbers of attached amoebae was apparent over the 48-h duration in undamaged hemibranchs in both treatment groups. There were no significant differences to mucous cell populations. Attachment of *Neoparamoeba* sp. to damaged gill filaments was significantly reduced ( $P < 0.05$ ) by 48 h post-exposure. These data further confirm and describe the primary pathogenic role of *Neoparamoeba* sp. and the early host response in AGD. Preliminary evidence suggests that lesions resulting from physical gill damage are not preferentially colonized by *Neoparamoeba* sp.

Adams MB, Nowak BF (2004) **Sequential pathology after initial freshwater bath treatment for amoebic gill disease in cultured Atlantic salmon, *Salmo salar* L.** J Fish Dis 27:163-173

Freshwater bathing is essential for control of amoebic gill disease (AGD) during the marine phase of the Tasmanian Atlantic salmon production cycle, a practice that is costly, production limiting and increasing in frequency. Although the pathogenesis of gill infection with *Neoparamoeba* sp. in naive Atlantic salmon, *Salmo salar*, is now understood, the progression of re-infection (post-treatment) required elucidation. Here, we describe the weekly histopathological progression of AGD from first to second freshwater bath. Halocline cessation and increased water temperature appeared to drive the rapid onset of initial infection prior to bathing. Freshwater bathing cleared lesions of attached trophozoites and associated cellular debris. Subsequent gill re-infection with *Neoparamoeba* sp. was evident at 2 weeks post-bath and had significantly increased ( $P < 0.001$ ), in severity by 4 weeks post-bath. No significant difference in gross pathology was observed until 4 weeks post-bath ( $P < 0.05$ ). The re-infective progression of AGD was characterized by localized host tissue responses juxtaposed to adhered trophozoites (epithelial oedema, hypertrophy and hyperplasia), non-specific inflammatory cell infiltration (macrophages, neutrophils and eosinophilic granule cells) and finally advanced hyperplasia with epithelial fortification. During the post-bath period, non-AGD lesions including haemorrhage, necrosis and regenerative hyperplasia were occasionally observed, although no evidence of secondary colonization of these lesions by *Neoparamoeba* sp. was noted. We conclude that pathogenesis during the inter-bath period was identical to initial infection although the source of re-infection remains to be established.

Bowman JP, Nowak B (2004) **Salmonid gill bacteria and their relationship to amoebic gill disease.** J Fish Dis 27:483-492

16S ribosomal RNA gene analysis was used to assess the bacterial community associated with Atlantic salmon, *Salmo salar* L., gills which were either affected by amoebic gill disease (AGD) or were AGD-negative, in order to determine the role that bacteria may play in the development of AGD. AGD-positive specimens were either infected in the laboratory with *Neoparamoeba pemaquidensis*, the causative agent of AGD, or were obtained from commercial salmon cages. Samples from laboratory fish maintained in sea water possessed a marine-type community while field samples which had been treated by a series of freshwater baths possessed a more diverse community which included variable proportions

of different bacterial ecotypes, including groups typically associated with soil, skin surfaces and faeces. Samples from fish infected with AGD in the laboratory and a sample from one of two salmon cage fish specimens were dominated by a phylotype belonging to the strictly marine bacterial genus *Psychroserpens* (family Flavobacteriaceae, phylum Bacteroidetes). The phylotype was not detected in any of the AGD-negative samples or in one of two AGD-positive samples obtained from fish subjected to temporary freshwater immersion. The possibility of certain *Psychroserpens* species as potential opportunistic pathogens associated with salmonid AGD is proposed.

Butler R, Nowak BF (2004) **A dual enzyme method for the establishment of long and medium-term primary cultures of epithelial and fibroblastic cells from Atlantic salmon gills.** J Fish Biol 65:1108-1125

A dual enzyme disaggregation method using collagenase and then trypsin was developed that allowed the reproducible initiation of primary cultures from Atlantic salmon *Salmo salar* gills. Cultures had both epithelial and fibroblast morphology and persisted for an average of 20 passages. Growth was dependent upon a minimum concentration of 5% foetal calf serum (FCS) for fibroblasts and 10% FCS for epithelial cells. Growth was mostly independent of substrate, although epithelial cells showed increased growth on type I collagen gels. Matrigel™ cell culture substrate produced reduced growth of fibroblasts and did not benefit epithelial cell growth. Epithelial cells reacted with monoclonal antibodies (MAbs) against mammalian cytokeratins, and fibroblast cells reacted with MAbs against mammalian fibronectin and type I collagen. The method also produced two long-term cultures: one epithelial and one fibroblast that have been designated RGE-2 and RGF respectively. (C) 2004 The Fisheries Society of the British Isles.

Butler R, Nowak BF (2004) **In vitro interactions between *Neoparamoeba* sp and Atlantic salmon epithelial cells.** J Fish Dis 27:343-349

*Neoparamoeba* sp., including the putative aetiological agent of amoebic gill disease in cultured fish (*N. pemaquidensis*), were incubated in vitro with an Atlantic salmon gill epithelium (RGE-2) cell line. Proliferation by the amoeba population was dependent upon culture osmolarity; no growth occurred at 330 mm kg(-1) but a sixfold increase was observed at 1000 mm kg(-1). At 780 mm kg(-1) there was a fourfold increase in the amoeba population but a concurrent decrease in RGE-2 cell density that was significantly greater than that caused by the high culture osmolarity alone. This apparent cytopathic effect (CPE) developed rapidly and resulted in complete cytolysis of the monolayer in 5 days. CPE occurred in multiple foci and presented as cell vacuolation, rounding and clumping, and the rapid clearance of large areas of the cell monolayer. The possibility that CPE is because of the presence of *Neoparamoeba* sp. derived cytolytic products is discussed in the context of the pathology of the disease in vivo and the occurrence of secreted cytopathogenic compounds in other amoeba species.

Douglas-Helders GM, Weir IJ, O'Brien DP, Carson J, Nowak BF (2004) **Effects of husbandry on prevalence of amoebic gill disease and performance of reared Atlantic salmon (*Salmo salar* L.).** Aquaculture 241:21-30

Improved husbandry has been identified as an area that may alleviate amoebic gill disease (AGD) on Tasmanian salmon farms. We report results of three trials that aimed to reduce AGD prevalence and/or minimise losses associated with AGD. In the first trial, cages were rotated between different sites and data compared to stationary cages that remained on a reference site; this arrangement was repeated over two consecutive years. The second trial

studied the effect of prophylactic freshwater bathing, while the third trial considered the effects of sea cage size. All trials evaluated the effect of treatment on AGD prevalence, fish biomass gain, and the percentage of mortalities. No significant reduction of AGD prevalence was detected in terms of *Neoparamoeba* presence on the gills as measured by the immunodot blot assay. However, fish from the rotated cages showed a significant longer period between freshwater baths ( $P = 0.037$ ), and the mean biomass in the rotated cages ( $P = 0.038$  in year 1 and  $P = 0.041$  in year 2), and the non-prophylactic bathed cages ( $P = 0.048$ ) was significantly higher at the end of the trials. The mortality rate was not affected by any of the treatments. The results of these trials suggest that impact of AGD on salmon industry can be offset by adjustment of husbandry methods.

Dyer AR, Barlow CG, Bransden MP, Carter CG, Glencross BD, Richardson N, Thomas PM, Williams KC, Carragher JF (2004) **Correlation of plasma IGF-I concentrations and growth rate in aquacultured finfish: a tool for assessing the potential of new diets.** *Aquaculture* 236:583-592

A recently developed radio immunoassay (RIA) for measuring insulin-like growth factor (IGF-I) in a variety of fish species was used to investigate the correlation between growth rate and circulating IGF-I concentrations of barramundi (*Lates calcarifer*), Atlantic salmon (*Salmo salar*) and Southern Bluefin tuna (*Thunnus maccoyii*). Plasma TGF-I concentration significantly increased with increasing ration size in barramundi and IGF-I concentration was positively correlated to growth rates obtained in Atlantic salmon ( $r(2)=0.67$ ) and barramundi ( $r(2)=0.65$ ) when fed a variety of diet formulations. IGF-I was also positively correlated to protein concentration ( $r(2)=0.59$ ). This evidence suggested that measuring IGF-I concentration may provide a useful tool for monitoring fish growth rate and also as a method to rapidly assess different aquaculture diets. However, no such correlation was demonstrated in the tuna study probably due to seasonal cooling of sea Surface temperature shortly before blood was sampled. Thus, some recommendations for the design and sampling strategy of nutritional trials where IGF-I concentrations are measured are discussed.

Glencross BD, Carter CG, Duijster N, Evans DR, Dods K, McCafferty P, Hawkins WE, Maas R, Sipsas S (2004) **A comparison of the digestibility of a range of lupin and soybean protein products when fed to either Atlantic salmon (*Salmo salar*) or rainbow trout (*Oncorhynchus mykiss*).** *Aquaculture* 237:333-346

This study compares the digestibility of a series of lupin and soybean protein products when fed to either rainbow trout or Atlantic salmon. The test ingredients in the study, from one of two key grain resources (lupins: *Lupinus angustifolius* and soybeans), represented various levels of processing of each grain in order to increase the protein content of the meals. A reference ingredient of enzymatically hydrolyzed casein (EHC) was also included in the study. The rainbow trout (266 +/- 18 g) were housed in freshwater tanks (250 l, salinity < 1 ‰, 22.1 +/- 1.8 degrees C) and acclimated to the diets for 6 days before faecal collection commenced. The Atlantic salmon (66 +/- 10 g) were housed in similar freshwater tanks (250 l, salinity < 1 ‰, 15 degrees C) and acclimated to the diets for at least 6 days before faecal collection commenced. Faeces were collected from each fish species using settlement collection methods. The digestibility of organic matter, phosphorus, energy and nitrogen was assessed using the diet-substitution method, with each test ingredient included in the diet at 30%. Several differences were observed between the two fish species in their capacity to digest nutrients and energy from each of the products. Organic matter and energy

digestibility of each of the ingredients was largely reflective of the protein content of each ingredient. Protein digestibilities were generally consistent between the two fish species with only lupin kernel meal having a significantly higher digestibility when fed to Atlantic salmon than rainbow trout and the soybean protein concentrate a significantly lower digestibility. Although limited differences in protein digestibility were noted among the ingredients when fed to rainbow trout, more substantial differences were noted when the same ingredients were fed to Atlantic salmon. The digestible energy value of the range of products examined was generally higher in Atlantic salmon than rainbow trout. A clear difference between the two fish species was their capacity to digest phosphorus from the ingredients, with several of the plant protein ingredients showing differences in phosphorus digestibility between the two fish species. Generally, both series of grain products have excellent potential as feed ingredients for either of these species. However, the digestive capacity of Atlantic salmon appears to more positively respond to the absence of dietary non-starch polysaccharide content than that of rainbow trout.

Gross K, Carson J, Nowak B (2004) **Presence of anti-Neoparamoeba sp antibodies in Tasmanian cultured Atlantic salmon, *Salmo salar* L.** J Fish Dis 27:81-88

Previous studies have indicated that when Atlantic salmon, *Salmo salar* L., are exposed to *Neoparamoeba* sp. the fish produce anti-*Neoparamoeba* sp. antibodies. It appears unlikely that these antibodies elicit any specific protection against amoebic gill disease (AGD) as fish with demonstrable activities have been affected by AGD. Experiments were conducted on Atlantic salmon cultured throughout Tasmania to assess the natural production of antibodies towards *Neoparamoeba* sp. Fish were sampled from areas where AGD was prevalent and from areas where there had been no reported cases. An enzyme-linked immunosorbent assay (ELISA) was used to measure anti-*Neoparamoeba* sp. antibody activities in serum. All fish from sea water had antibody activities greater than the negative control fish, including fish from areas with no reported cases of AGD. Time trial samples indicated that time after transfer to sea water did not appear to be a significant ( $P > 0.05$ ) factor in antibody activity, however location was ( $P < 0.05$ ). There was no agreement (corrected kappa value, 0.16) between the ELISA result and the isolation of *Neoparamoeba* sp. from the gills of the same fish. The results suggest that Atlantic salmon in seawater culture in Tasmania produce anti-*Neoparamoeba* sp. antibodies regardless of infection history, suggesting the presence of *Neoparamoeba* sp. in the environment.

Gross KA, Morrison RN, Butler R, Nowak BF (2004) **Atlantic salmon, *Salmo salar* L., previously infected with *Neoparamoeba* sp are not resistant to re-infection and have suppressed phagocyte function.** J Fish Dis 27:47-56

Previous studies have indicated that Atlantic salmon, *Salmo salar* L., affected by amoebic gill disease (AGD) are resistant to re-infection. These observations were based upon a comparison of gross gill lesion abundance between previously infected and naive control fish. Anecdotal evidence from Atlantic salmon farms in southern Tasmania suggests that previous infection does not protect against AGD as indicated by a lack of temporal change in freshwater bathing intervals. Experiments were conducted to determine if previous infection of Atlantic salmon with *Neoparamoeba* sp. would provide protection against challenge and elucidate the immunological basis of any protection. Atlantic salmon were infected with *Neoparamoeba* sp. for 12 days then treated with a 4-h freshwater bath. Fish were separated into two groups and maintained in either sea water or fresh water for 6 weeks. Fish were then transferred to one tank with a naive control group and challenged with *Neoparamoeba*



sp. Fish kept in sea water had lower mortality rates compared with first time exposed and freshwater maintained fish, however, these data are believed to be biased by ongoing mortalities during the seawater maintenance phase. Phagocyte function decreased over exposure time and freshwater maintained fish demonstrated an increased ability to mount a specific immune response. These results suggest that under the challenge conditions herein described, antigen exposure via infection does not induce protection to subsequent AGD.

Morrison RN, Crosbie PBB, Nowak BF (2004) **The induction of laboratory-based amoebic gill disease revisited.** J Fish Dis 27:445-449

Previous work in our laboratory defined a method of inducing laboratory-based amoebic gill disease (AGD) in Atlantic salmon, *Salmo salar* L. Gills of AGD-affected fish were scraped and the debris placed into fish-holding systems, eliciting AGD in naive Atlantic salmon. While this method is consistently successful in inducing AGD, variability in the kinetics and severity of infections has been observed. It is believed that the infections are influenced by inherently variable viability of post-harvest amoeba trophozoites. Here, a new method of experimental induction of AGD is presented that redefines the infection model including the minimum infective dose. Amoebae were partially purified from the gills of AGD-affected Atlantic salmon. Trophozoites were characterized by light microscopy and immunocytochemistry and designated *Neoparamoeba* sp., possibly *NeoParamoeba pemaquidensis*. Cells were placed into experimental infection systems ranging in concentration from 0 to 500 cells L<sup>-1</sup>. AGD was detected by gross and histological examination in fish held in all systems inoculated with amoebae. The number of gross and histological AGD lesions per gill was proportional to the inoculating concentration of amoebae indicating that the severity of disease is a function of amoeba density in the water column. The implications of these observations are discussed in the context of the existing AGD literature base as well as Atlantic salmon farming in south-eastern Tasmania.

Nowak B, Douglas-Helders M, Bagley C, Crosbie P, Adams M, Morrison R, Butler R, Carson J (2004) **Model Development for Epidemiology of Amoebic Gill Disease.** FRDC Project No 2001/245, Aquafin CRC Project 344. Tasmanian Aquaculture and Fisheries Institute, Launceston, Tasmania

Amoebic Gill Disease (AGD) is the main health problem affecting salmon industry in Southern Tasmania. To improve management of fish with AGD on the farms, the industry needs better understanding of AGD epidemiology. This will provide a basis on which to develop strategies for new treatment or vaccine application in the future. We have identified reservoirs of the amoeba causing AGD in Atlantic salmon in Tasmania. The amoebae were widespread and easily isolated from marine and estuarine sediments and cage netting. There was no apparent relationship between the presence of the amoebae in the environment and AGD prevalence in the cages. Development of methods to isolate amoebae from water samples and to accurately quantify the amoebae is needed if the relationship between the amoebae in the environment and AGD outbreaks is to be fully understood. However, computer simulations suggested that once fish in a cage are infected with AGD, they become the main source of amoebae and other reservoirs become insignificant.

Risk factors for AGD outbreaks were reviewed. New risk factors were identified on the basis of laboratory experiments, field trials, limited farm data and the industry's perception. Main environmental factors included high salinity and increased temperature. In vitro growth of the amoeba was affected by increased copper sulphate concentrations, low salinity and low cell

densities.

Effects of farm activities, such as movement of cages to a fallowed site or use of antifouling paint on cage netting, on AGD were evaluated. Movement of cages to a new site after bathing reduced the impact of AGD on these cages. While amoebae were isolated more often from the cages on which antifouling paints were used, it did not seem to affect AGD prevalence in these cages. A draft protocol of best husbandry techniques that reflected both industry practices and our current understanding of the disease was developed. Gross gill checks used by the industry to evaluate the need for freshwater bathing and the general gill status of the fish were compared to histological evaluations. This comparison confirmed that experienced farm personnel can identify gross lesions caused by AGD. This allows farm data, including gross gill scores to be used for AGD research. Sometimes, however the agreement between gross gill scores and histology worsens, most likely due to environmental factors affecting gross gill appearance. In general, AGD diagnosis based on gross gill score gives more positive results than histological diagnosis.

This project provided specialised training in introductory and advanced epidemiology, sampling design, design of field trials and data analysis to all stakeholders, including researchers, government veterinarians and industry. Potential for AGD data sharing and analysis for the whole industry was discussed. A trial AGD database was set up, however industry showed no interest in using it. A computer model was developed based on data from one farm collected during 2003. While the simulations provided interesting insights and identified knowledge gaps, the model could not be validated using data from the same farm from 2002, proving that it has serious limitations. Increased understanding of the disease outbreaks dynamics is needed before a predictive model of AGD can be developed.

Nowak B, Morrison R, Crosbie P, Adams M, Butler R, Bridle A, Gross K, Vincent B, Embar-Gopinath S, Carson J, Raison R, Villavedra M, McCarthy K, Broady K, Wallach M (2004) **Host-Pathogen Interactions in Amoebic Gill Disease**. FRDC Project No 2001/244, Aquafin CRC Project 342. Tasmanian Aquaculture and Fisheries Institute, Launceston, Tasmania

Prior to this project, our knowledge of Amoebic Gill Disease (AGD) was fundamentally limited. An improved understanding of host-pathogen interactions was required to provide a basis from which to develop effective strategies for future control and treatment of the disease. This was achieved by the development of new techniques and adaptation of existing ones for the study of ecto-parasitic gill disease. This project addressed the need for understanding disease development and progression. Sequential histopathology in both laboratory and field infections identified developmental stages of AGD, in terms of host response and pathogen proliferation and the disease's strong association with salinity and temperature. This work enabled the introduction of an AGD case definition, providing consistent and repeatable disease interpretation for future studies.

Nowak BF, Dawson D, Basson L, Deveney M, Powell MD (2004) **Gill histopathology of wild marine fish in Tasmania: potential interactions with gill health of cultured Atlantic salmon, *Salmo salar* L.** J Fish Dis 27:709-717

This study surveyed conditions in the gills of wild marine fish in Tasmania to determine potential interactions between wild and cultured fish. Wild marine fish of 12 species were captured from three Atlantic salmon farm sites and three reference sites around Tasmania. The survey concentrated on three species, red cod, *Pseudophycis bachus*, sand flathead, *Platycephalus bassensis*, and jack mackerel, *Trachurus declivus*. Seventy-six per cent of

salmon pens contained wild fish species. The number of species found in a pen ranged from one to nine and the number of individuals ranged from one to 23. Trichodinids were prevalent and occurred on seven of the 13 species examined. Trichodina occurred on the gills of all but one specimen of red cod. Monogenean gill flukes were observed on all three major species sampled and were abundant on sand flathead. Other parasites and conditions observed in the survey included metacercariae of digenean trematodes, epitheliocystis and cysts of unknown origin. Infestations of trichodinids on red cod and monogenean gill flukes on sand flathead were significantly more intense at farm sites than at reference sites. Atlantic salmon sampled at the same time from the farms were only affected by amoebic gill disease and isopods.

Powell M, Carson J, van Gelderen R (2004) **Experimental induction of gill disease in Atlantic salmon *Salmo salar* smolts with *Tenacibaculum maritimum***. Dis Aquat Org 61:179-185

An experimentally induced bacterial infection of marine Atlantic salmon *Salmo salar* smolt gills was developed using strains of *Tenacibaculum maritimum* originally isolated from disease outbreaks in Tasmania. The gills of salmon were inoculated with a high concentration of bacteria ( $4 \times 10^{11}$  cells per fish) of either strain 00/3280 or 89/4747 *T. maritimum*. Gentle abrasion of the gills was used to enhance the progression of gill disease. One strain (00/3280) was highly pathogenic, causing morbidity and mortality within 24 h post-inoculation, and produced acute focal branchial necrosis associated with significant increases in plasma osmolality and lactate concentration compared with controls (non-inoculated) or strain 89/4747-inoculated fish. There were no differences in the whole body net ammonium flux between control (non-inoculated) and strain 00/3280-inoculated fish. Gill abrasion resulted in acute telangiectasis and focal lamellar hyperplasia in all fish regardless of bacterial inoculation. This work provides the basis of a challenge model suitable for investigating the pathophysiological processes associated with acute branchial necrosis in marine fish, suggesting that osmoregulatory and possibly respiratory dysfunction are the primary consequences of infection.

Sajjadi M, Carter CG (2004) **Dietary phytase supplementation and the utilisation of phosphorus by Atlantic salmon (*Salmo salar* L.) fed a canola-meal-based diet**. Aquaculture 240:417-431

The effect of phytase supplementation to a canola-meal-based diet on phosphorus utilisation in Atlantic salmon was studied in a two-by-two factorial design. Diets were prepared without phytase or inorganic phosphorus supplementation, with phytase, with supplemental inorganic phosphorus and with both phytase and supplemental inorganic phosphorus. Available phosphorus was set below requirement and the total phosphorus set to meet requirements for salmonids. After 12 weeks, there were no significant differences in survival, feed intake and weight gain between diets. There was an interaction effect between supplements on bone ash, bone phosphorus and whole-body phosphorus so that adding phytase, inorganic phosphorus, or both resulted in significantly ( $P < 0.05$ ) higher values for these parameters. An interaction effect was also observed for phosphorus digestibility, phosphorus retention efficiency and phosphorus load. Phosphorus digestibility and retention efficiency were significantly ( $P < 0.05$ ) higher, and phosphorus load was significantly ( $P < 0.05$ ) lower in fish fed the phytase supplemented diet compared with diets containing supplemental inorganic phosphorus. In conclusion, phytase increased phosphorus

availability, therefore reducing the need to add inorganic phosphorus and reducing phosphorus waste from plant-meal-based diets for Atlantic salmon.

Sajjadi M, Carter CG (2004) **Effect of phytic acid and phytase on feed intake, growth, digestibility and trypsin activity in Atlantic salmon (*Salmo salar*, L.)**. *Aquacult Nutr* 10:135-142

In the majority of experiments, the effects of phytic acid (with or without phytase) are not separated from the effects of adding plant meals containing phytic acid. A 12-week experiment was conducted with Atlantic salmon (28.9 g) to determine the separate and combined effects of phytic acid and phytase on feed intake, trypsin activity, digestibility and growth. Diets were prepared without phytic acid and phytase; with 2000 U phytase kg<sup>-1</sup> diet; with 10 g sodium phytate kg<sup>-1</sup> diet; and with 10 g sodium phytate and 2000 U phytase kg<sup>-1</sup> diet. The basal diet contained sufficient phosphorus and other minerals to meet salmonid requirements. The addition of phytic acid had no significant effect on feed intake or weight gain, it significantly ( $P < 0.05$ ) reduced protein digestibility although there was no reduction in trypsin activity. Phytase inclusion neutralized the effect of phytic acid on protein digestibility. Phytase had no effect on feed intake but significantly enhanced growth whether included with or without phytic acid. Feed efficiency ratio was significantly improved for fish fed the diet containing both phytase and phytic acid but not separately. The significance of this experiment was to separate the direct effects of phytase and the direct effects of phytic acid, added in a pure form, from effects due to other components in ingredients containing phytic acid.

Wong FYK, Carson J, Elliott NG (2004) **18S ribosomal DNA-based PCR identification of *NeoParamoeba pemaquidensis*, the agent of amoebic gill disease in sea-farmed salmonids**. *Dis Aquat Org* 60:65-76

*NeoParamoeba pemaquidensis* is a parasomal amoeboid protozoan identified as the agent of amoebic gill disease (AGD) in Atlantic salmon *Salmo salar* reared in sea-pens in Tasmania, Australia, and coho salmon *Oncorhynchus kisutch* farmed on the west coast of the USA. Outbreaks of AGD caused by immunologically cross-reactive paramoebae have also been reported in sea-farmed salmonids in several other countries. Complete 18S rDNA sequences were determined for respective paramoebae isolated from infected gills of salmon from Tasmania and Ireland, and *N. pemaquidensis* isolates from the USA and UK, including representative free-living isolates. Alignments over 2110 bp revealed 98.1 to 99.0% sequence similarities among isolates, confirming that paramoebae implicated in AGD in geographically distant countries were homologous and belonged to the same species, *N. pemaquidensis*. The results supported previous findings that *N. pemaquidensis* exists as a widely distributed, amphizoic marine protozoan. Partial 18S rDNA sequences were obtained for the ultra-structurally similar species, *N. aestuarina*, and for the morphologically similar but non-parasomal amoeba *Pseudoparamoeba pagei*. *N. aestuarina* had 95.3 to 95.7% sequence similarities with *N. pemaquidensis* strains, which distinguished 2 closely related but separate species. *NeoParamoeba* spp. were not analogous to *P. pagei* or to other marine *Gymnamoebia*. We designed 4 oligonucleotide primers based on elucidated 18S rDNA sequences and applied them to single-step and nested 2-step PCR protocols developed to identify *N. pemaquidensis* to the exclusion of apparently closely related and non-related protistan taxa. Nested PCR was able to detect the AGD parasite from non-purified, culture-enriched net microfouling samples from Atlantic salmon sea-pens in Tasmania, and confirmed that *N. pemaquidensis* was also responsible for AGD in chinook

salmon *O. tshawytscha* in New Zealand. Our sequence and PCR analyses have now shown that AGD affecting 3 different salmonid species farmed in 4 countries are associated with *N. pemaquidensis*. A species-specific diagnostic PCR provides for the first time, a highly specific detection and identification assay for *N. pemaquidensis* that will facilitate future ecological and epidemiological studies of AGD.

2003

Adams MB, Nowak BF (2003) **Amoebic gill disease: sequential pathology in cultured Atlantic salmon, *Salmo salar* L.** J Fish Dis 26:601-614

Amoebic gill disease (AGD) affects the marine culture phase of Atlantic salmon, *Salmo salar* L., in Tasmania. Here, we describe histopathological observations of AGD from smolts, sampled weekly, following transfer to estuarine/marine sites. AGD was initially detected histologically at week 13 post-transfer while gross signs were not observed for a further week post-transfer. Significant increases ( $P < 0.001$ ) in the proportion of affected gill filaments occurred at weeks 18 and 19 post-transfer coinciding with the cessation of a halocline and increased water temperature at the cage sites. The progression of AGD histopathology, during the sampling period, was characterized by three phases. (1) Primary attachment/interaction associated with extremely localized host cellular alterations, juxtaposed to amoebae, including epithelial desquamation and oedema. (2) Innate immune response activation and initial focal hyperplasia of undifferentiated epithelial cells. (3) Finally, lesion expansion, squamation-stratification of epithelia at lesion surfaces and variable recruitment of mucous cells to these regions. A pattern of preferential colonization of amoebae at lesion margins was apparent during stage 3 of disease development. Together, these data suggest that AGD progression was linked to retraction of the estuarine halocline and increases in water temperature. The host response to gill infection with *Neoparamoeba* sp. is characterized by a focal fortification strategy concurrent with a migration of immunoregulatory cells to lesion-affected regions.

Bransden MP, Carter CG, Nichols PD (2003) **Replacement of fish oil with sunflower oil in feeds for Atlantic salmon (*Salmo salar* L.):** effect on growth performance, tissue fatty acid composition and disease resistance. Comp Biochem Phys B 135:611-625

Dietary sunflower oil (SFO) was used to gradually replace fish oil (FO) in six diets (which also contained fish meal) for Atlantic salmon parr (initial mass: 21.7 g). The effect on growth performance, tissue fatty acid profiles and disease resistance was monitored after 63 days. At the conclusion of the trial, no significant differences were detected in growth between any of the feeds. Fatty acid composition of whole carcass, dorsal muscle and liver generally reflected that of the diets. Forty percent of the FO could be replaced by SFO before tissue 22:6n-3 was significantly reduced, although other essential and non-essential fatty acids were more susceptible to change. Significant differences were detected in cumulative mortality of Atlantic salmon challenged with *Vibrio anguillarum* at the trials conclusion, although this was not correlated to the inclusion level of SFO. Despite the changes observed to the tissue fatty acid profile, there was no significant effect on growth suggesting that SFO is a suitable alternative to FO in diets for Atlantic salmon parr when fish meal is also included.

Bridle AR, Butler R, Nowak BF (2003) **Immunostimulatory CpG oligodeoxynucleotides increase resistance against amoebic gill disease in Atlantic salmon, *Salmo salar* L.** J Fish Dis 26:367-371

Amoebic gill disease (AGD) severely affects sea-cage cultured populations of Atlantic salmon, *Salmo salar* L., in Tasmania (Munday, Foster, Roubal & Lester 1990). The AGD caused by the pathogen, *Neoparamoeba pemaquidensis* (Page) (Kent, Sawyer & Hedrick 1988; Howard & Carson 1993), has been an enduring problem in Tasmanian salmonid culture. Cases of AGD are not limited to Tasmania, as the disease has been diagnosed in cultured marine fish throughout the world (Nowak, Carson, Powell & Dykova 2002). Freshwater bathing is currently the preferred form of treatment for both the culture of Atlantic salmon in Australia and turbot, *Scophthalmus maximus* (L.), culture in Spain (Nowak et al. 2002). The effectiveness of freshwater bathing as a method for treating AGD is limited as *N. pemaquidensis* is not completely eradicated from the gills of the fish (Parsons, Nowak, Fisk & Powell 2001). In addition, the need for a perennial supply of fresh water and the labour costs required to freshwater bathe the sea-cages mean that an alternative treatment strategy may prove more cost-effective. Vaccination against AGD is an ideal treatment strategy but so far trials have been unsuccessful (Zilberg & Munday 2001). However, this does not mean that all immunological aspects of AGD treatment and control are futile. Manipulation of the innate immune response through the administration of immunostimulants may be an alternative strategy for the control and treatment of AGD. Oligodeoxynucleotides (ODNs) containing cytosine-phosphodiester-guanine (CpG) motifs are potent immunostimulants that may be beneficial in an AGD control and treatment strategy. Bacterial DNA, unlike vertebrate DNA, contains frequent unmethylated CpG motifs that have the ability to activate mammalian immune cells causing them either to proliferate or produce cytokines (Krieg, Yi, Matson, Waldschmidt, Bishop, Teasdale, Koretzky & Klinman 1995; Klinman, Yi, Beaucage, Conover & Krieg 1996). Similarly, *in vitro* studies in fish have shown immune cell recognition of CpG-ODNs, subsequent immune cell proliferation and cytokine production (Jørgensen, Johansen, Stenersen & Sommer 2001a; Jørgensen, Zou, Johansen & Secombes 2001b; Tassakka & Sakai 2002). However, there have been no studies of the *in vivo* ability of CpG-ODNs to enhance resistance to disease in fish. Therefore, in this paper we report that intraperitoneal (i.p.) administration of CpG-ODNs is able to increase resistance to AGD in Atlantic salmon.

Carson J, Wilson T (2003) **Development of selective enrichment culture-polymerase chain reaction (SEC-PCR) for the detection of bacterial pathogens in covertly infected farmed salmonid fish.** FRDC Project No 99/201. Tasmanian Aquaculture and Fisheries Institute

Bacterial disease is a major cause of stock loss in aquaculture. The severity of infection may range from acute to chronic through to benign. This latter condition, termed covert infection, is insidious, as fish may appear to be outwardly healthy but during periods of stress, these carriers may breakdown leading to spread of infection and development of a disease outbreak.

Several bacterial pathogens, known to exist in Australia and the cause of significant disease episodes in Atlantic salmon and rainbow trout, can cause covert infections including: atypical *Aeromonas salmonicida*, *Lactococcus garvieae*, *Tenacibaculum maritimum* and *Yersinia ruckeri*.

Early detection of covertly infected fish is considered desirable as it provides a means of determining a suitable disease control strategy such as imposing movement restrictions to prevent the spread of disease, changing management practices to avoid stress or determining the spread of disease in a population at risk of infection. The standard method for identifying carriers is to stress a cohort of fish using a combination of heat and

immunosuppression to force covertly infected fish to breakdown with disease. This form of testing is undesirable for animal welfare considerations, is difficult to accomplish and takes over three weeks to generate results.

The research described in this report has led to the development of a replacement test to identify covertly infected fish. Called Selective Enrichment Culture PCR Enzyme Hybridization Assay (SPE), the laboratory based test only requires the use of skin mucus or faecal samples and positive results are obtained within six days. The primary step in SPE uses selective enrichment culture to amplify the target pathogen. Media for the salmonid pathogens had been developed previously except for *A. salmonicida*. A selective enrichment medium was developed for the atypical salmonid biovar of *A. salmonicida* and was shown to have a good level of selectivity when tested with over 400 strains of normal flora bacteria commonly associated with salmonids.

Following enrichment culture, DNA is extracted and the target pathogen detected using the DNA amplification technique PCR or RT-PCR coupled with a DNA probe hybridization step to verify positive reactions. Within the amplification process, a PCR contamination control step is included as a measure to reduce false positive reactions. A rapid two step method for the extraction of DNA and RNA from enrichment culture media using glass microfibre filter plates was developed. Formatted for high throughput testing, 96 samples can be processed simultaneously. Optimised PCR and RT-PCR protocols for the four pathogens could detect as little as 4fg of DNA or RNA, equivalent to one bacterial cell. Coupled with the high throughput DNA/RNA extraction method, the detection level by PCR or RT-PCR ranged from 20 to 80 cells ml<sup>-1</sup> of culture medium. Products of PCR and RT-PCR were detected through hybridization with a secondary verification DNA probe. This was achieved using a biphasic PCR format followed by in-situ probe hybridization undertaken as a two step process in the one reaction well. High throughput processing was retained using 96 well format plates. PCR contamination control was implemented using the amplicon inactivator isopsoralen IP-10. Integrated into PCR process as a post-amplification step, following UV activation, it was possible to inactivate at least 6x10<sup>7</sup> amplicons, sufficient as a routine control measure. Field validation of SPE established that the test did not generate unexpected false positive reactions and test specificity was determined to be >99%. Test sensitivity was better than expected and case definitions had to be established for test interpretation. For farms with recent history of disease, RT-PCR was used as it provides an indication of covert infection with live pathogen. SPE in the PCR format was restricted to testing for evidence of exposure to pathogens as the test detects live carriage as well as DNA remnants from dead cells remaining from recent but past infections. From field surveys of farmed salmonids, live carriage levels ranged from 0-18% for *A. salmonicida*, 2% for *L. garvieae*, 1 % for *T. maritimum* and 4% for *Y. ruckeri*.

Carter CG, Bransden MP, Lewis TE, Nichols PD (2003) **Potential of thraustochytrids to partially replace fish oil in Atlantic salmon feeds.** *Mar Biotechnol* 5:480-492

The replacement of fish oil with a dried product made from thraustochytrid culture, a marine microorganism, in canola-oil-based diets for Atlantic salmon was investigated. Salmon (37 g) were fed for 51 days on diets containing only canola oil, canola oil and fish oil, or canola oil and the thraustochytrid. There were no significant differences in final weight (106.1 +/- 1.1 g), weight gain (69.6 +/- 1.1 g), feed consumption (16.5 +/- 0.2 mg dry matter g<sup>(-1)</sup> d<sup>(-1)</sup>), feed efficiency ratio (1.15 +/- 0.03 g g<sup>(-1)</sup>), or productive protein value (51.2% +/- 1.7%) between the diets. Nor were there any significant differences in whole-body chemical composition, organ somatic indices, or measures of immune function. However, following

transfer to seawater and 2 challenges with *Vibrio anguillarum*, cumulative mortality was significantly lower in fish fed some fish oil than in those fed the 2 diets containing no fish oil. In conclusion, the thraustochytrid had no detrimental effects on the performance of salmon but, at the current inclusion of 10%, failed to confer the same effect as fish oil under challenging conditions.

Carter CG, Lewis TE, Nichols PD (2003) **Comparison of cholestane and yttrium oxide as digestibility markers for lipid components in Atlantic salmon (*Salmo salar* L.) diets.**

Aquaculture 225:341-351

The study aimed to test the similarity between apparent digestibility coefficients (ADC) calculated using either yttrium oxide or cholestane. Atlantic salmon were fed three diets containing a different mix of oil sources: diets contained only canola oil (CO), canola oil and fish oil (CFO), or canola oil and thraustochytrid meal (CTH) (a marine microorganism with potential as an alternative oil source). Both markers were concentrated by the same amount in the faeces compared to the diets. ADC values using cholestane tended to be higher than yttrium, and there was a significant marker effect for crude protein, energy, total lipid and triacylglycerol. Although statistically significant the marker effect was due to numerically small differences in ADC values and probably of limited biological significance. ADC values for the majority of fatty acids did not show marker effects. Consequently, it was concluded that the broad similarity in the digestibility of lipid components between markers validated the use of cholestane for calculating lipid digestibility in Atlantic salmon.

Clark G, Powell M, Nowak B (2003) **Effects of commercial freshwater bathing on reinfection of Atlantic salmon, *Salmo salar*, with Amoebic Gill Disease.** Aquaculture 219:135-142

Fish with Amoebic Gill Disease (AGD) were examined over a 10-day period following commercial freshwater bathing to assess the time to reinfection. Samples were taken from fish before freshwater bathing and then 1, 3, 5 and 10 days after bathing to determine the number of amoebae present on the gills. Freshwater bathing significantly reduced the number of amoebae on the gills, with an 86 +/- 9.1 % reduction in the number of live amoebae found on the gills after freshwater bathing. However, amoeba numbers returned to pre-bath levels 10 days after bathing. There was no significant effect on number of AGD lesions/filament, the mean ranged from 0.08, 3 days after bathing, to 0.14, 5 days after bathing. However, the number of *NeoParamoeba pemaquidensis* dramatically dropped in histological sections from 0.53 per AGD lesion before the bath to 0 per AGD lesion 1 day after the bath and then remained significantly lower, reaching 0.08 per AGD lesion 10 days after the bath. The number of mucous cells changed, with Alcian blue (AB) (pH 1) positive cells decreasing immediately after bathing. Results of this study show that commercial freshwater bathing is effective at removing amoebae from the gills of fish, however, reinfection can occur within a week.

Crosbie PBB, Nowak BF, Carson J (2003) **Isolation of *NeoParamoeba pemaquidensis* page, 1987 from marine and estuarine Sediments in Tasmania.** Bull Eur Assoc Fish Pathol 23:241-244

To gauge environmental ubiquity of *NeoParamoeba pemaquidensis*, the causative agent of amoebic gill disease (AGD) in farmed Atlantic salmon in Tasmania, sediments in both marine and estuarine sites, including those with no history of AGD, were screened for presence of the organism. With one exception *N. pemaquidensis* was detected in all locations.



Douglas-Helders GM, Handler J, Carson J, Nowak BF (2003) **Assessment over time of the infectivity of gill-derived and seawater dispersed NeoParamoeba pemaquidensis (Page, 1987)**. Bull Eur Assoc Fish Pathol 23:35-39

A laboratory infection trial tested if NeoParamoeba pemaquidensis, the protozoan responsible for AGD, remained infectious for up to 14 days when out of contact with host tissues. In the infection trial, 14 Atlantic salmon (*Salmo salar*) were exposed to gill-derived paramoebae, which had been out of contact with hosts for up to 14 days. At the conclusion of the trial fish tested immunodot blot positive and were positive for histology. This implies that infection occurs from water to fish and the zone of infection around salmon farms may be very extensive.

Douglas-Helders GM, O'Brien DP, McCorkell BE, Zilberg D, Gross A, Carson J, Nowak BF (2003) **Temporal and spatial distribution of paramoebae in the water column - a pilot study**. J Fish Dis 26:231-240

Amoebic gill disease is the main disease affecting the salmonid industry in Tasmania, but no information on the distribution of the causative pathogen, NeoParamoeba pemaquidensis, in the aquatic environment is available. This pilot study aimed to determine temporal and spatial distributions of paramoebae species in the water column, using an immuno-dot blot technique. Water samples were collected from inside fish cages at various depths (0.5, 5.5 and 11.0 m) in both summer and winter, as well as various distances (0, 0.5, 240, 280, 750 and 1100 m) away from the sea cage and farming site. Paramoebae densities were estimated using the most probable number technique (MPN). Temperature, salinity, dissolved oxygen, turbidity, nitrite and nitrates, and bacterial counts were measured for each water sample. Data were analysed using a residual maximum likelihood test and significant associations between paramoebae densities and environmental factors were analysed. Results showed that densities were significantly higher in summer ( $P = 0.017$ ), at 5.5 m depth ( $P = 0.029$ ), and reduced to the lowest density at 1100 m away from the cage sites ( $P = 0.008$ ). Bacterial counts, turbidity and temperature were found to be significantly associated with paramoebae densities.

Douglas-Helders GM, Tan C, Carson J, Nowak BF (2003) **Effects of copper-based antifouling treatment on the presence of NeoParamoeba pemaquidensis Page, 1987 on nets and gills of reared Atlantic salmon (*Salmo salar*)**. Aquaculture 221:13-22

Amoebic gill disease (AGD) is the main disease affecting the salmon industry in Australia. Little information is available on the epidemiology of AGD and the biology of NeoParamoeba pemaquidensis [Page, 1987], the disease-causing organism of AGD. In previous studies, *N. pemaquidensis* was found on biofouled netting of sea cages, and a reduction in AGD prevalence was achieved with increasing number of net changes. Presently, it is not known if *N. pemaquidensis* on netting is able to induce AGD. To reduce biofouling on nets, antifouling paints are commonly used on Tasmanian salmon farms. This study investigated the effects of a copper-based antifouling paint on the *N. pemaquidensis* densities on nets and the AGD prevalence of Atlantic salmon reared in these nets. Four sea cages stocked with 5-9 kg/m<sup>3</sup> Atlantic salmon were used in this study. Two nets were coated with a copper-based antifouling paint and two nets were not treated and used as a control. Fish were sampled every 2 weeks for 10 weeks. A gross gill score was determined and gill mucus samples were taken for dot blot analysis to determine the presence of *N. pemaquidensis* for each fish. Biofouling samples from netting were inoculated onto 75% malt yeast agar culture plates, and presence of *N. pemaquidensis* assessed using conventional culture techniques,

followed by indirect immunofluorescent antibody test (IFAT). The presence of *N. pemaquidensis* was confirmed from culture-enriched biofouling samples from weeks 2 and 8 were tested using nested PCR. Results suggest that copper paint treated cages had significantly higher paramoebae ( $P=0.002$ ) and AGD ( $P=0.014$ ) prevalence compared to the control cages. No treatment effect was found on the intensity of infection, determined by gross gill scores ( $P=0.243$ ). At the end of the study, the paramoebae prevalence of net samples was 58.5% (S.E. 1.5) and AGD prevalence was 42.5% (S.E. 2.5) for copper treated nets, while no paramoebae were found on control nets and AGD prevalence was 35.0% (S.E. 5.0). Nets could be the source of *N. pemaquidensis* infection of fish with AGD, and therefore copper paint treated nets could be a risk factor for AGD.

Powell MD, Nowak BF (2003) **Acid-base and respiratory effects of confinement in Atlantic salmon affected with amoebic gill disease.** J Fish Biol 62:51-63

Amoebic gill disease (AGD) in cultured salmonids causes severe multifocal hyperplastic lesions in the gills with the potential to influence respiratory and acid-base physiology Atlantic salmon *Salmo salar* affected with AGD were Surgically implanted with dorsal aortic catheters and, following recovery, were confined for 5 min ( $n = 16$ ) or left undisturbed ( $17 = 8$ ). Confinement caused an acute extracellular acidosis that was corrected in 6 h amongst surviving fish. There was a gradual increase in plasma lactate concentrations that peaked at 1 h post-confinement then declined by 9h recovery In a second experiment, AGD-affected fish were confined then recovered either in a tank of static water ( $n 9$ ) or while being forced to swim at 1.5 body lengths  $s^{-1}$  ( $n = 6$ ). There was no significant difference between fish recovered by swimming and those in static water in terms of recovery of the acute extracellular acidosis and lactate accumulations coincident with exhaustive exercise. Confinement severely compromised the survival of AGD-affected Atlantic salmon, although Survivors appeared to recover similarly to other studies. Forced swimming of AGD-affected Atlantic salmon following confinement did not facilitate recovery and is unlikely to be a useful strategy for mitigating the effects of stressful episodes such as crowding and fish movement and commercial handling.

2002

Carter CG, Foale M, Hauler RC, Attard M, Edwards S, Bransden MP, Nowak BF, Farhangi M, Lewis TE, Nichols PD (2002) **Aquaculture Feed Development for Atlantic Salmon (*Salmo salar*).** In: Carter CG, Hauler RC, Foster C (eds) FRDC Project No 1998/322. Tasmanian Aquaculture and Fisheries Institute

The research provided data of strategic importance on the feed requirements of a major farmed Australian fish species, Atlantic salmon, and advanced the study of fish nutrition, particularly our understanding of essential amino acid requirements. This was achieved through collaboration between Skretting Australia, the major manufacturer of salmonid feeds in Australia, and the Nutrition Group at the Tasmanian Aquaculture and Fisheries Institute. The research generated information that will be essential in the evaluation and further development of feeds for Atlantic salmon and is also of relevance for rainbow trout. The focus was on Tasmanian conditions, this is important because they are different to those in which Atlantic salmon are farmed elsewhere in the world.

Douglas-Helders GM, Dawson DR, Carson J, Nowak BF (2002) **Wild fish are not a significant reservoir for *Neoparamoeba pemaquidensis* (Page, 1987).** J Fish Dis 25:569-574

Amoebic gill disease (AGD), caused by the protozoan *Neoparamoeba pemaquidensis* (Page, 1987) is the most important disease affecting salmon farms in Tasmania. Reservoirs for this protozoan parasite are largely unknown. This study investigated wild fish as a potential reservoir of *N. pemaquidensis*. A total of 325 wild fish, comprising 12 different fish species, were caught from and around salmon farms and examined for the presence of AGD. None of the wild fish were infected with AGD. In a laboratory trial, seahorse, *Hippocampus abdominalis*, greenback flounder, *Rhombosolea tapirina*, and Atlantic salmon, *Salmo salar*, were challenged with *N. pemaquidensis*. *Neoparamoeba pemaquidensis* was detected on the gills on 10 of 15 (66.7%) flounder, nine of 24 (37.5%) seahorses, and six of six (100%) Atlantic salmon. However, paramoebae positive flounder and seahorse lacked the characteristic AGD gill pathology. It is concluded that AGD does not appear in wild fish and wild fish do not seem to be a reservoir of the pathogen.

Fisk DM, Powell MD, Nowak BF (2002) **The effect of Amoebic Gill Disease and hypoxia on survival and metabolic rate of Atlantic salmon (*Salmo salar*)**. Bull Eur Assoc Fish Pathol 22:190-194

AGD affected fish showed reduced survival following a 50% oxygen saturation hypoxic challenge (21.4% survival) compared with 88.9% survival of non-affected fish. The metabolic rates of AGD affected and non-affected fish were not significantly different under normoxic conditions. AGD-affected fish showed a significant reduction in metabolic rate under hypoxia compared with normoxia. These results suggest that hypoxia may not be the most significant factor leading to mortality with AGD but rather that there maybe some scope for metabolic compensation.

Powell MD, Forster ME, Nowak BF (2002) **Apparent vascular hypertension associated with Amoebic Gill Disease affected Atlantic salmon (*Salmo salar*) in Tasmania**. Bull Eur Assoc Fish Pathol 22:328-333

The dorsal aortic (DAP), ventral aortic (VAP) blood pressures and heart rate were measured in Atlantic salmon affected with AGD in a clinical outbreak. DAP and VAP were high compared with published values for other salmonid species. Fish subjected to a 3-h freshwater bath and returned to seawater (the treatment of choice for AGD control) had a significantly lower dorsal aortic pressure, with values comparable to those for other salmonids. Heart rate was not affected by freshwater exposure. These data suggest that there may be a vascular hypertension associated with AGD in Atlantic salmon. Although the source of the hypertension remains to be identified, this finding may help to explain the poor post-stress survival of AGD-affected salmon.

Powell MD, Nowak BF, Adams MB (2002) **Cardiac morphology in relation to amoebic gill disease history in Atlantic salmon, *Salmo salar* L.** J Fish Dis 25:209-215

Fish from cages with histories of heavy and light amoebic gill disease (AGD) outbreaks were harvested and the morphology, histology and activities of lactate dehydrogenase determined. Although fish with a history of heavy AGD were smaller, their heart somatic indices were similar to those of fish with a history of light AGD. However, morphometrically the ratios of ventricle axis length and width and axis length and height were significantly higher, and there was an overall thickening of the muscularis compactum in the ventricle of fish with heavy AGD history. There was no difference in the lactate dehydrogenase activity of the ventricle muscle in the two fish groups. These results suggest that the change in ventricle shape associated with AGD was a possible compensation for an increased afterload where the lengthening of the ventricle was compensated for by an increase in

muscle thickness, but without any overall ventricular hypertrophy or gain in ventricular mass. This suggests that AGD may be associated with cardiovascular compromise in affected fish.

Tan CKF, Nowak BF, Hodson SL (2002) **Biofouling as a reservoir of *Neoparamoeba pemaquidensis* (Page, 1970), the causative agent of amoebic gill disease in Atlantic salmon.** *Aquaculture* 210:49-58

Amoebic gill disease (AGD) is currently the most important disease affecting the Tasmanian salmonid industry and is caused by a marine amoeba, *Neoparamoeba pemaquidensis* (Page, 1970). In this study biofouling communities on salmon cages were surveyed for the presence of the disease agent over a period of 4 months. Malt-yeast-seawater (MYS) agar plates were used to culture *N. pemaquidensis* with its presence confirmed by immunofluorescent antibody test (IFAT). Positive percentages of categorised samples ranged from 0% to 55%. The survey detected the presence of *N. pemaquidensis* on a number of macrofouling species (in particular bryozoan *Scrupocellaria bertholetti* and solitary ascidian *Ciona intestinalis*), and in microfouling and water samples. High percentages of positive IFATs occurred in microfouling aggregates, the solitary ascidian, *C. intestinalis*, and centrifuged water samples. No positive IFATs occurred from samples of *Caprella* sp. The presence of *N. pemaquidensis* was sporadic and varied in species and over sampling month. Experimental exposure of Atlantic salmon, *Salmo salar*, to lightly fouled netting was conducted to assess the potential for microfouling to act as a source of infection. No signs of the disease were detected in fish exposed to lightly fouled netting treatments, while 100% of positive control fish were infected and had an average of 4.24 +/- 1.79 amoebae per field of view in IFAT of mucus smears. When combined with *N. pemaquidensis* loads in the water column, the loads of amoebae in biofouling communities may contribute to disease outbreaks. Thus, biofouling should be considered a risk factor for AGD outbreaks.

2001

Adams MB, Nowak BF (2001) **Distribution and structure of lesions in the gills of Atlantic salmon, *Salmo salar* L., affected with amoebic gill disease.** *J Fish Dis* 24:535-542

Gills of Atlantic salmon, *Salmo salar* L., with amoebic gill disease (AGD), were analysed by routine histology to identify lesion morphology and distribution patterns. Numbers of lesions occurring dorsally, medially and ventrally in the gill filaments were recorded as was lesion size, proximity to the gill arch and the degree of pathological severity involved. The mean number of lesions and pathological severity in the dorsal region of the second left gill arch were significantly higher than that found ventrally ( $P < 0.01$ ). There were no significant differences between gill regions in lesion size or proximity of lesions to the gill arch. Serially sectioned lesions revealed interlamellar cysts to be spherical to ovate in shape and fully enclosed within a wall of epithelium. Small to medium size cysts sometimes contained necrotic amoebae. Inflammatory cells, morphologically identified as neutrophils and macrophages, were occasionally seen infiltrating medium sized cysts. Larger cysts were mostly clear of any cellular debris.

Bransden MP, Carter CG, Nowak BF (2001) **Effects of dietary protein source on growth, immune function, blood chemistry and disease resistance of Atlantic salmon (*Salmo salar* L.) parr.** *Anim Sci* 73:105-113

Many studies with fin fish have demonstrated the potential to use alternative dietary protein sources to fish meal based on growth responses, although these trials mostly neglect to

determine if such protein sources affect immune function. This study investigated the effect of fish meal replacement with dehulled lupin meal (LPN) or hydrolysed poultry feather meal (FTH). Atlantic salmon (*Salmo salar* L.) parr were supplied isonitrogenous and isoenergetic diets with 40% of the dietary protein provided by LPN or FTH, or 400 g/kg of the dietary protein provided equally by LPN and FTH (MIX). A diet mainly containing fish-meal protein acted as a control (CON). Growth, immune function, blood chemistry and disease resistance were assessed after 56 days. Significant differences ( $P < 0.05$ ) in weight gain were detected between Atlantic salmon given the CON and FTH diets, whilst those salmon given LPN and MIX did not differ from any other. Productive protein values were significantly lower ( $P < 0.01$ ) for salmon on FTH compared with those on CON and MIX. Immune function (as assessed by lysozyme, antiprotease, neutrophil oxygen radical production and plasma total immunoglobulin) and blood chemistry (as assessed by plasma total protein and glucose) were not significantly ( $P < \text{greater than} > 0.05$ ) affected by any diet. Mortality rates of Atlantic salmon challenged with *Vibrio anguillarum* were not influenced by diet. These data suggest Atlantic salmon could be supplied diets with the fish meal component reduced to supply approximately 600 g/kg of the total protein, with the remaining 400 g/kg supplied by dehulled lupin meal or a combined dehulled lupin and hydrolysed poultry feather meal without any adverse effects on growth, immune function or blood chemistry.

Douglas-Helders M, Saksida S, Raverty S, Nowak BF (2001) **Temperature as a risk factor for outbreaks of Amoebic Gill Disease in farmed Atlantic salmon (*Salmo salar*)**. Bull Eur Assoc Fish Pathol 21:114-116

Amoebic Gill Disease (AGD) outbreaks in Atlantic salmon have recently occurred below the lower temperature limit previously recognised for *Neoparamoeba pemaquidensis*. This observation challenges the role of ambient water temperature as one of the prime risk factors for AGD.

Douglas-Helders M, Carson J, Howard T, Nowak B (2001) **Development and validation of a new dot blot test for the detection of *Paramoeba pemaquidensis* (Page) in fish**. J Fish Dis 24:273-280

In this study, the development of a dot blot assay to assess amoebic gill disease (AGD) using non-lethal gill mucus samples is described and its performance validated by comparing the assay with the indirect fluorescent antibody rest (IFAT), the 'gold standard' test. The agreement between the two tests was high, with a positive predictive value of 95% and negative predictive value of 93%, with a corrected kappa value of 0.88. The sensitivity and specificity of the test were 97 and 91%, respectively. The dot blot is both sensitive and specific for *Paramoeba pemaquidensis* and is formatted so that large numbers of samples can be conveniently analysed.

Hauler RC, Carter CG (2001) **Lysine deposition responds linearly to marginal lysine intake in Atlantic salmon (*Salmo salar* L.) parr**. Aquacult Res 32:147-156

This study investigated lysine utilization at marginal lysine intake (mg day<sup>-1</sup>) in Atlantic salmon (*Salmo salar* L.) parr fed diets similar to those used in dose-response lysine requirement experiments. Duplicate tanks of salmon were fed Diet PL20.1 (20.1 g lysine kg<sup>-1</sup>) and 19.8 MJ DE kg<sup>-1</sup>) containing 54% of the lysine in the crystalline (free) form at four intake levels for 50 days. Feed intake levels were measured at 0.59, 0.85, 1.10 and 1.16 (satiation,)%BW day<sup>-1</sup>. In addition, duplicate tanks of salmon were fed to satiation with Diets FML9.1 (9.1 g lysine kg<sup>-1</sup>) and FML20.7 (20.7 g lysine kg<sup>-1</sup>) in which lysine was derived almost entirely from fish meal. Feed intakes of FML9.1 (0.70%BW day<sup>-1</sup>) and

FML20.7 (1.21%BW day<sup>-1</sup>) were significantly lower and higher ( $P < 0.001$ ) than the satiation intake of the Diet PL20.1, respectively. Over all dietary treatments, lysine deposition was dependent on lysine intake (LI, mg day<sup>-1</sup>) as described by the equation: lysine deposition (mg day<sup>-1</sup>) = 0.708LI - 0.035 ( $r^2 = 0.97$ ,  $n = 12$ ,  $P < 0.001$ ). Addition of marginal lysine intakes from Atlantic salmon dose-response lysine requirement literature resulted in an equivalent relationship. The linear relationship suggests that lysine utilization remains constant at marginal lysine intake over different dietary formulations and life-stages. Consequently, the lysine requirement (allowance) of Atlantic salmon would be more appropriately estimated by a factorial approach, assuming constant efficiency of lysine utilization and the addition of obligatory lysine loss (maintenance). Efficiency of lysine utilization and obligatory lysine loss was estimated to be 71% and 0.05 mg day<sup>-1</sup> from the current experiment and 78% and 0.10 mg; day<sup>-1</sup> with the addition of the literature data. Based on the constant lysine utilization observed in this study, it is proposed there is a need to re-evaluate lysine requirements expressed as a dietary concentration.

Morrison RN, Nowak BF, Carson J (2001) **The histopathological effects of a levamisole-adjuvanted *Vibrio anguillarum* vaccine on Atlantic salmon (*Salmo salar* L.).**

Aquaculture 195:23-33

Atlantic salmon (*Salmo salar* L.) were administered a levamisole-adjuvanted or non-adjuvanted *Vibrio anguillarum* vaccine by either bath or intraperitoneal (IP) injection. The potential side effects of vaccination were investigated at a histological level, with the skin, gills, anterior kidney and spleen all examined. At a gross level, no pathological changes were evident in any organs. Observations of histological structures found that the skin, anterior kidney and spleen were not affected by vaccination. However pathological changes were observed in the gills of fish treated with the adjuvanted vaccine by both routes of administration. Pathology of the gills was multifocal and included hyperplasia of mucous cells, lamellar oedema, proliferation of chloride cells, inflammation and necrosis. Hyperplasia and hypertrophy of epithelial cells resulted in lamellar fusion. The abundance of lamellar associated mucous cells significantly increased in the gills of fish from groups administered the levamisole-adjuvanted vaccine (IP and bath) ( $P < 0.05$ ). However, the effect of the treatments on lamellar fusion was not significant ( $P > 0.05$ ).

Nowak B (2001) **Qualitative evaluation of risk factors for amoebic gill disease in cultured Atlantic salmon.** Risk Analysis in Aquatic Animal Health, Proceedings:148-154  
 Marine salmonid farming was established in Australia less than twenty years ago. Its success has been due to its disease-free status, since most of the infectious diseases infecting salmonid culture in other countries are absent in Australia. Amoebic gill disease (AGD) is the main disease affecting Atlantic salmon culture in Tasmania. Outbreaks of this disease have been reported in the United States of America, Ireland, France, New Zealand and Chile. While salinity and temperature have been identified as the main environmental factors influencing the prevalence of AGD, a number of other factors, both environmental and host-specific, may be significant. Risk is the likelihood of the disease occurrence and the likely magnitude of the consequences of an adverse effect on aquatic animal health. Risk assessment is the evaluation of the risk, while risk management is the process of identifying, selecting and implementing measures which can be applied to reduce the level of risk. The aim of this review is to discuss risk factors, which may influence the prevalence of the disease.

Parsons H, Nowak B, Fisk D, Powell M (2001) **Effectiveness of commercial freshwater bathing as a treatment against amoebic gill disease in Atlantic salmon.** *Aquaculture* 195:205-210

Amoebic gill disease (AGD) is the most serious health problem in Atlantic salmon culture in Tasmania, Australia. This disease is caused by an amoeboid protozoan, *Paramoeba pemaquidensis* and has resulted in seacage mortalities as high as 50%. Current treatment involves fish being bathed in freshwater for periods of 2-3 h. The aim of this project was to determine the effectiveness of commercial freshwater bathing. Gill samples were collected from Atlantic salmon before and after routine freshwater bathing. Each fish was weighed, measured (fork length), gross AGD score determined, gill smears stained with "Diff Quick" and trypan blue and gill arches examined using routine histology. Freshwater bathing significantly reduced the prevalence of characteristic mucoid patches on the gills, presence of paramoebae on gill smears and the number of paramoebae per lesion in histological sections ( $P < 0.05$ ). Trypan blue staining of gill smears revealed that 27% of the paramoebae were still alive after 2-h freshwater bathing, although the numbers appeared to be lower than before freshwater bathing. Paramoebae were commonly found (71.17%) in cysts formed by fused gill lamellae within AGD lesions. Before the bathing, only 31.9% of paramoebae were present within the cysts and the remaining parasites were present on the surface of the hyperplastic lesions. Results of this study showed that freshwater bathing is effective in the removal of the majority of paramoebae associated with fish infected with AGD. However, alterations in bathing procedure or an alternative treatment may be required to achieve the total removal of paramoebae from gills of Atlantic salmon.

Parsons HJ, Nowak BF, Powell MD, Dix T (2001) **Changes in water quality during commercial freshwater bathing of Atlantic salmon (*Salmo salar*) in Tasmania - implications for treatment of Amoebic Gill Disease.** *Bull Eur Assoc Fish Pathol* 21:71-76

Changes in water quality of commercial freshwater baths used to treat Atlantic salmon (*Salmo salar*) with Amoebic Gill Disease (AGD) were examined at three aquaculture sites. The bathing time ranged from 2.4 to 3.7 hours. Water pH was significantly greater before than after the bathing. Farm site had a statistically significant effect on water pH changes. Ammonia values increased slightly after bathing from 0 to 0.25 mg/l at two of the sites, while nitrate and nitrite levels remained undetectable. Sodium, potassium and chloride levels differed between sites and baths. The magnitude of the changes was minimal indicating the ionic balance of the fish is not adversely affected by the freshwater bathing.

Percival SB, Lee PS, Carter CG (2001) **Validation of a technique for determining apparent digestibility in large (up to 5 kg) Atlantic salmon (*Salmo salar* L.) in sea cages.** *Aquaculture* 201:315-327

This study was undertaken to assess whether collection of faeces by a stripping method was suitable for measuring digestibility in large seawater Atlantic salmon of up to 5 kg. Experiments were conducted to determine the effects on the apparent digestibility coefficient (ADC) of faecal collection technique, urine and mucus contamination of faecal samples, immediate repeated sampling on individuals, stripping pressure during the collection of faeces, dorso-flexion of the tail prior to faecal sample collection, variation between individual fish and section of gut sampled. Faecal collection by rectal suction and dissection was compared with the stripping collection method. Rectal suction produced comparable results to stripping; however, ADC for crude protein was significantly ( $P < 0.05$ ) lower after dissection. It is proposed that the loose consistency of the faeces produced by large salmon

on commercial feed causes it to redistribute along the gut after euthanasia. Urine and mucus contamination, stripping pressure, immediate repeated sampling from individuals and dorso-flexion of the tail did not significantly affect ADC. However, the ADC for organic matter ( $P < 0.01$ ) and crude protein ( $P < 0.05$ ) were significantly lower when faeces were stripped from near the anus to the anus compared with from the pelvic fin region to the anus. There was no relationship between fish size and ADC for gross energy or crude protein. The stripping method used appeared to be suitable for collecting faeces from large seawater Atlantic salmon (300-5000 g) for the purpose of calculating apparent digestibility. A robust and practical procedure that takes account of several factors that can cause major variation in data is suggested.

Powell MD, Parsons HJ, Nowak BF (2001) **Physiological effects of freshwater bathing of Atlantic salmon (*Salmo salar*) as a treatment for amoebic gill disease.** *Aquaculture* 199:259-266

The physiological effects of bathing marine net-pen reared Atlantic salmon with freshwater the treatment for amoebic gill disease (AGD) in Tasmania was evaluated. Acute exposure of AGD affected salmon to hyperoxic freshwater for at least 2 h resulted in a decrease in gill succinic dehydrogenase activity and increases in the total number of mucous cells on the gills. Plasma ions, respiratory and acid-base variables were unaffected by acute hyperoxic and normoxic exposure. Thus, it was concluded that freshwater bathing as a treatment for AGD poses little risk of side effects, but also no benefit with regard to the physiological status of the salmon. Reduced gill enzyme activity may restrict prolonged bathing times, suggesting that care should be taken to ensure that the hyper-ionoregulatory capacity of the salmon is not compromised.

2000

Bransden MP, Carson J, Munday BL, Handler JH, Carter CG, Nowak BF (2000) **Nocardiosis in tank-reared Atlantic salmon, *Salmo salar* L.** *J Fish Dis* 23:83-85  
 Nocardiosis is an infection caused by aerobic Grampositive, branching, filamentous rods of the genus *Nocardia*. The organism is resident in both soil and plants (Austin & Austin 1993; Frerichs 1993), and is closely related to *Mycobacterium* spp. Nocardiosis caused by *Nocardia asteroides* and *N. seriolae* (previously *N. kampachi*) has been reported in several finfish species, both freshwater and marine. The first reported incidence was recorded by Valdez & Conroy (1963) in neon tetras, *Hyphessobrycon innesi* (Myers), and subsequently in other fish species, some of commercial significance including rainbow trout, *Oncorhynchus mykiss* (Walbaum) (Snieszko, Bullock, Dunbar & Pettijohn 1964), brook trout, *Salvelinus fontinalis* (Mitchill), (Campbell & MacKelvie 1968) and yellowtail, *Seriola quinqueradiata* (Temminck & Schlegel; Kubota, Kariya, Nakamura & Kira 1968). Despite two of these incidences occurring in salmonids, infection by *Nocardia* spp. in Atlantic salmon, *Salmo salar* L., has not previously been reported.

Carter CG, Houlihan DF, He ZY (2000) **Changes in tissue free amino acid concentrations in Atlantic salmon, *Salmo salar* L., after consumption of a low ration.** *Fish Physiol Biochem* 23:295-306

Atlantic salmon (*Salmo salar* L.) with an average weight of 411 +/- 16 g were fed after a period of 7 days without food and the free amino acid concentrations in the pylorus, liver and white muscle measured before and at 3, 6, 9, 15 and 24 h after feeding. There were few significant postprandial changes in tissue free amino acid concentrations. In the white



muscle, concentrations of six indispensable amino acids were significantly ( $p < 0.05$ ) higher after 9 (Ile, Leu, Phe, Thr) or 15 h (Val, Met). Individual feed intake was measured and there were significant positive correlations between amino acid intake and amino acid concentrations in white muscle free pools for total amino acids ( $p < 0.001$ ), total indispensable amino acids ( $p < 0.001$ ) and individual indispensable amino acids (Ile, Leu, Lys, Met, Phe, Val). These relationships were due to relatively low feed intake (0.28% body weight) that followed 7 days without feeding. The indispensable amino acid profile of the white muscle free pool was compared with that of standard proteins (the feed, whole body and white muscle), as well as with indispensable amino acids requirements. At different times one of two indispensable amino acids, Phe (at 0, 3, 6, 9, 15 h after feeding) or Trp (at 24 h after feeding), was present at the lowest relative concentrations compared to the other indispensable amino acids. This showed that although changes in tissue free amino acid concentrations following feeding were small the amino acid profile (relative concentrations) in the white muscle free pool changed. It is proposed that the lowest relative concentration of an indispensable amino acid in the white muscle free pool should be considered in relation to its potential to limit the efficiency of protein synthesis and retention.

Douglas-Helders M, Nowak B, Zilberg D, Carson J (2000) **Survival of *Paramoeba pemaquidensis* on dead salmon: Implications for management of cage hygiene.** Bull Eur Assoc Fish Pathol 20:167-169

Amoebic gill disease (AGD) is the most serious disease problem in Atlantic salmon aquaculture in Tasmania at present. Little is known however, about the sources or reservoirs of *Paramoeba pemaquidensis*, the causative agent of AGD. This study evaluated the possibility of mortalities being a reservoir of *P. pemaquidensis* that could infect live naive fish as well as uninfected dead fish. Using Immunofluorescent antibody test (IFAT) for *P. pemaquidensis* on gill mucus smears it was determined that paramoebae remain on infected gills for at least 30 hours after death of the host and that during this time the number of paramoebae appear to increase. In addition it was established that paramoebae from dead infected fish can colonise the gills of previously uninfected dead fish thereby potentially increasing the bio-burden of paramoebae on infected farms.

Morrison RN, Nowak BF, Carson J (2000) **The effect of levamisole as an adjuvant on the humoral immune response of Atlantic salmon (*Salmo salar* L.).** Bull Eur Assoc Fish Pathol 20:101-105

Pre and post-smelt Atlantic salmon (*Salmo salar* L.) were administered a levamisole adjuvanted *Vibrio anguillarum* vaccine by bath or intraperitoneal (IP) injection. A significant serum anti-*V. anguillarum* antibody response was elicited in groups of fish administered an IP injection of bacterium only. Fish (pre and post-smelt) treated with the levamisole adjuvanted vaccine (IP and bath) showed a suppressed response relative to the respective positive (vaccinated) control groups. No detectable antibody response was elicited in fish not treated or treated with a placebo. Results from this trial suggest that levamisole as an adjuvant has a narrow range of efficacy.

Nowak B, Clark A, Pankhurst T, Speare D (2000) **Langhans' giant cells in the gills of an Atlantic salmon.** Aust Vet J 78:191-192

Metacercarial infection in fish usually results in cyst formation with a characteristic lack of host response,<sup>1</sup> at least partly due to encapsulation by fibrous tissue. These cysts can develop in almost any fish tissue, their distribution depending on the species of trematode and fish.<sup>1</sup> Digenean metacercariae are uncommon in cultured fish unless the other hosts

needed for their life cycle are present (mollusc intermediate host and fish-eating bird final host). In seawater net pen reared salmonids, metacercariae of four of the trematodes have caused problems, including mortality: neascus type (affecting skin), Diplostomum sp (skin, fins, cornea and gills), Cryptocotyle lingua (eye) and Stephanostomum tenue (heart).<sup>2</sup> A single metacercaria of unidentified species was found in the gills of only one fish during a histological survey of the gills of 2659 seawater netpen-reared Atlantic salmon (*Salmo salar*), cultured in Tasmania from 1995 until 1997.

Powell MD, Fisk D, Nowak BF (2000) **Effects of graded hypoxia on Atlantic salmon infected with amoebic gill disease.** J Fish Biol 57:1047-1057

Atlantic salmon *Salmo salar* with amoebic gill disease (AGD) were exposed to a graded hypoxia (135-40 mmHg water PO<sub>2</sub>) and blood samples analysed for respiratory gases and pH at 119, 79.5 and 40 mmHg water PO<sub>2</sub>. There were no differences in the rate of oxygen uptake between infected and control fish. However, arterial PO<sub>2</sub>, and pH were significantly lower in the infected fish whereas PCO<sub>2</sub> was significantly higher in infected fish compared with controls prior to hypoxia and at 119 mmHg water PO<sub>2</sub>. At 79.5 and 40 mmHg water PO<sub>2</sub> saturation, there were no significant differences in blood PO<sub>2</sub> or pH although blood PCO<sub>2</sub> was elevated in AGD affected fish at 50% hypoxia (79.5 mmHg water PO<sub>2</sub>). The elevated levels of PCO<sub>2</sub> in fish affected by AGD resulted in a persistent respiratory acidosis even during hypoxic challenge. These data suggest that even though the fish were severely affected by AGD, the presence of AGD while impairing gas transfer under normoxic conditions, did not contribute to respiratory failure during hypoxia.

1999

Blyth PJ, Kadri S, Valdimarsson SK, Mitchell DF, Purser GJ (1999) **Diurnal and seasonal variation in feeding patterns of Atlantic salmon, *Salmo salar* L., in sea cages.** Aquacult Res 30:539-544

Atlantic salmon, *Salmo salar* L., in sea cages exhibit feeding patterns that vary both diurnally and seasonally. Hitherto, there are no data reporting feed rate and its variation through a complete annual cycle. Here we present data from Scotland showing diurnal and interseasonal variation in feeding patterns and feeding rates of Atlantic salmon fed daily to satiation from shortly after transfer to seawater until harvest about 11 months later. A major feeding peak regularly occurred soon after dawn, and feeding rates remained high for approximately one hour. Over the remainder of the day, the fish fed at a lower but steady rate. Relative feed intake varied over the trial, being initially high in summer followed by a sharp decline in autumn, and then further declining until fish reached harvest size at the beginning of the following summer. Further investigations of the relationship between variation in circannual feeding patterns and environmental parameters should now be carried out to improve the understanding of the mechanism behind these patterns.

Carter CG, Hauler RC (2000) **Fish meal replacement by plant meals in extruded feeds for Atlantic salmon, *Salmo salar* L.** Aquaculture 185:299-311

The replacement of fish meal protein with soybean meal (SB) or protein concentrates made from narrow-leafed lupin (LP) or field peas (PP) was investigated in extruded feeds for Atlantic salmon. Salmon (47 g) were fed for 63 days on extruded feeds containing each of the plant meals to replace 25% and 33% of the fish meal protein and performance compared against a nutritionally balanced control and a commercial salmon feed formulation (extruded under the same conditions). There were no significant differences in weight gain between

the control and feeds containing the plant proteins. The commercial leed produced significantly higher weight gain than the control feed and LP at both replacement levels. Feed consumption was significantly higher for LP at 33%, but there were no other significant differences between the other feeds. Feed efficiency ratio (FER) and productive protein value (PPV) were highest for PP and SE and not affected by inclusion level, whereas they were significantly lower for LP at 33% inclusion. The weight gain and feed efficiency ratio data showed that soybean meal and pea protein concentrate had the best potential for replacing at least 33% of the fish meal protein in extruded salmon feeds and that lupin protein concentrate was less well utilised at the higher inclusion level. These results support the use of processed plant meals as important replacement protein sources for fish meal in extruded feeds for Atlantic salmon.

Clark A, Nowak BF (1999) **Field investigations of amoebic gill disease in Atlantic salmon, *Salmo salar* L., in Tasmania.** J Fish Dis 22:433-443

Amoebic gill disease (AGD) is the most serious health problem in Atlantic salmon cultured in Tasmania. Our field investigation examined prevalence of AGD during 2 years, every year for up to 7 months after transfer to sea water. The relationship between environmental factors and AGD prevalence was determined. Additionally, effects of adding levamisole to freshwater baths were investigated in a field trial. AGD was recorded on all farms, except for farm A, which did not move salmon from a brackish site to a full-salinity site during the study. The prevalence showed a bimodal distribution with the first larger peak in summer (usually in January) and the second smaller peak in autumn (between March and May). During both years the prevalence of AGD was significantly greater in January than any other month. Sampling month and the interaction between farm and month had a statistically significant effect on AGD prevalence. AGD was recorded at a minimum temperature of 10.6 degrees C and minimum salinity of 7.2 ppt. There was a positive relationship between the time since the freshwater bath and the prevalence of AGD for the first 30 days after the bath, with a dramatic increase in the AGD prevalence about 3 weeks after the bath. After 30 days, there was no statistically significant relationship between AGD prevalence and days since the last bath, except for the second bath. The addition of levamisole to the freshwater bath did not significantly increase the time between treatments. The relationship between diagnosis on the basis of gross signs and histological diagnosis was significant, however, the gross diagnosis was unreliable within the lower range, with 31.8% false negatives and 15.9% false positives and kappa value of 0.2742.

Nowak BF, Clark A (1999) **Prevalence of epitheliocystis in Atlantic salmon, *Salmo salar* L., farmed in Tasmania, Australia.** J Fish Dis 22:73-78

Amoebic gill disease (AGD) is the most serious health problem in Atlantic salmon cultured in Tasmania. Our field investigation examined prevalence of AGD during 2 years, every year for up to 7 months after transfer to sea water. The relationship between environmental factors and AGD prevalence was determined. Additionally, effects of adding levamisole to freshwater baths were investigated in a field trial. AGD was recorded on all farms, except for farm A, which did not move salmon from a brackish site to a full-salinity site during the study. The prevalence showed a bimodal distribution with the first larger peak in summer (usually in January) and the second smaller peak in autumn (between March and May). During both years the prevalence of AGD was significantly greater in January than any other month. Sampling month and the interaction between farm and month had a statistically significant effect on AGD prevalence. AGD was recorded at a minimum temperature of 10.6 degrees C

and minimum salinity of 7.2 ppt. There was a positive relationship between the time since the freshwater bath and the prevalence of AGD for the first 30 days after the bath, with a dramatic increase in the AGD prevalence about 3 weeks after the bath. After 30 days, there was no statistically significant relationship between AGD prevalence and days since the last bath, except for the second bath. The addition of levamisole to the freshwater bath did not significantly increase the time between treatments. The relationship between diagnosis on the basis of gross signs and histological diagnosis was significant, however, the gross diagnosis was unreliable within the lower range, with 31.8% false negatives and 15.9% false positives and kappa value of 0.2742. In this paper, the present authors report the prevalence of epitheliocystis in Atlantic salmon farmed in Australia and discuss factors which may influence its occurrence.

Rungruangsak-Torrissen K, Carter CG, Sundby A, Berg A, Houlihan DF (1999)

**Maintenance ration, protein synthesis capacity, plasma insulin and growth of Atlantic salmon (*Salmo salar* L.) with genetically different trypsin isozymes.** Fish Physiol Biochem 21:223-233

Growth was found to be associated with the changes of trypsin activity in the pyloric caecal tissues and the level of plasma insulin in Atlantic salmon (*Salmo salar* L.). A decrease in trypsin activity accompanied by an increase in plasma insulin was detected one month before an enhanced growth was observed. There were significant relationships between weight specific consumption rate, plasma insulin levels and fish growth. The correlation of weight specific consumption rate was higher with growth rate ( $R^2=0.7$ ,  $p < 0.0001$ ) than with plasma insulin concentration ( $R^2=0.4$ ,  $p < 0.0001$ ).

When the comparison was made between Atlantic salmon carrying and lacking the trypsin variant TRP-2\*92, the fish with the variant had lower maintenance ration ( $p < 0.05$ ), higher capacity for protein synthesis in the white muscle ( $p < 0.02$ ), and a greater ability to utilize the feed at a restricted ration than the fish without the variant. In Atlantic salmon lacking the variant, both plasma insulin concentrations and growth rates were significantly lower ( $p < 0.05$ ) in the fish fed 0.5% bw day<sup>-1</sup> than those fed 1% bw day<sup>-1</sup>. Whilst the growth rates of TRP-2\*92 salmon fed the different rations became similar one month after similar levels of plasma insulin were observed between them. The TRP-2\*92 salmon may be defined as a high protein growth efficiency fish with low protein turnover rate.

Genetic variation in trypsin isozyme pattern affects feed utilization, plasma insulin levels and growth in Atlantic salmon.

Zilberg D, Nowak B, Carson J, Wagner T (1999) **Simple gill smear staining for diagnosis of amoebic gill disease.** Bull Eur Assoc Fish Pathol 19:186-189

Amoebic gill disease (AGD) caused by a *Paramoeba* spp. is the major disease affecting cultured Atlantic salmon in Tasmania. Primary diagnosis of the disease on the farm relies on gross morphology, and it is then confirmed in the laboratory with an immunofluorescence antibody test (IFAT). In this study we evaluated the potential use of a rapid method for the detection of AGD using a Quick Dip(R) stain and compared it to the established IFAT. Quick Dip(R) staining revealed 96% agreement with IFAT in the determination whether a sample is positive or negative for AGD, with sensitivity and specificity values of 88.23% and 92.85%, respectively and a kappa value of 0.7628. Mean number of *Paramoeba* spp. per field of view positively correlated in the two methods ( $r=0.972$ ), although it was significantly greater when IFAT was used. Quick Dip(R) stained *Paramoeba* spp. in a mucus smear is identified by its morphological features. It measures 15 to 20  $\mu$  m, appearing dark blue with a darker blue

and purple-stained internal organelles. The study suggests that Quick Dip(R) can be used as a fast method for the diagnosis of AGD.

1998

Carter CG (1998) **Fish Meal Replacement in Aquaculture Feeds for Atlantic Salmon.** Report to Fisheries Research and Development Corporation. pp. 136. ISBN 0-85901-800-8  
If aquaculture is to continue to expand in Australia cost-effective diets based on Australian agricultural ingredients urgently need to be developed. The replacement of fish meal as the protein source of choice is a global research priority driven by a declining supply of fish meal and rapidly expanding aquaculture and aquaculture feed industries. This report describes the progress achieved with Atlantic salmon to develop suitable methods, replace fish meal with plant meals and to trial additives for increasing nutrient utilisation.

1997

Clark A, Nowak B, Handlinger J, Munday BL, Percival S (1997) **Clubbing and necrosis gill (CNG) syndrome in sea-caged Atlantic salmon, *Salmo salar* L, in Tasmania: An initial report.** J Fish Dis 20:59-68

In autumn 1993, an outbreak of gill disease occurred in Atlantic salmon, *Salmo salar* L., smelts farmed in the Huon estuary in southern Tasmania, Australia. Histologically, the presumptive primary lesion appeared as focal, sometimes full thickness, gill necrosis, which showed no host reaction in the initial stages. Healing of these damaged areas gave rise to conspicuous expanded tips or clubbing of gill filaments. The term clubbing and necrosis gill (CNG) syndrome is suggested to include both these histological features. Fusion of lamellae proximally along the filament, often with vascular dilation, was also a feature of the condition. Similar lesions have been described previously in the literature, but the initial necrosis followed by the very prominent clubbing of filaments was sufficiently different from previous descriptions to warrant designation of this condition as a distinct entity.

Hindell MA, Pemberton D (1997) **Successful use of a translocation program to investigate diving behavior in a male Australian fur seal, *Arctocephalus pusillus doriferus*.** Mar Mamm Sci 13:219-228

This study reports some of the first foraging behaviour data collected for male fur seals. A nonbreeding male Australian fur seal, *Arctocephalus pusillus doriferus*, captured at a commercial salmon farm in southern Tasmania, Australia, was relocated 450 km from the site of capture. The animal was equipped with a geolocating time-depth recorder that recorded diving behavior and approximate location for the 14.4 d that it took the seal to travel down the east coast of Tasmania and be recaptured at the salmon farm. During its time at sea, the seal spent most of its time over the relatively shallow shelf waters. It spent 30% of its time ashore on a number of different haul-out sites. The deepest dive was 102 m and the maximum duration was 6.8 min. "Foraging" type dives made up 31.2% of the time at sea and had a median duration of 2.5 min and a median depth of 14 m. The seal performed these dives more commonly during the latter part of its time at sea, while it was on the east coast. Unlike other fur seal species studied to date, there was no evidence of a diurnal foraging pattern; it made dives at all times of the day and night.

1996

McCarthy ID, Carter CG, Houlihan DF, Johnstone R, Mitchell AI (1996) **The performance of all-female diploid and triploid Atlantic salmon smolts on transfer together to sea water.** J Fish Biol 48:545-548

In transfer to sea water for 45 days, the return of appetite was later and growth rates tended to be lower for triploid Atlantic salmon, *Salmo salar*, reared together with diploid Atlantic salmon. All mortalities comprised of triploid salmon (29%) and were attributable to failed smelt syndrome. No correlation was found between the growth of diploid or triploid fish in fresh water and their subsequent growth on transfer to sea water.

1995

Schmidtke LM, Carson J (1995) **Characteristics of Flexibacter-Psychrophilus Isolated from Atlantic Salmon in Australia.** Dis Aquat Org 21:157-161

*Flexibacter psychrophilus* (syn. *Cytophaga psychrophila*) was isolated in Tasmania, Australia, from farmed Atlantic salmon *Salmo salar* with moderate to severe erosion of the fins; there was no evidence of skin lesions. The mortality level in the population of affected fish was less than 0.01% wk<sup>-1</sup>, but the morbidity level was in excess of 80%. The phenotype of the Australian isolates is in good agreement with strains from Europe and North America and differs only in that the Australian isolates produced a brown pigmentation on tyrosine agar and did not hydrolyse Tween 80. The growth in vitro of all isolates was inhibited by acriflavine, ampicillin, and oxolinic acid at concentrations in excess of 0.5 µg ml<sup>-1</sup> and by oxytetracycline at 1.56 µg ml<sup>-1</sup>; none of the isolates were inhibited by sulphamethazine or trimethoprim at 25 µg ml<sup>-1</sup>.

1994

Carter CG, McCarthy ID, Houlihan DF, Johnstone R, Walsingham MV, Mitchell AI (1994) **Food-Consumption, Feeding-Behavior, and Growth of Triploid and Diploid Atlantic Salmon, *Salmo-Salar* L, Parr.** Can J Zool 72:609-617

To test whether triploid Atlantic salmon (*Salmo solar* L.) parr were at a disadvantage compared with diploid parr the growth and feeding behaviour of individual parr in separate and mixed groups of diploid and triploid fish were studied in two experiments. At the end of the first experiment (separate groups) the diploid parr had significantly higher mean specific growth rates than the triploid parr. This was due to significantly higher growth during the first 40 days of the experiment; growth was not significantly different over the last 52 days. The consumption rates of individual parr were measured using radiography. There were no consistent differences in consumption rates between triploid and diploid parr in mixed or separate groups. However, at the start of the experiment the mean consumption rates of the triploid parr were significantly lower than those of the diploid parr and could explain their lower growth rates. There were no differences in the amount of damage to the caudal fin between triploid and diploid parr in either experiment. However, in a mixed group, damage to the dorsal fin of triploid parr was more severe than to the dorsal fin of diploid parr, which suggested that triploid parr in mixed groups were more likely to be the recipients of agonistic actions than the diploid parr. However, the specific growth rates of the diploid and triploid parr in mixed groups were not significantly different. It was concluded that under certain circumstances triploid and diploid Atlantic salmon parr may exhibit differences in growth and feeding behaviour.

1993

Carter CG, Houlihan DF, Buchanan B, Mitchell AI (1993) **Protein-Nitrogen Flux and Protein-Growth Efficiency of Individual Atlantic Salmon (*Salmo-Salar L*)**. *Fish Physiol Biochem* 12:305-315

Protein-nitrogen flux (the proportions of consumed and absorbed protein-nitrogen partitioned into protein synthesis and growth) was examined in Atlantic salmon, *Salmo salar L*. Salmon were held in groups and fed high or low rations or starved. Individual food consumption rates were measured using radiography. Fish varied widely in protein growth efficiency (protein growth divided by protein consumption), but this did not correlate with consumption rate, digestive capacity (as measured by absorption efficiency, trypsin levels and pyloric caecal size) or feeding hierarchy rank. Protein synthesis rates, measured in whole-animals, were linearly correlated with protein consumption and assimilation. There was a significant correlation between protein growth efficiency and the efficiency of retention of synthesised proteins. The capacity for protein synthesis and RNA activity were positively correlated with rates of food consumption and growth but were not correlated with protein growth efficiency. It was concluded that individual differences in protein growth efficiency related to differences in synthesis retention efficiency, but not to differences in the capacity for protein synthesis, RNA activity, digestive capacity or feeding hierarchy rank.

1992

Carter CG, Houlihan DF, Mccarthy ID (1992) **Feed-Utilization Efficiencies of Atlantic Salmon (*Salmo-Salar L*) Parr - Effect of a Single Supplementary Enzyme**. *Comp Biochem Phys A* 101:369-374

1. Salmon *Salmo salar* parr were fed three rations of a control diet and one ration of a diet containing a supplementary enzyme, alpha-amylase.
2. X-Radiography was used to estimate the consumption rates of individual fish.
3. Consumption-growth relationships for wet weight, dry weight, nitrogen and carbon were established for the two diets.
4. The supplementary enzyme had no statistically significant effect on the consumption-growth relationships or feed utilization efficiency.

## **Environmental Interactions and Management**

2019:

Bannister J, Sievers M, Bush F, Bloecher N (2019) **Biofouling in marine aquaculture: a review of recent research and developments**. *Biofouling*:1-18

Biofouling in marine aquaculture is one of the main barriers to efficient and sustainable production. Owing to the growth of aquaculture globally, it is pertinent to update previous reviews to inform management and guide future research. Here, the authors highlight recent research and developments on the impacts, prevention and control of biofouling in shellfish, finfish and seaweed aquaculture, and the significant gaps that still exist in aquaculturalists' capacity to manage it. Antifouling methods are being explored and developed; these are centred on harnessing naturally occurring antifouling properties, culturing fouling-resistant genotypes, and improving farming strategies by adopting more sensitive and informative monitoring and modelling capabilities together with novel cleaning equipment. While no simple, quick-fix solutions to biofouling management in existing aquaculture industry situations have been developed, the expectation is that effective methods are likely to evolve as aquaculture develops into emerging culture scenarios, which will undoubtedly influence the path for future solutions.

Bush F, Macleod C, Ross J (2019) **Marine Biofouling in Salmon Farming: Issues and Controls. Emerging issues**. Institute for Marine and Antarctic Studies, Hobart, Tasmania [Internal review]

The colonisation and growth of fouling organisms on salmon farm infrastructure can result in a number of environmental and animal welfare problems, and even infrastructural damage which can in turn have economic implications affecting the profitability of the aquaculture practices (Fitridge et al. 2012). This review considers the issues associated with biofouling of salmon farm infrastructure, identifies options for management and considers these in the context of both existing regulatory controls and practical industry application.

Condie, S.A., Oliver, E.C.J., Hallegraeff, G.M. (2019). **Environmental drivers of unprecedented *Alexandrium catenella* dinoflagellate blooms off eastern Tasmania, 2012–2018**. *Harmful Algae* 87, 101628

Blooms of the highly toxic dinoflagellate *Alexandrium catenella* (previously referred to as *tamarensis* group 1) were first detected off eastern Tasmania in 2012 and have since been responsible for incidences of human paralytic shellfish poisoning and extended closures (up to 25 weeks) of mussel, oyster, scallop, abalone and rock lobster industries (up to 150 mg/kg PST in mussels). Investigation of meteorological and oceanographic influences indicate that the annually recurrent winter-spring blooms (June-Oct) occur within a narrow water temperature window (10-15 degrees C) under two distinct sets of conditions: (1) following high rainfall and land run-off, under relatively light winds; and (2) following periods of anomalously low air temperatures and associated cooling of shallow coastal waters, again under relatively light winds. The common driver of blooms appears to be the development of stratification in coastal waters, via salinity and/or temperature gradients. We propose a framework for evaluating the risk of *Alexandrium* with the aim of developing a forecasting capability, and compare these environmental conditions with historic data to understand the recent advent of these blooms.



Cummings CR, Lea MA, Lyle JM (2019) **Fur seals and fisheries in Tasmania: an integrated case study of human-wildlife conflict and coexistence**. *Biol Conserv* 236:532-542

Cultivating more harmonious ways of interacting with top predators is a major challenge in sustainably managing and developing fisheries. In-depth, interdisciplinary case studies represent important tools for high-lighting emergent properties in complex human-predator relationships. In this study we integrate original social research with detailed secondary historic and natural-scientific information on a long-standing case of human-wildlife conflict: the relationship between fur seals and fisheries in Tasmania. Stakeholders were targeted and surveyed via anonymous questionnaire about their experiences and perceptions of seal-fishery interactions and seals in the ecosystem. The most frequently cited outcomes of interactions for both commercial and recreational fishers were damaged gear, lost catch, and damaged catch. Most fishers indicated that they believed population-level controlled culling or targeted removal of problem individuals would be the most effective strategies to manage and reduce interactions. In contrast, the general public and resource/environmental managers indicated strong preferences for non-lethal forms of management, with culling the lowest ranked strategy in terms of perceived effectiveness. Perceptions of ongoing rapid population increase evident in fishing sub-groups contrast with available seal population data. Such discrepancy suggests that reported increasing seal-fishery interactions may be more reflective of behavioural change, with seals becoming habituated to certain fishing activities. Areas of promise identified for future research and management focus on: technical mitigation to minimise direct interactions, building tolerance in fishing communities, and targeted ecological research to disentangle the effects of pinniped abundance, distribution (including seasonal population flux between breeding regions), and habituation on interactions. Documenting the contemporary status of this relationship is an integral step in managing such conflicts.

Ross J, Wild-Allen K, Andrewartha J, Beard J, Moreno D, Macleod C (2019) Understanding oxygen dynamics and the importance for benthic recovery in Macquarie Harbour - Progress Report July 2019. FRDC 2016/067: Environmental Research in Macquarie Harbour. Institute for Marine and Antarctic Studies, Hobart, Tasmania

This report provides an ongoing update on the status of dissolved oxygen (DO) and benthic conditions in Macquarie Harbour. It follows on from the results previously outlined in the IMAS reports released throughout 2017 and 2018. These reports first described the deterioration of benthic and water column conditions in spring 2016, early signs of faunal recovery in the following autumn, when oxygen levels had improved, and the subsequent decline in benthic conditions when oxygen concentrations in middle and bottom waters returned to very low levels in spring 2017. In the most recent report we described a continuation of this cycle; an increase in faunal numbers in mid-2018 following improved oxygen concentrations in the middle and bottom waters through the summer of 2017/18. This report presents the results and preliminary interpretation of a repeat survey of benthic communities in January 2019 and DO monitoring data up until late May 2019.

Trainer, V.L., Moore, S.K., Hallegraef, G., Kudela, R.M., Clement, A., Mardones, J.I., Cochlan, W.P. (2019). **Pelagic harmful algal blooms and climate change: Lessons from nature's experiments with extremes Harmful Algae**  
<https://doi.org/10.1016/j.hal.2019.03.009>

Time series now have sufficient duration to determine harmful algal bloom (HAB) responses

to changing climate conditions, including warming, stratification intensity, freshwater inputs and natural patterns of climate variability, such as the El Niño Southern Oscillation and Pacific Decadal Oscillation. Against the context of time series, such as those available from phytoplankton monitoring, dinoflagellate cyst records, the Continuous Plankton Recorder surveys, and shellfish toxin records, it is possible to identify extreme events that are significant departures from long-term means. Extreme weather events can mimic future climate conditions and provide a “dress rehearsal” for understanding future frequency, intensity and geographic extent of HABs. Three case studies of extreme HAB events are described in detail to explore the drivers and impacts of these oceanic outliers that may become more common in the future. One example is the chain-forming diatom of the genus *Pseudo-nitzschia* in the U.S. Pacific Northwest and its response to the 2014-16 northeast Pacific marine heat wave. The other two case studies are pelagic flagellates. Highly potent *Alexandrium catenella* group 1 dinoflagellate blooms (up to 150 mg/kg PST in mussels; 4 human poisonings) during 2012-17 created havoc for the seafood industry in Tasmania, south-eastern Australia, in a poorly monitored area where such problems were previously unknown. Early evidence suggests that changes in water column stratification during the cold winter spring season are driving new blooms caused by a previously cryptic species. An expansion of *PseudoChattonella* cf. *verruculosa* to the south and *A. catenella* to the north over the past several years resulted in the convergence of both species to cause the most catastrophic event in the history of the Chilean aquaculture in the austral summer of 2016. Together, these two massive blooms were colloquially known as the “Godzilla-Red tide event”, resulting in the largest fish farm mortality ever recorded worldwide, equivalent to an export loss of USD\$800 million which when combined with shellfish toxicity, resulted in major social unrest and rioting. Both blooms were linked to the strong El Niño event and the positive phase of the Southern Annular Mode, the latter an indicator of anthropogenic climate change in the southeastern Pacific region. For each of these three examples, representing recent catastrophic events in geographically distinct regions, additional targeted monitoring was employed to improve the understanding of the climate drivers and mechanisms that gave rise to the event and to document the societal response. Scientists must be poised to study future extreme HAB events as these natural experiments provide unique opportunities to define and test multifactorial drivers of blooms.

Weltz K, Lyle JM, Bell JD, Semmens JM (2019) **Dietary analysis reveals the vulnerability of the endangered Maugean skate (*Zearaja maugeana*) to benthic changes in Macquarie Harbour.** *Marine and Freshwater Research* 70:745-753

Species occupying high trophic positions with a small population size, limited diet and restricted range are vulnerable to extinction. The endangered Maugean skate (*Zearaja maugeana*) in Macquarie Harbour is potentially threatened by changing benthic environmental conditions (declining dissolved oxygen concentrations and decreased macrofaunal diversity). To inform investigations on potential indirect impacts of benthic environmental changes on the Maugean skate and its prey, non-lethal techniques were used to identify dietary composition and trophic position of the species. The Maugean skate occupies a high trophic position (3.70) in the food web, with both stable-isotope (median 86% contribution to isotopic signatures, credible interval (CI) = 44–100%) and stomach-content analysis showing crustaceans, particularly brachyuran crabs (58.5% index of relative importance), as the dominant prey type. Aquaculture feed from salmonid fish farms operating in the harbour was not found to be important in skate diet. This study showed that

the Maugean skate consumes a small number of benthic prey species, highlighting its vulnerability to changing environmental conditions in the harbour.

White CA, Woodcock SH, Bannister RJ, Nichols PD (2019) **Terrestrial fatty acids as tracers of finfish aquaculture waste in the marine environment**. *Reviews in Aquaculture* 11:133-148

Waste from open-cage aquaculture flows directly into the marine environment from uneaten feeds, faecal material and dissolved nutrients. Sustainable management outcomes are regularly based on the dispersal patterns of the waste, with biochemical tracing a key tool in understanding the footprint of aquaculture. We examined the use of fatty acid (FA) analysis to trace aquaculture waste for this purpose, with the aim of identifying specific biomarkers for environmental applications, as well as identifying challenges that are regularly encountered. Overall, the widespread use of terrestrial-based oils in the production of marine aquaculture feeds has increased the use of FA biomarkers to trace aquaculture waste across benthic and pelagic systems, in vertebrates, invertebrates and environmental samples such as sediment and seston. A combination of linoleic acid (LA), oleic acid (OA) and  $\alpha$ -linolenic acid (ALA), which are dominant C18 FA in terrestrial seed and animal oils, is the most commonly used biomarkers, along with overall shifts in FA profile related to diet. A challenge regularly encountered, particularly in lower trophic species, was the capacity of an organism to biomodify or selectively spare certain dietary FA, which can mask assimilation of aquaculture-derived FA. In these species, controlled feeding studies are needed to properly understand FA metabolism and to ensure correct interpretation of FA data collected from wild samples. Overall, as FA biomarkers have the capacity to link aquaculture waste in the environment with potential effect, they are a valuable and increasingly applied tool for examining the overall influence of aquaculture in marine ecosystems.

Witkowski SJ, Carter CG, Grunenwald M, Hadley S, MacLeod CKA, Townsend AT, Adams LR (2019) **Development and assessment of novel endogenous markers in commercial aquafeeds to measure apparent digestibility in large Atlantic salmon (*Salmo salar*) on salmon farms**. *Aquaculture* 508:192-198

As salmon aquaculture continues to grow, understanding aquafeed conversion is imperative for making informed strategic growth planning and management decisions. Accurately measuring the apparent digestibility (AD) of commercial aquafeeds on farms requires greater understanding and method development, including the validation of one or more inert endogenous AD markers. Laboratory and commercial experiments assessed the potential of elements that naturally occur in aquafeeds as potential endogenous AD markers. Two commonly used and validated AD markers, acid insoluble ash (an endogenous marker) and ytterbium oxide (an exogenous marker) were compared using protein AD calculated from a range of endogenous elements. Using a Percentage Similarity Analysis, comparison of protein digestibility calculated with recognised AD markers demonstrated lutetium (Lu) was the most accurate endogenous AD marker. Identification of Lu as an effective endogenous AD marker in commercial salmon aquafeeds will facilitate the straightforward measurement of AD under commercial conditions. This work has important applications in salmon aquaculture where protein AD is fundamental to growth efficiency.

2018

Bush F (2018) **A brief review - Intertidal monitoring methods. Emerging issues**. Institute for Marine and Antarctic Studies, Hobart, Tasmania [Internal review]

This brief internal review identifies intertidal monitoring methods which may be of interest in monitoring salmon environmental interactions with the intertidal zone.

Bush F (2018) **A review of salmon farm regulatory environmental monitoring practices worldwide. Best Practice Reviews.** Institute for Marine and Antarctic Studies, Hobart, Tasmania [Internal review]

The purpose of this literature review is to provide an up to date account of the regulatory environmental monitoring which is being carried out in association with salmon farms and its context within management frameworks. Within the first section monitoring practices in Norway, Chile, Scotland, Canada, New Zealand and Tasmania within the context of existing management frameworks are described. In the second section these practices are discussed.

Bush F, Macleod C, Bannister R (2018) **Trigger Levels and their use in the Environmental Management of Salmon Farming. Best Practice Reviews.** Institute for Marine and Antarctic Studies, Hobart, Tasmania [Internal review]

The aims of this paper are firstly to review the use of benthic and water quality impact indicators and triggers in the compliance monitoring and management of salmon farms in Tasmania and for select countries worldwide (Norway, Scotland, Chile, Canada and New Zealand). In doing this, the role of environmental condition descriptors (e.g. enrichment or impact stages) within the management process will also be considered, more specifically their characterisation and use. Where existing examples directly relating to salmon farming are absent, other monitoring case studies will be considered to add to existing knowledge. Secondly, management actions adopted in response to triggers will be reviewed in the different jurisdictions and finally, the advantages and disadvantages of different management frameworks will be discussed.

Crawford C, Harwin S (2018) **Reassessment of intertidal macroalgal communities near to and distant from salmon farms and an evaluation of using drones to survey macroalgal distribution.** FRDC Project No 2014-241. Institute for Marine and Antarctic Studies, Hobart, Tasmania

The salmon farming industry has significantly expanded in the last decade in South-eastern Tasmania both in production (total production from Tasmania now estimated at 55,000 tonnes per annum) and in number and location of farms. Along with this expansion has been an increasing concern from the general community about the effects of salmon farms on the environment. This includes a reported spread of 'nuisance' algae in the intertidal zone; however, this has not been verified scientifically. As one of the main effects of salmon farming is an increase in nutrients to the environment from waste products, a possible flow-on effect could be a proliferation of macroalgal beds near salmon farming operations. However, a survey of intertidal areas in the Huon and D'Entrecasteaux Channel region in 2002/03 found no clear patterns of macroalgal abundance with distance from salmon farms, (Crawford et al 2006), and it was recommended that any future studies focus on surveying the abundance of key species in the intertidal, *Ulva* spp. and *Hormosira banksii*, which are widely distributed and readily identified.

In this project researchers from the Institute for Marine and Antarctic Studies at the University of Tasmania repeated the survey of intertidal macroalgae conducted in 2002/03 to assess whether abundances have significantly changed at these sites. We also investigated monitoring macroalgal abundance at a larger spatial scale using Unmanned Aerial Vehicles (UAVs), commonly known as drones. Since the previous survey in 2002/03, the technology

for UAVs has significantly advanced and they are now a much more cost effective option. However, the techniques and quantitative analyses required to monitor intertidal algal assemblages using drones have not been assessed.

This report discusses the results of the surveys assessing the percentage cover of intertidal macroalgae at different spatial scales using standard quadrat sampling and UAVs (drones), and discusses issues and limitations of monitoring in the intertidal zone in southern Tasmania.

Hadley S, Macleod C, Ross DJ (2018) **Modelling dispersion of *Mya japonica* on East coast of Tasmania**. Institute for Marine and Antarctic Studies, Hobart, Tasmania [Advice note]

*Mya japonica* a species of soft shell clam has been discovered in an area of the Prosser River on the east coast of Tasmania. This species is not native to Tasmania and considered a potential threat to the local environment. This document models the dispersal of clam larvae using area around the Prosser River as a starting point. CONNIE3 (<http://www.csiro.au/connie/>), a particle tracking software, was used to model the dispersal of the larval clams. The parameterisation used in order for CONNIE 3 to simulate the spread of *Mya japonica* (particles) was provided by DPIPWE and IMAS. The location of the release points was also provided by DPIPWE; however, these points are restricted by the constraints of the model grid.

Hadley S, Wild-Allen K, Johnson C, Macleod C (2018) **Investigation of broad scale implementation of integrated multitrophic aquaculture using a 3D model of an estuary**. Mar Pollut Bull 133:448-459

A 3D ecosystem model was used to quantify changes in water quality brought about by salmon aquaculture in the D'Entrecasteaux Channel and Huon Estuary in southeast Tasmania. Macroalgae-based integrated multitrophic aquaculture (IMTA) was simulated and showed that IMTA is capable of reducing the increased chlorophyll concentration attributable to fish farming by up to 10–15% in large areas of the region, during the season of highest production. Kelp farms (*Macrocystis pyrifera*) recovered between 6 and 11% of the dissolved inorganic nitrogen (DIN) input by salmon aquaculture over a nine month period, with DIN remediation increasing linearly with farm size. Under a ten-fold increase in aquaculture to very high loads, a much lower remediation effect was found for both chlorophyll and DIN. Model results indicate that IMTA could have an important impact on reducing negative effects of finfish aquaculture on water quality providing that stocking rates are not too high.

Hallegraeff G, Bolch C, Campbell K, Condie S, Dorantes-Aranda J, Murray S, Turnbull A, Ugalde S (2018) **Improved understanding of Tasmanian harmful algal blooms and biotoxin events to support seafood risk management**. FRDC Project No 2014/032. Institute for Marine and Antarctic Studies, Hobart, Tasmania

During October 2012, a shipment of Blue Mussels (*Mytilus galloprovincialis*) from the poorly monitored east coast of Tasmania, Australia, was tested by Japanese import authorities and found to be contaminated with unacceptable levels of Paralytic Shellfish Toxins (PSTs; 10 mg/kg). Subsequently local oysters, scallops, clams, the viscera of abalone and rock lobsters were also found to be contaminated. This led to a global product recall of all Australian shellfish exported to Japan and loss to the local economy of AUD 23M. A review of this critical incident (Campbell et al. 2013) identified:

1. Failure of plankton monitoring to provide timely results and failure to detect Alexandrium;
2. Failure of seafood risk assessment by not recognizing the risk of a new mussel farming venture in a poorly monitored area;
3. Failure of PST monitoring by relying only on plankton monitoring as a first screen rather than including shellfish testing.

The analytical turnaround from Tasmanian biotoxin meat analyses conducted at the National Biotoxin Lab at Advanced Analytical Pty Ltd in Sydney is currently in the order of 7-10 days; but during the peak of the 2012 PST event, this sometimes expanded up to 3 weeks, with sample collection/ transport contributing to over 50% of the turnaround time. Such delays in advice to lease-holders are frustrating and can lead to losses of already harvested stocks when retrospectively found to be tainted with PST toxins. Improvements in understanding of Tasmanian harmful algal bloom biology, ecology and toxicology are needed to support seafood biotoxin risk management. Project aims/objectives:

1. Develop, calibrate and adopt screening techniques for rapid detection and evaluation of toxins;
2. Use state-of-the art molecular techniques to elucidate the genetic population structure and biology of toxic Alexandrium tamarense - group algae;
3. Oceanographic modelling of time periods and zones at risk, and coordinated data capture to enable prediction of biotoxin event development;
4. Perform a desktop seafood risk assessment as a prelude for a future stage 2 proposal focusing on Paralytic Shellfish Toxin foodweb transfer.

Hallegraeff G, Dorantes-Aranda J, Turnbull A, Fitzgibbon, Q., Gardner, C. (2018) **Improved risk management of paralytic shellfish toxins in Southern Rock Lobster**. FRDC Project No 2017/086. Institute for Marine and Antarctic Studies, Hobart, Tasmania

The \$84 M lobster industry has been impacted by seasonal Tasmanian closures (up to 5+ months) due to PST contamination of hepatopancreas (HP) notably in the St Helens and Maria Island regions (up to 4 mg STX.diHCl/kg), and up to Flinders Island, with an estimated lost revenue cost of 780k. While only trace levels of PSTs have been detected to date in lobster tail meat, HP contamination poses significant trade barriers for key markets such as China and Hong Kong. While cooking of lobsters did not release biotoxins into the cooking water, HP is a sought after foodstuff (mainly used as a dipping sauce for tail meat) and consumed by 16% of fishers on the Tasmanian east coast and 22% on the west coast, but this has since decreased due to public health warnings. Limited evidence suggests that PST contamination of lobsters may also occasionally occur in South Australia and Victoria. The monitoring, management and mitigation approaches for biotoxin risks in crustaceans (as spelled out in the DPIPWE Rock Lobster Biotoxin Plan and Decision Protocol) remain poorly developed. The current approach uses bivalve PST monitoring as a trigger for lobster testing, with a very limited number (5) animals tested at a prohibitive cost of 5k, which if positive (>0.8 mg/kg STX eq) leads to lengthy closures of large fishing zones. The proposed work will refine monitoring tools to reduce the costs associated with PST biotoxin risk management of Southern Rock Lobster, including the application of cheaper and faster (and hence more frequent and more reliable) PST testing and also explore whether tests can be conducted in a non- destructive manner using haemolymph as a proxy. In addition, tank studies of PST in lobster and field studies on the variation in toxicity, and the validation of a rapid test kit will help to inform the effectiveness of the current geographical zones in the

management plan. Potential impact of PST on lobster vigour will also be investigated using a combination of tank exposure and blood biomarker studies, the latter once calibrated also applied to Tasmanian field surveys.

Ross J, Macleod C (2018) MF266 **Franklin Lease Recovery Assessment**. Institute for Marine and Antarctic Studies, Hobart, Tasmania

As a result of the substantial expansion of *Beggiatoa* bacteria and significant loss of fauna observed in September/October 2016 at the Franklin Lease (MF266), Tassal Pty Ltd were directed to destock the lease; this was completed by mid-April 2017. Tassal were also directed to complete a postharvest survey in May 2017 to provide a reference with which to assess subsequent recovery. This was to be followed with two further surveys that would assess the extent and rate of recovery, and determine whether the sediments had recovered sufficiently to provide for the possibility of restocking; these additional surveys were conducted in January and April 2018. The Director, EPA has asked IMAS to review the post-harvest and post-fallow environmental reports submitted by Tassal, together with any relevant additional information collected by IMAS in Macquarie Harbour, and provide feedback focused on the following terms of reference:

- What do the results of the Tassal environmental surveys show with regard to recovery of the sediments in and around MF266 following the impact identified in Spring/Summer 2016/2017?
- How do the Tassal results compare with the results of IMAS research transects on the lease (where relevant) and can the IMAS research in Macquarie Harbour (including external control sites) provide any further information/context around the characterisation of MF266 with regard to organic enrichment and recovery?
- Based on benthic community composition and sediment chemistry, can IMAS provide any advice regarding potential assimilative capacity of the lease (including comparative capacity of different areas within the lease)?
- Are the components of the post-harvest and post-fallow surveys adequate for characterising the status of the lease and monitoring impacts and recovery from organic enrichment?
- Does IMAS have additional recommendations regarding ongoing monitoring of the lease to address any critical knowledge gaps and inform management decisions (spatial, temporal, parameters etc.)?

Ross J, Macleod C (2018) **Understanding oxygen dynamics and the importance for benthic recovery in Macquarie Harbour - Progress Report February 2018**. FRDC 2016/067: Environmental Research in Macquarie Harbour. Institute for Marine and Antarctic Studies, Hobart, Tasmania

This report provides an update on the status of dissolved oxygen (DO) and benthic conditions in Macquarie Harbour. It follows on from the results reported in the IMAS reports released in January, May and September 2017 which described a deterioration of benthic and water column conditions in Macquarie Harbour in spring 2016 and some early signs of faunal recovery observed in May 2017. This report presents the results and preliminary interpretation of DO monitoring data up until the beginning of January 2018 and a repeat survey of benthic communities in October 2017. This work is part of the research project (FRDC Project 2016-067: Understanding oxygen dynamics and the importance for benthic

recovery in Macquarie Harbour to address these needs) funded by the Fisheries Research Development Corporation with the support of both industry and government (EPA and DPIPWE).

Ross J, Wild-Allen K, Andrewartha J, Macleod C (2018) **Understanding oxygen dynamics and the importance for benthic recovery in Macquarie Harbour - Progress Report June 2018**. FRDC 2016/067: Environmental Research in Macquarie Harbour. Institute for Marine and Antarctic Studies, Hobart, Tasmania

This report provides an update on the status of dissolved oxygen (DO) and benthic conditions in Macquarie Harbour. It follows on from the results outlined in the IMAS reports released in January, May, September 2017 and January 2018. These reports described a deterioration of benthic and water column conditions in Macquarie Harbour in spring 2016, early signs of faunal recovery observed in May 2017 and a subsequent decline in benthic conditions in spring 2017 when oxygen concentrations in middle and bottom waters returned to very low levels. Oxygen concentrations in the middle and bottom waters have since improved through the summer of 2017/18 due to recharge events that commenced in late spring 2017. This report presents the results and preliminary interpretation of a repeat survey of benthic communities in January 2018 and DO monitoring data up until the beginning of May 2018. This work is part of the research project (FRDC Project 2016-067: Understanding oxygen dynamics and the importance for benthic recovery in Macquarie Harbour to address these needs) funded by the Fisheries Research Development Corporation with the support of both industry and government (EPA and DPIPWE); the scope and funding for the project was recently extended for a further two years (until April 2020).

Ross J, Wild-Allen K, Andrewartha J, Stehfest K, Macleod C (2018) **Understanding oxygen dynamics and the importance for benthic recovery in Macquarie Harbour - Progress Report December 2018**. FRDC 2016/067: Environmental Research in Macquarie Harbour. Institute for Marine and Antarctic Studies, Hobart, Tasmania

This report provides an update on the status of dissolved oxygen (DO) and benthic conditions in Macquarie Harbour. It follows on from the results outlined in the IMAS reports released in January, May, September 2017 and January and June 2018. These reports described a deterioration of benthic and water column conditions in Macquarie Harbour in spring 2016, early signs of faunal recovery observed in May 2017 and a subsequent decline in benthic conditions in spring 2017 when oxygen concentrations in middle and bottom waters returned to very low levels. In the June 2018 report we highlighted that oxygen concentrations in the middle and bottom waters improved through the summer of 2017/18 due to recharge events that commenced in late spring 2017. This report presents the results and preliminary interpretation of a repeat survey of benthic communities in June 2018 and DO monitoring data up until the beginning of October 2018. This work is part of the research project (FRDC Project 2016-067: Understanding oxygen dynamics and the importance for benthic recovery in Macquarie Harbour to address these needs) funded by the Fisheries Research Development Corporation with the support of both industry and government (EPA and DPIPWE).

Weltz K, Lyle JM, Semmens JM, Ovenden JR (2018) **Population genetics of the endangered Maugean skate (*Zearaja maugeana*) in Macquarie Harbour, Tasmania**. *Conserv Genet* 19:1505-1512

The Maugean skate (*Zearaja maugeana*) has only been recorded in two remote and isolated



estuaries on the west coast of Tasmania, Australia. While the population status in one of these estuaries (Bathurst Harbour) is uncertain, it is likely that Macquarie Harbour now represents the sole remaining habitat for this species. Environmental conditions, in particular dissolved oxygen levels and benthic biodiversity, in Macquarie Harbour have deteriorated in recent years, impacted by increased nutrient inputs from an expanding salmonid aquaculture industry. These environmental changes are believed to pose a threat to the persistence of the Maugean skate. In assessing the risks for this rare and range-restricted species, it is vital to consider genetic information when developing management strategies. Both mitochondrial and microsatellite markers showed that the species has low genetic diversity; with no detectable genetic diversity in over 3000 base pairs surveyed from the mitochondrial genome, low average microsatellite heterozygosity ( $0.35 \pm 0.11$ ), a low average number of alleles per locus ( $2.1 \pm 0.4$ ) across eight microsatellite loci and no overall population structure within the microsatellite loci ( $F_{st} = -0.002$ ,  $p = 0.718 \pm 0.012$ ). There was also evidence of a recent bottleneck or founder event, which may explain the low observed genetic diversity. While the species may have existed with low genetic diversity for many generations, the results of this study represent a flag for conservation concern for the Maugean skate. Given that Macquarie Harbour may be its last remaining habitat, any threats to the species resulting in local extinction could equate to global loss of this unique skate species.

White CA, Bannister RJ, Dworjanyn SA, Husa V, Nichols PD, Dempster T (2018) **Aquaculture-derived trophic subsidy boosts populations of an ecosystem engineer.** *Aquaculture Environment Interactions* 10:279-289

Environmental management of coastal aquaculture is focused on acute impacts of organic and nitrogenous wastes close to farms. However, the energy-rich trophic subsidy that aquaculture provides may create cascades with influences over broader spatial scales. In a fjord region with intensive fish farming, we tested whether an ecosystem engineer, the white urchin *GracilEchinus acutus*, was more abundant at aquaculture sites than control sites. Further, we tested whether diets influenced by aquaculture waste altered reproductive outputs compared with natural diets. Urchins formed barrens at aquaculture sites where they were 10 times more abundant ( $38 \text{ urchins m}^{-2}$ ) than at control sites ( $4 \text{ urchins m}^{-2}$ ). Urchins were on average 15 mm larger at control sites. In the laboratory, urchins fed aquafeed diets had 3 times larger gonad indices than urchins fed a natural diet. However, their reproduction was compromised. Eggs from females fed an aquafeed diet had 13% lower fertilisation success and 30% lower larval survival rates at 10 d compared with females fed a natural diet. A reproductive output model showed that enhanced numbers of 10 d old larvae produced by the dense aquaculture-associated aggregations of *G. acutus* will supersede any detrimental effects on reproduction, with larval outputs from aquaculture sites being on average 5 times greater than control sites. The results show that aquaculture waste can act as a trophic subsidy in fjord ecosystems, stimulating aggregations of urchins and promoting the formation of urchin barrens. Where finfish aquaculture is concentrated, combined effects on the wider environment may produce ecosystem-level consequences.

Bell J, Ross J, Mardones J, Wild-Allen K, Macleod C (2017) **Huon Estuary/D'Entrecasteaux Channel nutrient enrichment assessment: Establishing the potential effects of Huon Aquaculture Company P/L nitrogen inputs.** Institute for Marine and Antarctic Studies, Hobart, Tasmania

Aquaculture, like most farming practices, has the potential to impact the environment

through the introduction of nutrients; nitrogen (N) in particular can have an impact as it generally limits primary productivity of coastal marine systems. N enters the environment as overfeed, faeces and urine. Approximately 85% of the N released is dissolved (urine) and immediately available to primary producers in the water column. The remainder is released as particulate material (faeces and feed), which settles onto the seabed where sedimentation processes break down the particulates, consuming oxygen in the process, and releasing various forms of N back into the water column, providing an additional nutrient source to feed primary productivity. This, along with naturally occurring forms of N, can lead to nutrient enhancement and potentially eutrophication and algal blooms.

Nutrient inputs from the salmonid aquaculture industry in the D'Entrecasteaux Channel and Huon region are regulated through a feed cap, to help ensure levels remain environmentally sustainable. In the Huon River and Port Esperance Marine Farming Development Plan (MFDP) area and the D'Entrecasteaux Channel MFDP area each salmonid company has been allocated a Total Permissible Dissolved Nitrogen Output (TPDNO) limit. This limit and the period over which it applies has been determined in accordance with the provisions of Management Controls contained in each of the MFDPs. Dissolved nitrogen outputs that can be discharged within the MFDP area must not exceed the prescribed limit during any 12-month period. In July 2015, Huon Aquaculture Company (HAC) reported to government that it had exceeded its TPDNO limit in the Huon/Port Esperance MFDP area. Further, it was also clear that they would continue to exceed this limit for some time. As a result the present study was commissioned to 1) document the nature, timing and location of HACs N exceedance, 2) assess the extent of any adverse ecological effects using available monitoring data, and 3) evaluate the potential risk of adverse effects using modelling. The findings of the resultant report would then be used to help determine what (if any) management response might be needed to reverse any observed negative impacts in the Huon Estuary/Port Esperance MFDP area. The primary resource available for this assessment is data collected for the Broadscale Environmental Monitoring Program (BEMP). The BEMP is a comprehensive environmental monitoring program that was designed to track broadscale changes in the system; both natural and in response to changes in salmon aquaculture inputs and other sources of nutrients. It is a legislative requirement of finfish aquaculture license holders in the D'Entrecasteaux Channel and Huon/Esperance MFDP areas. As a result, a range of nutrient, water chemistry and algal composition data are gathered monthly throughout the year and fortnightly during summer.

Bush F (2017) **Monitoring Sulphide vs. Redox. Best Practice Reviews.** Institute for Marine and Antarctic Studies, Hobart, Tasmania [Internal review]

Sulphide concentration and redox potential are both regularly used in assessments of benthic impact from fish farms however the regulatory requirement to measure, and methods employed to measure these parameters, can vary among the salmon producing countries. This document aims to provide some information on the background of why we measure these parameters together with some practical information relating to sampling and analysis techniques. In addition it provides a review of regulatory requirements to measure redox potential and sulphide concentrations and the methods employed in Norway, Scotland, Canada, Chile and New Zealand.

Condie S, Gorton R, Hadley S, Little R, Macleod C, Ogier E, Proctor W, Ross DJ, Sporcic M, Wild-Allen K (2017) **INFORMD2: Risk-based tools supporting consultation, planning and adaptive management for aquaculture and other multiple-uses of the coastal**

**waters of southern Tasmania.** FRDC 2012/024. CSIRO Oceans and Atmosphere, Hobart, Tasmania

INFORMD2 had an ambitious agenda aimed at supporting environmentally sustainable development of the Tasmanian salmon industry in southern Tasmania and helping to establish a broader social license for that development. This was achieved by developing four new products to assist in planning and ongoing management of aquaculture leases:

- (i) A new approach to identifying community, government and industry values (Your Marines Values (2013), YMV) that has facilitated a more informed engagement processes and greater trust between participants.
- (ii) A new biogeochemical model for the waters of the Derwent Estuary, Huon River and D'Entrecasteaux Channel. This model has been validated in detail and is now being used by stakeholders to test scenarios for planning and water quality impact assessment.
- (iii) A publicly accessible online decision support tool (CONNIE) that can be used to identify waterborne interactions between aquaculture and other marine activities and assets. This facility is now being used extensively to identify impact zones and quantify pathogen risks.
- (iv) A new online decision support tool (MAREE) to be used by government and industry for rapid assessment of the impacts of marine and coastal activities on local water quality. Examples include the impacts of nutrient and sediment loads associated with stocking of salmon leases; sewage treatment plants, other industrial discharges; and altered land-use in local catchments.

These models and tools integrate a diverse body of information and understanding relating to the marine environment of southern Tasmania. Together with ongoing monitoring programs, they are helping to place southern Tasmania as a global leader in environmental management of aquaculture.

Dorantes-Aranda, J. J., Campbell, K., Bradbury, A., Elliott, C., Harwood, D., Murray, S., Ugalde, S., Wilson, K., Burgoyne, M., Hallegraef, G. (2017) . **Comparative performance of four immunological test kits for the detection of Paralytic Shellfish Toxins in Tasmanian shellfish.** *Toxicon* 125, 110-119

Blooms of the toxic dinoflagellate *Alexandrium tamarense* (Group 1) seriously impacted the Tasmanian shellfish industry during 2012 and 2015, necessitating product recalls and intensive paralytic shellfish toxin (PST) product testing. The performance of four commercial PST test kits, Abraxis<sup>TM</sup>, Europroxima<sup>TM</sup>, Scotia<sup>TM</sup> and Neogen<sup>TM</sup>, was compared with the official AOAC LC-FLD method for contaminated mussels and oysters. Abraxis and Europroxima kits underestimated PST in 35-100% of samples when using standard protocols but quantification improved when concentrated extracts were further diluted (underestimation  $\leq$  18%). The Scotia kit (cut off 0.2-0.7 mg STX-diHCl eq/kg) delivered 0% false negatives, but 27% false positives. Neogen produced 5% false negatives and 13% false positives when the cut off was altered to 0.5-0.6 mg STX-diHCl eq/kg, the introduction of a conversion step eliminated false negatives. Based on their sensitivity, ease of use and performance, the Neogen kit proved the most suitable kit for use with Tasmanian mussels and oysters. Once formally validated for regulatory purposes, the Neogen kit could provide shellfish growers with a rapid tool for harvesting decisions at the farm gate. Effective rapid screening preventing compliant samples undergoing testing using the more expensive and time consuming LC-FLD method will result in significant savings in analytical costs.

Dorantes-Aranda, J., Tan, J., Hallegraef, G., Campbell, K., Ugalde, S., Harwood, T., Bartlett, J., Campas, M., Crooks, S. Gerssen, A., Harrison, K. Huet, A., Jordan, T. Koeberl,

M., Monaghan, T., Murray, S., Nimmagadda, R., Ooms, C., Quinlan, R., Shi, F., Turner, A., Yakes, B., and Turnbull, A. (2017). **Detection of Paralytic Shellfish Toxins in Mussels and Oysters using the Qualitative Neogen™ Lateral Flow Immunoassay: Collaborative Study.** Journal of AOAC International 101(2). DOI: <https://doi.org/10.5740/jaoacint.17-0221>

Paralytic shellfish toxins (PSTs) in bivalve molluscs represent a public health risk and are controlled via compliance with a regulatory limit of 0.8 mg saxitoxin (STX) center dot 2HCl equivalents per kilogram of shellfish meat (eq/kg). Shellfish industries would benefit from the use of rapid immunological screening tests for PSTs to be used for regulation, but to date none have been fully validated. An interlaboratory study involving 16 laboratories was performed to determine the suitability of the Neogen test to detect PSTs in mussels and oysters. Participants performed the standard protocol recommended by the manufacturer and a modified protocol with a conversion step to improve detection of gonyautoxin 1&4. The statistical analysis showed that the protocols had good homogeneity across all laboratories, with satisfactory repeatability, laboratory, and reproducibility variation near the regulatory level. The mean probability of detection (POD) at 0.8 mg STX center dot 2HCl eq/kg using the standard protocol in mussels and oysters was 0.966 and 0.997, respectively, and 0.968 and 0.966 using the modified protocol. The estimated LOD in mussels was 0.316 mg STX center dot 2HCl eq/kg with the standard and 0.682 mg STX center dot 2HCl eq/kg with the modified protocol, and 0.710 and 0.734 mg STX center dot 2HCl eq/kg for oysters, respectively. The Neogen test may be acceptable for regulatory purposes for oysters in accordance with European Commission directives in which the standard protocol provides, at the regulatory level, a probability of a negative response of 0.033 on 95% of occasions. Its use for mussels is less consistent at the regulatory level due to the wide prediction interval around the POD.

Hadley S, Macleod C, Ross DJ (2017) **Modelling risk of disease transmission between farming zones in Storm Bay.** Institute for Marine and Antarctic Studies, Hobart, Tasmania [Advice note]

This document investigates the modelled likelihood of particles (as a proxy for pathogens) spreading between the proposed farming zones in Storm Bay. CONNIE3 was used to model connectivity between zones, and the modelling approach was similar to that used in Nutrient Dispersion Modelling advice note (2017) for DPIPWE. The parameterisation used in order for CONNIE 3 to simulate the spread of infectious material (particles) was provided by DPIPWE. The location and size of the farming zones for each company (Huon Aquaculture, Petuna and Tassal) were provided by DPIPWE.

Hadley S, Macleod C, Ross DJ (2017) A summary of the improvements in the latest version of DEPOMOD (NewDEPOMOD) as compared with earlier versions (DEPOMOD/AutoDEPOMOD). Institute for Marine and Antarctic Studies, Hobart, Tasmania [Advice note]

DEPOMOD is a software tool created to predict the level and extent of deposition of particulate waste material and associated benthic impacts resulting from open cage finfish aquaculture based on user defined farm (e.g. feeding rates, configuration) and environmental information (e.g. currents, bathymetry). This tool was recently redeveloped into a more accurate version, NewDEPOMOD. This report presents an overview of the

changes to the model, as well as the meaning and default values of the important user defined parameters.

Hadley S, Macleod C, Ross J (2017) **Implementation of NewDEPOMOD for modelling the depositional footprint from salmon aquaculture in Storm Bay**. Institute for Marine and Antarctic Studies, Hobart, Tasmania [Advice note]

As part of the IMAS Sustainable Marine Research Collaboration Agreement (SMRCA) we were commissioned to i) provide an initial summary/ "Advice Note" of improvements in NewDEPOMOD and what this might mean for application in Tasmania (see above), ii) to provide a depositional footprint for an existing farm in Storm Bay using DEPOMOD/ New DEPOMOD and iii) based on those initial modelled outputs provide an "Advice Note" on initial sensitivity analysis/ model application protocols. This report addresses points ii) and iii). In terms of sensitivity (iii) we focus on changes in the forcing (or input) data rather than changes in model parameter values.

Hallegraeff, G., Dorantes-Aranda, J.J., Mardones, J., Seger, A. (2017). **Review of Progress in our Understanding of Fish-Killing Microalgae: Implications for Management and Mitigation**. In Proceedings ICHA17 Conference, pp. 148-153.

Fish-killing algal species are responsible for much greater global economic impacts than HAB species leading to seafood biotoxin contamination. Yet the precise mechanisms of how microalgae kill fish remain poorly understood. Progress has been hindered by the use of widely different bioassay systems and lack of analytical methods to quantify and characterize so-called "ichthyotoxins". All high biomass blooms even of nontoxic phytoplankton can cause significant stress for finfish contained in intensive aquaculture systems. Highly potent fish-killers include the taxonomically unrelated flagellate groups *Cochlodinium*, *Karenia*, *Chattonella*, *PseudoChattonella*, *Heterosigma*, *Prymnesium* which all readily lyse upon impact on the sensitive gill tissues of fish. A key mechanism for fish-gill damage being proposed is HAB cell lysis releasing free fatty acids (EPA, DHA, OPA) which in synergism with reactive oxygen species generate labile (min to hrs) lipid peroxidation products. Cell lysis is critical for *Karlodinium* and *Alexandrium* ichthyotoxicity, and high ROS producing strains (eg. *Chattonella*, Chilean *A. catenella*) cause greatest gill damage. With perhaps a single exception (*Florida Karenia brevis*), none of these ichthyotoxins are of human health significance, meaning that recently killed fish are still fit for human consumption. Finely ground bentonite clays at environmentally acceptable concentrations can effectively mop up ichthyotoxins and offer great potential as a HAB emergency response tool.

Keeley NB, Macleod CK, Taylor D, Forrest R (2017) **Comparison of three potential methods for accelerating seabed recovery beneath salmon farms**. *Aquaculture* 479:652-666

Fish production from sea-cages is a globally significant and expanding industry, but farm production can be constrained due to localised but extreme seabed enrichment, which requires the farm to be rested for extended periods. This study compares the effectiveness of three potential techniques for accelerating seabed recovery in highly enriched sediments. Benthic changes induced by in-situ 'harrowing' (heavy raking of the seabed), 'irrigation' with oxygenated surface-water, and simulated sediment 'removal' are described in relation to passive recovery. Treatment effectiveness was assessed after four months based on physico-chemical and biological analyses of sediments, changes in benthic respiration in mesocosm experiments, and an assessment of the instantaneous water column effects

induced during treatment. Results indicated significant sediment plumes associated with reduced dissolved oxygen levels, particularly during 'removal', but the magnitude and duration of the changes were negligible in an ecological effects context. Two treatments, 'harrowing' (HA) and 'irrigation' (IR), had little impact on seabed condition, particularly when compared with the natural recovery that occurred over the study period. Whereas, the 'removal' (RE) treatment (exposing the underlying sediment) significantly improved the physico-chemical and biological properties, and appeared to facilitate benthic recolonization. These findings suggest that, removal of degraded surface sediments has the potential to accelerate seabed recovery and can be a useful management strategy where trace metal concentrations (e.g. copper and zinc) have become unacceptably elevated. However, commercial-scale implementation would be contingent upon: i) further evaluation of water column effects associated with larger-scale treatments, and ii) the ability to safely dispose of the sediments.

Ross J, Macleod C (2017) **Interim Synopsis of Benthic and Water Column Conditions. Environmental Research in Macquarie Harbour.** Institute for Marine and Antarctic Studies, Hobart, Tasmania

In the first part of the synopsis we provide a brief summary of the key research projects and their findings with a focus on the ecology and condition of the Harbour benthic and water column environments. This provides important context for the subsequent presentation and discussion of the latest observations<sup>1</sup> and current status of the Macquarie Harbour environment. We finish with a discussion of suggested research priorities aimed at improving our understanding of the current situation that will assist management in the short to long term. It is important to acknowledge that this is an 'interim' synopsis of what we, the authors, believe are the key research findings in the context of the current observations of the Harbour environment. It is not an exhaustive and detailed presentation of all available science. Further detail can be found in the respective reports.

Ross J, Macleod C (2017) **Understanding oxygen dynamics and the importance for benthic recovery in Macquarie Harbour - Progress Report May 2017.** FRDC 2016/067: Environmental Research in Macquarie Harbour. Institute for Marine and Antarctic Studies, Hobart, Tasmania

This report provides an update on the status of dissolved oxygen and benthic conditions in Macquarie Harbour. It follows on from the results reported in the IMAS report released in January 2017 which described the deterioration of benthic and water column conditions in Macquarie Harbour in spring 2016. This report presents the results and preliminary interpretation of oxygen monitoring data up until the end of March 2017, and a repeat survey of benthic communities in January/February 2017. This ongoing work forms part of a research project (FRDC Project 2016-067: Understanding oxygen dynamics and the importance for benthic recovery in Macquarie Harbour to address these needs) funded by the Fisheries Research Development Corporation with the support of both industry and government (EPA and DPIWWE).

Ross J, Wild-Allen K, Macleod C (2017) **Understanding oxygen dynamics and the importance for benthic recovery in Macquarie Harbour - Progress Report September 2017.** FRDC 2016/067: Environmental Research in Macquarie Harbour. Institute for Marine and Antarctic Studies, Hobart, Tasmania

This report provides an update on the status of dissolved oxygen and benthic conditions in Macquarie Harbour. It follows on from the results reported in the IMAS reports released in

January and May 2017 which described the deterioration of benthic and water column conditions in Macquarie Harbour in spring 2016 and subsequent observations in early 2017, respectively. This report presents the results and preliminary interpretation of dissolved oxygen (DO) monitoring data up until the end of August 2017, a repeat survey of benthic communities in May/June 2017 and findings from CSIRO's oxygen tracer modelling. This work is part of the research project (FRDC Project 2016-067: Understanding oxygen dynamics and the importance for benthic recovery in Macquarie Harbour to address these needs) funded by the Fisheries Research Development Corporation with the support of both industry and government (EPA and DPIPWE).

Swadling KM, Eriksen RS, Beard JM, Crawford CM (2017) **Marine currents, nutrients and plankton in the coastal waters of south eastern Tasmania and responses to changing weather patterns.** FRDC Project No 2014/031. Institute for Marine and Antarctic Studies, Hobart, Tasmania

This report is an evaluation of one of the largest longer term data sets of water quality collected in a major waterway in Australia in recent years. Storm Bay at the mouth of the River Derwent, Tasmania, was sampled monthly for five years 2009-2015 for water quality – physical characteristics, nutrients, and phyto- and zoo-plankton - by the Institute for Marine and Antarctic Studies at the University of Tasmania. The project was conducted primarily for the Tasmanian salmon aquaculture industry and is the first major baseline assessment of water quality before salmon farming commences in a new region. The report is also of importance to other users of coastal waters in southeastern Tasmania, including commercial and recreational fishers, because it describes the major oceanic currents and weather patterns that influence water quality and productivity in the region. It also examines changes that have occurred over the previous three decades.

Turnbull, A., Tan, J., Ugalde, S., Hallegraef, G., Campbell, K., Harwood, T. and Dorantes-Aranda, J. (2017). **Single-Laboratory Validation of the Neogen™ Qualitative Lateral Flow Immunoassay for the Detection of Paralytic Shellfish Toxins in Mussels and Oysters.** Journal of AOAC International 101(2). DOI: <https://doi.org/10.5740/jaoacint.17-0135>

Detection of paralytic shellfish toxins (PSTs) in bivalve shellfish by analytical methods is complicated and costly, requiring specific expertise and equipment. Following extensive blooms of *Alexandrium tamarense* Group 1 in Tasmania, Australia, an investigation was made into commercially available screening test kits suitable for use with the toxin profiles found in affected bivalves. The qualitative Neogen rapid test kit, with a modified protocol to convert gonyautoxins GTX1&4 and GTX2&3 into neosaxitoxin and saxitoxin (STX), respectively, with higher cross-reactivities, was the best fit-for-purpose. This validation study of the test kit and the modified protocol was undertaken following AOAC INTERNATIONAL guidelines for the validation of qualitative binary chemistry methods. The validation used four different PST profiles representing natural profiles found in Australia and in Europe: two in a mussel matrix and two in an oyster matrix. The test kit was shown to have appropriate selectivity of the toxin analogs commonly found in bivalve shellfish. The matrix and probability of detection (POD) study showed that the rapid test kit used with the modified protocol was able to consistently detect PST at the bivalve regulatory level of 0.8 mg STX center dot 2HCl eq/kg, with a POD estimated via the binomial logistic regression of 1.0 at 0.8 mg STX center dot 2HCl eq/kg in all tested profiles in both matrixes. The POD at 0.4 mg STX center dot 2HCl eq/kg was 0.75 and 0.46 for the two toxin profiles in an oyster matrix

and 0.96 and 1.0 for the two toxin profiles in a mussel matrix. No significant differences in the PODs of the PSTs at the regulatory level were found between production lots of the test kits. The results suggest the method is suitable to undergo a collaborative validation study.

Weltz K, Lyle JM, Ovenden J, Morgan JAT, Moreno DA, Semmens JM (2017) **Application of environmental DNA to detect an endangered marine skate species in the wild.** Plos One 12 (6) e0178124.

Environmental DNA (eDNA) techniques have only recently been applied in the marine environment to detect the presence of marine species. Species-specific primers and probes were designed to detect the eDNA of the endangered Maugean skate (*Zearaja maugeana*) from as little as 1 L of water collected at depth (10–15 m) in Macquarie Harbour (MH), Tasmania. The identity of the eDNA was confirmed as *Z. maugeana* by sequencing the qPCR products and aligning these with the target sequence for a 100% match. This result has validated the use of this eDNA technique for detecting a rare species, *Z. maugeana*, in the wild. Being able to investigate the presence, and possibly the abundance, of *Z. maugeana* in MH and Bathurst harbour (BH), would be addressing a conservation imperative for the endangered *Z. maugeana*. For future application of this technique in the field, the rate of decay was determined for *Z. maugeana* eDNA under ambient dissolved oxygen (DO) levels (55% saturation) and lower DO (20% saturation) levels, revealing that the eDNA can be detected for 4 and 16 hours respectively, after which eDNA concentration drops below the detection threshold of the assay. With the rate of decay being influenced by starting eDNA concentrations, it is recommended that samples be filtered as soon as possible after collection to minimize further loss of eDNA prior to and during sample processing.

White CA, Bannister RJ, Dworjanyn SA, Husa V, Nichols PD, Kutti T, Dempster T (2017) **Consumption of aquaculture waste affects the fatty acid metabolism of a benthic invertebrate.** Sci Total Environ 586:1170-1181

Trophic subsidies can drive widespread ecological change, thus knowledge of how keystone species respond to subsidies is important. Aquaculture of large carnivorous fish generates substantial waste as faeces and lost feed, providing a food source to mobile benthic invertebrates. We used a controlled feeding study combined with a field survey to better understand the interaction between salmon aquaculture and the sea urchin, *Echinus acutus*, a dominant mobile invertebrate in Norwegian fjords. We tested if diets affected urchin fatty acid composition by feeding them one of three diet treatments (“aquafeed”, “composite” and “natural”) for 10 weeks. To test if proximity to fish farms altered *E. acutus* fatty acid composition, populations were sampled at 10 locations in Hardangerfjord and Masfjord (Western Norway) from directly adjacent and up to 12 km from farms. Fatty acids were measured in gonads and eggs in the diet experiment and in gonads and gut contents from wild animals. Urchins directly assimilated aquaculture waste at farm sites, as evidenced by elevated linoleic acid (LA), oleic acid (OA) and  $\sum$  LA, OA in their tissues. The diet experiment highlighted the biosynthetic and selective dietary sparing capacity of *E. acutus* in both gonads and eggs, with evidence for the elongation and desaturation of eicosapentaenoic acid (EPA) and arachidonic acid (ARA) from C18 fatty acid precursors. Elevated biosynthesis of non-methylene interrupted (NMI) fatty acids, in particular 20:3 $\Delta$ 7,11,14 and 20:2  $\Delta$ 5,11, were also linked to a high C18 fatty acid, low  $\geq$ C20 long-chain polyunsaturated fatty acid (LC-PUFA) diet. Fatty acid composition of gonads of wild urchins indicated a highly variable diet. The study indicates that the generalist feeding ecology of *E. acutus*, coupled



with extensive biosynthetic capacity, enables it to exploit aquaculture waste as an energy rich trophic subsidy.

White CA, Nichols PD, Ross DJ, Dempster T (2017) **Dispersal and assimilation of an aquaculture waste subsidy in a low productivity coastal environment**. *Mar Pollut Bull* 120:309-321

To understand dispersal and assimilation of aquaculture waste subsidies in a naturally low-productivity environment, we applied a novel, rapid transmethylation technique to analyse sediment and biota fatty acid composition. This technique was initially validated at Atlantic salmon farms in Macquarie Harbour, Australia, where sediments were collected at farm and control locations. Subsequently, sediment, benthic polychaete and zooplankton were sampled at sites 0, 50, 250, 500 and 1000 m distant from multiple cages. Results demonstrated an acute deposition zone up to 50 m from cages and a diffuse zone extending 500 m from cages. Changes in sediment concentration of linoleic acid, oleic acid and total fatty acids were effective tracers of farm deposition. Bacterial biomarkers indicated that aquaculture waste stimulates bacterial productivity in sediments, with elevated biomarker concentrations also detected in benthic polychaetes. Overall, fatty acid analysis was a sensitive technique to characterize the benthic footprint of aquaculture influence.

2016

Bell J, Lyle J, Semmens J, Awruch C, Moreno D, Currie S, Morash A, Ross J, Barrett N (2016) **Movement, habitat utilisation and population status of the endangered Maugean skate and implications for fishing and aquaculture operations in Macquarie Harbour**. FRDC 2013/008. Institute for Marine and Antarctic Studies, Hobart, Tasmania  
This study, undertaken by the Institute for Marine and Antarctic Studies, represents the first major investigation of the ecology and biology of the endangered Maugean Skate (*Zearaja maugeana*). Maugean Skates are only known from two estuarine systems located on the west coast of Tasmania (Macquarie Harbour and Bathurst Harbour), suggesting that the species has one of the most restricted distributions of any elasmobranch. The Maugean Skate is afforded a degree of protection in the World Heritage listed Bathurst Harbour, a no-take marine protected area, although they appear to be rare in this location. In Macquarie Harbour they appear to be relatively abundant but are subjected to multiple impacts due to human activities: they are a by-catch in recreational gillnets, and salmonid aquaculture operations are widespread and expanding in Macquarie Harbour. It is not known how these activities, coupled with the environmental variability in Macquarie Harbour, impact on the Maugean Skate population.

Due to the issues outlined above, the present study aimed to:

1. determine the distribution, habitat utilisation and movement of the Maugean Skate in Macquarie Harbour;
2. determine the key biological characteristics of Maugean Skate, including population size, reproductive dynamics and feeding habits;
3. describe the spatial and temporal dispersal patterns of aquaculture escapees;
4. assess the potential impacts of current and proposed marine farming operations on the Maugean Skate population; and
5. evaluate strategies to reduce the probability of encountering Maugean Skate whilst fishing (gillnetting) for escapees

In relation to the behaviour of escapees, Atlantic Salmon and Rainbow Trout dispersed rapidly upon release, moving widely and generally randomly throughout Macquarie Harbour.

Several Rainbow Trout and a single Atlantic Salmon showed an affinity for regions near aquaculture leases suggesting at least some of these individuals were feeding on aquaculture overfeed. The vast majority did not, however, survive or remain in the harbour for longer than about two months following release. As a general observation, Rainbow Trout tended to survive slightly longer than Atlantic Salmon. While about 25% of the escapees were recaptured by recreational fishers (in gillnets), most are assumed to have died of natural causes (starvation and possibly predation). About 20% of the salmonids did, however, leave the harbour either by moving out to sea (mainly Atlantic Salmon) or entering the Gordon River (Rainbow Trout). The fate of these individuals could not be assessed.

Hadley S, Jones E, Johnson C, Wild-Allen K, Macleod C (2016) **A Bayesian inference approach to account for multiple sources of uncertainty in a macroalgae based integrated multi-trophic aquaculture model.** Environ Model Software 78:120-133

A Bayesian inference method was employed to quantify uncertainty in an Integrated Multi-Trophic Aquaculture (IMTA) model. A deterministic model was reformulated as a Bayesian Hierarchical Model (BHM) with uncertainty in the parameters accounted for using “prior” distributions and unresolved time varying processes modelled using auto-regressive processes. Observations of kelp grown in 3 seeding densities around salmon pens were assimilated using a Sequential Monte Carlo method implemented within the LibBi package. A low to medium seeding density results in the most efficient removal of excess nutrients in this simple system.

Hadley S, Wild-Allen K, Johnson C, Macleod C (2016) **Quantification of the impacts of finfish aquaculture and bioremediation capacity of integrated multi-trophic aquaculture using a 3D estuary model.** J Appl Phycol 28:1875-1889

In this study, a coupled 3D hydrodynamic, sediment, and biogeochemical model was used to simulate an idealized temperate test estuary. A macroalgal-based IMTA model was applied within the estuarine model, to examine the spatial pattern of phytoplankton production arising from increasing levels of finfish aquaculture and the capacity of *Macrocystis pyrifera* to bioremediate the impacts of nutrification. IMTA scenario results demonstrated a strong spatial variability in the capacity of *M. pyrifera*-based IMTA to reduce water column chlorophyll concentration. This demonstration of the use of IMTA to improve system wide water quality is valuable for regional planners and managers as it provides an analysis and quantification of a method to achieve estuarine health and economic benefit.

Macleod C, Ross DJ, Hadley S, Henriquez L, Barrett N (2016) **Clarifying the relationship between salmon farm nutrient loads and changes in macroalgal community structure/distribution.** FRDC 2011/042. Fisheries Research & Development Corporation, Hobart, Tasmania

This project was developed in response to questions raised by the salmon industry about the potential for adverse impacts on macroalgae as a result of the additional nutrient inputs from farming operations in marine systems. Two student projects were identified with the intention of further exploring the relationship between salmon farm nutrient loads and changes in macroalgal community structure and distribution. The research took two different but complementary directions, which comprised the body of research for two PhD students. The first project was primarily concerned with how additional nutrient inputs added directly to a subtidal reef system might affect macroalgal ecology and performance. The second project was a desk based modelling study focused on determining whether growing macroalgae in conjunction with salmon farming is a viable mechanism for reducing nutrient loads within a

stylized waterbody, what species might respond most effectively to salmon farm derived nutrients, whether there could be additional benefits associated with such macroalgal culture (such as a secondary commercial crop or further environmental benefits associated with the growth of threatened/ ecologically valued species) and where the optimum location would be for growing such species. The outcomes of this research are described in more detail in the following report, both theses and any papers published or in press at the time this report was submitted have been attached as appendices.

Macleod CK, Eriksen RS, Chase Z, Aritz SE (2016) **Chapter 13: Chemical pollutants in the marine environment: causes, effects, and challenges.** In: Solan M, Witeley NM (eds) *Stressors in the Marine Environment: Physiological and ecological responses; societal implications.* Oxford University Press, United Kingdom

Chemicals are now commonplace in the marine environment; with chemical pollutants found in every region, from the Arctic to the tropics and from the smallest estuary to the depths of the oceans. The majority of these pollutants are terrestrial in origin, where they originally had some specific benefits and purpose. However, in the marine environment they can often have adverse and quite unexpected impacts. This chapter will discuss the various types of chemical pollutant found in our marine and coastal systems, and consider how those might affect marine fauna, flora, and ecosystem processes. The chapter will also explain the many and varied ways in which these chemicals (intentionally or unintentionally) can find their way into our oceans and coastal habitats. To end, the chapter will address options for management, monitoring, and regulation and highlight knowledge gaps and target areas of research.

Macleod C, Ross DJ, Carter C, Gardner C, Barrett N, Hallegraaf G, Bolch C, Lea M (2016) **Submission to the Marine Farming Planning Review Panel Independent Assessment of salmon farming at Okehampton Bay.** Institute for Marine and Antarctic Studies, Hobart, Tasmania [Advice note]

We hope that this submission provides the Panel with an understanding of the research that has been undertaken by IMAS and its predecessors, commonly and in collaboration with other organisations, to inform both management and monitoring of salmon farming in Tasmania as a whole as well as UTAS/ IMAS research information and knowledge specifically relevant to the environmental management of salmon farming in Okehampton Bay specifically.

Revell A, Ross J, Thompson A (2016) **Investigating Dissolved Oxygen Drawdown in Macquarie Harbour.** CSIRO Oceans and Atmosphere, Hobart, Tasmania

There have been a number of recent reports on, and reviews of, oxygen in Macquarie Harbour (e.g. MHDOWG 2014, ADS 2015, Knight et al. 2015). These identified some potentially large and unquantified processes that are likely to be important in the oxygen balance in Macquarie Harbour. Here we review some observations of dissolved oxygen in Macquarie Harbour and report on the first measurements of biological oxygen demand (BOD) from the water column. Although spatially variable BOD was ~ 2 times benthic respiration. A comparison between long term net drawdown and measured gross rates of oxygen consumption highlights the role of surface exchange in maintaining oxygen levels above the halocline while extended periods without mixing lead to significant periods of low dissolved oxygen in the mid-water column. Elevated levels of labile organic carbon with fatty acid profiles characteristic of farm derived material were detected at a depth of 3m close to

the cages and coincident with increased rates of oxygen consumption when compared to the far field site. Based on this study it is recommended that sufficient measurements of BOD be conducted to determine the spatial variability of oxygen drawdown and farm derived organic material throughout the Harbour. In addition a simple model should be developed to better understand processes important to oxygen cycling; particularly mixing which resupplies most of the oxygen. This combination would provide a superior capability to manage oxygen in Macquarie Harbour for the benefit of its many users, the environment and its ecology.

Ross DJ, Revill AT, Bodrossy L, Nichols PD (2016) **Particulate Organic Matter - Source, Composition and Degradation (Objective 2)**. MHTWG Pelagic DO Study. Institute for Marine and Antarctic Studies, Hobart, Tasmania

A key objective of this study was to try and use biomarkers to identify and track the influence of aquafeed waste in the water column. The concentration of linoleic acid and oleic acid and their contribution to the fatty acid profile provided a clear signature of aquafeed waste in the environment, however, only in the cage itself. At the closest distance sampled to stocked cages, 100m, there was no detectable aquafeed fatty acid signature. This is perhaps not that surprising when considering the highly diffusive nature of the water column, the relatively low stocking densities at the time of sampling and the difficulty in matching sampling with feeding and fish extraction. The highly labile nature of aquafeed further adds to this challenge. It is also important to consider that indirect pathways i.e. emissions of dissolved inorganic nutrients (e.g. ammonium) and the subsequent incorporation into organic matter would not be detected using fatty acids. Given the likely importance of nitrifying organisms in the harbour, future work should consider measuring the N isotope of ammonium to trace this pathway. In this study we did intend to measure the isotopic signature of particulate organic nitrogen, but unfortunately concentrations were too low and not amenable to analysis.

Ross J, McCarthy A, Davey A, Pender A, Macleod C (2016) **Understanding the Ecology of Dorvilleid Polychaetes in Macquarie Harbour: Response of the benthos to organic enrichment from finfish aquaculture**. FRDC 2014/038. Institute for Marine and Antarctic Studies, Hobart, Tasmania

The expansion of salmon farming in Macquarie Harbour is contingent upon ecologically sustainable development. Underpinning such development are management regimes designed to regulate and minimise the impact of farming on the benthic environment. The management protocols implemented in Macquarie Harbour were developed based on farming practices and conditions in southern Tasmania and were established with the aim of ensuring that any impacts were not severe, reversible and could be constrained within defined boundaries. However, the response of the benthic invertebrate communities, and most notably Dorvilleid polychaetes, in Macquarie Harbour to enrichment from salmonid aquaculture has been inconsistent with expectations. The benthic communities in Macquarie Harbour, and their response to organic enrichment, differ from that previously observed in southern Tasmanian regions. In particular, the response of the Dorvilleid polychaetes in Macquarie Harbour was quite different to that observed in other polychaete species in southern Tasmania and highlighted the need for focused research on their ecology and behaviour in response to organic enrichment in Macquarie Harbour. This report describes the work conducted by the Institute of Marine and Antarctic Studies (University of Tasmania) to address this knowledge gap. In the first part of the study, the international literature was reviewed to establish the current state of understanding regarding Dorvilleid ecology, and in particular, how they respond to organic enrichment. The second part of the study comprised

a targeted field survey at selected leases to identify the relationship between Dorvilleids and sediment condition, and to characterise the environmental conditions associated with major changes in Dorvilleid distribution and abundance. The results were compared with previous surveys (including baseline surveys undertaken throughout the harbour) to investigate whether there have been any broad scale changes in the benthic ecology. The findings are discussed in the context of benthic monitoring requirements for fish farm management in Macquarie Harbour.

Valentine JP, Jensen M, Ross DJ, Riley S, Ibbott S (2016) **Understanding broad scale impacts of salmonid farming on rocky reef communities.** FRDC 2014/042. Fisheries Research & Development Corporation, Hobart, Tasmania

The strategic growth of the Tasmanian Salmonid Industry is contingent upon ecologically sustainable development. Current Tasmanian production is not meeting domestic demand, and the industry is in the process of expanding farming operations to meet strategic growth targets. While local scale impacts are well understood, the extent of broad scale environmental impacts from finfish farming needs to be better understood, especially in relation to nutrient emissions and their potential ecosystem effects on macroalgal community assemblages on rocky reefs. This report describes the results of analyses and surveys designed to examine patterns of change and characterise reef assemblages in south eastern Tasmania, where there is increasing concern as the salmonid aquaculture sector expands into more exposed waterways that overlap with traditional wild fishing sectors (such as abalone and rock lobster). The first part of the study used the existing Marine Protected Area (MPA) monitoring dataset to examine patterns of change in macroalgal communities in south eastern Tasmania between 1992 and 2015. The second component of the study included a field survey of rocky reefs, incorporating the MPA sites, along with additional sites chosen to better represent industry expansion into new growth areas. The study used a collaborative approach, with field work and data analysis carried out by Aquenal and Marine Solutions. Scientific support and supply of the MPA dataset was provided by IMAS. This report describes the approach and results of the time series analysis and reef characterisation. Complexities associated with reef assessment and options for future monitoring are also addressed.

White CA, Dworjanyn SA, Nichols PD, Mos B, Dempster T (2016) **Future aquafeeds may compromise reproductive fitness in a marine invertebrate.** Mar Environ Res 122:67-75  
Aquaculture of higher trophic level species is increasingly dependent on the use of terrestrial oil products. The input of terrestrially derived n-6 polyunsaturated fatty acids (PUFA) into marine environments has subsequently increased, with unknown consequences for recipient species. We exposed a sea urchin, *Heliocidaris erythrogramma* to three experimental diets for 78 days: a high n-3 PUFA marine imitation treatment, a high n-6 PUFA “future aquafeed” treatment and an intermediate “current aquafeed” treatment. Female urchins fed the high n-6 PUFA diet produced larvae with lower survival rates than all other treatments. Males fed the high n-6 PUFA diet produced no viable sperm. Fatty acid composition in reproductive material revealed comprehensive biosynthetic and dietary sparing capabilities in *H. erythrogramma*. Despite this, the ratio of n-6 PUFA to n-3 PUFA in reproductive tissue increased significantly with diet. We suggest alterations to this ratio is the likely mechanism of negative impact on larval development.

2015

Hadley S, Wild-Allen K, Johnson C, Macleod C (2015) **Modeling macroalgae growth and nutrient dynamics for integrated multi-trophic aquaculture.** J Appl Phycol 27:901-916

To test a variety of suitable macroalgae species and management scenarios for IMTA, a numerical model is developed to quantify the remediation of dissolved nutrients and production of macroalgae near a nutrient source. Results show that of the three species studied, *Macrocystis pyrifera* removed 75 % of dissolved inorganic nitrogen (DIN) input from a point source, while *Porphyra umbilicalis* and *Ulva lactuca* removed 5 %. Choice of macroalgae species greatly affects the success of IMTA and that both harvesting and farm arrangements (depth of cultivation and hydrodynamic conditions) can be used to greatly optimize bioremediation.

Keeley NB, Forrest BM, Macleod CK (2015) **Benthic recovery and re-impact responses from salmon farm enrichment: Implications for farm management.** Aquaculture 435:412-423

This paper describes a two-year study of spatial and temporal patterns and processes in the benthos in response to the removal of salmon cages from a sheltered coastal embayment, coupled with the simultaneous reintroduction of cages at an adjacent location. Significant recovery was evident at the fallowed site in the first six months; however, the macrofaunal assemblage remained impacted at the conclusion of the study. By comparison, the reintroduction of a fully operational farm overwhelmed the macro benthic community within three months, with anoxic and near-azoic conditions developing. Both removal and reintroduction of the farms triggered alternating oscillations of geochemical and biological variables, which were attributed to effects on sediment chemistry from organic loading, 'boom and bust' cycles of opportunistic taxa in response to food supply, and the associated variations in metabolic potential. The study also revealed interesting spatial dynamics in the benthos and some useful indicators of different stages of recovery and re-impact. It is concluded that farm reintroductions should aim to gradually increase production; allowing time for the benthos to adapt to the additional organic flux, and be maintained at a level that avoids macrofaunal collapse. The sediment's ability to cope with organic inputs from fish farming, and hence the duration of the recovery period, is contingent on the organic load in each farming cycle and the extent to which the sediment community is allowed to recover. Understanding the influence of each of these on sediment processes is important for sustainable long-term management of farming operations.

Macleod C, Kelly B, Hortle M, Hulls J, Main A, Elliott N (2015) **An evaluation of the options for the expansion of salmonid aquaculture in Tasmanian waters.** FRDC 2011/735.

Institute for Marine and Antarctic Studies, Hobart, Tasmania

This project is the final stage of the evaluation of the options for the expansion of salmonid aquaculture in Tasmanian waters and provides a decision support approach which provides guidance for the identification of the types (and sources) of information that would be needed to support an aquaculture development application in the coastal zone. The project also provides a framework for presentation and analysis of the resultant information such that it can be readily reviewed and assessed by various stakeholders in order to make informed decisions on both site suitability and potential interactions that would allow for the optimal site selection. A template for comparison and assessment of the risks and information needs for aquaculture development/ expansion proposals is provided. The resultant simple but effective planning and decision support framework provides a functional mechanism for

community engagement and communication and should support the development application process and improve planning decisions.

Oh ES, Edgar GJ, Kirkpatrick JB, Stuart-Smith RD, Barrett NS (2015) **Broad-scale impacts of salmon farms on temperate macroalgal assemblages on rocky reefs**. Mar Pollut Bull 98:201-209

Intensive fish culture in open sea pens delivers large amounts of nutrients to coastal environments. Relative to particulate waste impacts, the ecological impacts of dissolved wastes are poorly known despite their potential to substantially affect nutrient-assimilating components of surrounding ecosystems. Broad-scale enrichment effects of salmonid farms on Tasmanian reef communities were assessed by comparing macroalgal cover at four fixed distances from active fish farm leases across 44 sites. Macroalgal assemblages differed significantly between sites immediately adjacent (100m) to fish farms and reference sites at 5km distance, while sites at 400m and 1km exhibited intermediate characteristics. Epiphyte cover varied consistently with fish farm impacts in both sheltered and exposed locations. The green algae *Chaetomorpha* spp. predominated near fish farms at swell-exposed sites, whereas filamentous green algae showed elevated densities near sheltered farms. Cover of canopy-forming perennial algae appeared unaffected by fish farm impacts.

Ross J, Hartstein N, Macleod C (2015) **Characterising benthic pelagic interactions in Macquarie Harbour - organic matter processing in sediments and the importance for nutrient dynamics**. FRDC 2012/047, Hobart, Tasmania

This study assessed the impacts of organic enrichment from salmon farming on nitrogen cycling processes in the sediments in Macquarie Harbour and assessed the sediment's capacity to recover during fallowing. This research has improved our understanding of sediment processes in Macquarie Harbour and has enabled sediment function to be more appropriately represented in the environmental model. It is clear that the Macquarie Harbour ecosystem and the associated biogeochemical processes are different from that previously described for other systems in Tasmania. Consequently, any changes in environmental conditions and our understanding of system dynamics would require the model to be revised. From a sediment monitoring perspective, bulk identifiers of organic matter source (C:N ratio and  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signature) together with measured rates of respiration appeared to be good environmental indicators of the footprint of farm derived organic matter and sediment function respectively. Anaerobic processes and the production of reduced compounds were important in the overall benthic biogeochemistry of the harbour. Measurement of sediment function at some, but not all, sites showed patterns consistent with expectations during fallowing and stocking. This suggests that drivers other than stocking (e.g. changes in diet, conversion ratios, feeding regimes, bottom water conditions) are playing a significant role in determining sediment condition. A greater understanding of the drivers of sediment function in response to different stages of farming activity is likely to improve the effectiveness of farm based management of stocking and fallowing regimes. Interestingly, in the second half of the study a significant decline in bottom water oxygen conditions was evident. The causes of this decline and the implications for broader ecosystem dynamics warrants further investigation, and as such, may require the model to be revised for future use.

Keeley NB, Macleod CK, Hopkins GA, Forrest BM (2014) **Spatial and temporal dynamics in macrobenthos during recovery from salmon farm induced organic enrichment: When is recovery complete?** Mar Pollut Bull 80:250-262

This study documents eight years of benthic recovery at a highly impacted salmon farm. Substantial recovery occurred in the first 2 years, and was assessed to be complete after 5 years. However, minor differences were still evident, along with some on-going benthic instability, attributable to medium-scale spatial movements and successional patterns of macrobenthos. Quantifying the endpoint of 'recovery' proved challenging due to: lack of a widely accepted definition, inherent variability in recovering sediments, differing trajectories of impact and reference sites, and statistical challenges. More complex biotic indices and metrics incorporating multiple variables were the most robust indicators. Statistical tests for 'parallelism' in the trajectories of Cage and Reference sites proved useful, but results were contingent upon how the method was applied, and should therefore be used in conjunction with data-visualisation methods. The study highlights the importance of a predetermined recovery endpoint, and using multiple indicators and a weight-of-evidence assessment approach.

Lucieer V, Pender A (2014) **An environmental assessment of a proposed amendment to the marine farming zone at Zuidpool Rock in the D'Entrecasteaux Channel (Assessment 2)**. Institute for Marine and Antarctic Studies, Hobart, Tasmania

An environmental assessment of a proposed marine farming zone extension at Zuidpool Rock, in the D'Entrecasteaux Channel, was conducted in February 2014. This assessment is comprised of two parts, encompassing Zuidpool Rock northern and southern sites and have been distinguished as Assessments 1 and 2, with the results being presented in two separate reports. This report addresses Assessment 2. Two sites were surveyed in this assessment. The northern site covered 2.51 km<sup>2</sup> (251 ha) and the southern site covered 2.81 km<sup>2</sup> (281 ha). Both sites were orientated in a north westerly direction. The benthic habitats within the zone assessment area for the northern site comprised of silt (73.6%) and silty sand (26.4%) and the southern site, silt 69.8% and silty sand (30.2%). Environmental data on substrate type, habitat distribution, bathymetry, and benthic flora and fauna were assessed. Within the northern proposed zone the substrate was characterized by silt and silty sand. The bathymetry ranged from 24 m to 39 m and was characterized by a deep channel to 54 m in the south west of the site which was flanked by two shallower banks, indicative of a drowned river valley. The southern proposed site was characterized by silty sand and sand and a depth strata ranging from 26 m on the southern end of the site with the deepest point found at the bottom of a steep embankment at 46 m. The Northern Zuidpool site was characterized brown silt on the surface with some evidence of layering to grey silt. All of the New Zealand screwshells (*Maoriculpus roseus*) that were sampled in the Northern site were dead. Amphipods and squat lobsters (*Munida haswelli*) were common across the region. Brittle stars (*Amphiura elandiformis*), polychaetes and squat lobsters (*Munida haswelli*) were noted in all of the grab samples. Heart urchins (*Echinocardium cordatum*) and Ghost shrimp (Family: Callianassidae) were also noted. The Southern Zuidpool site was characterised by brown silt and yellow silty sand substrate with shell grit, evidence of active bioturbation; live and dead New Zealand screwshells (*Maoriculpus roseus*), brittle stars, squat lobsters (*Munida haswelli*) and polychaete worms were common across the whole site. Both of the sites, North and South contained sediment 'pock mark' features; these seabed features are common in many parts of the channel. The pock marks that appear in the center of the Zuidpool North site and to the northern part of the Zuidpool South site are identical and are around 20 m in diameter and 1- 2 m deep.



Lucieer V, Pender A (2014) **An environmental assessment of a proposed amendment to the marine farming zone at Zuidpool Rock in the D'Entrecasteaux Channel (Assessment 1)**. Institute for Marine and Antarctic Studies, Hobart, Tasmania

An environmental assessment of a proposed marine farming zone extension at Zuidpool Rock, in the D'Entrecasteaux Channel, was conducted in February 2014. This assessment is comprised of two parts, encompassing Zuidpool Rock northern and southern sites and have been distinguished as Assessments 1 and 2, with the results being presented in two separate reports. This report addresses Assessment 1. The site covered 2.76 km<sup>2</sup> (276 ha) and was orientated in a north westerly direction. The benthic habitats within the zone assessment area comprised of silt (60.7%) and silty sand (39.3%). Environmental data on substrate type, habitat distribution, bathymetry, and benthic flora and fauna were assessed. Within the proposed zone the substrate was characterized by silt and silty sand. The bathymetry ranged from 23 m to 54 m and was characterized by a deep channel to 54 m in the south west of the site which was flanked by two shallower banks, indicative of a drowned river valley. The silty sand substrate was brown to grey brown in colour with shell grit, evidence of active bioturbation; live and dead New Zealand screwshells (*Maoriculpus roseus*), brittle stars, squat lobsters (*Munida haswelli*) and polychaete worms were common across the whole site.

Lucieer V, Pender A (2014) **An environmental assessment of a proposed marine farming zone extension at Browns Point, in the D'Entrecasteaux Channel**. Institute for Marine and Antarctic Studies, Hobart, Tasmania

An environmental assessment of a proposed marine farming zone extension at Browns Point, in the D'Entrecasteaux Channel, was conducted in February 2014. The site covered 6 km<sup>2</sup> (600.92 ha) and was orientated in a north easterly direction perpendicular to the coastline. The benthic habitats within the zone assessment area comprised of reef (1.5%) and silty sand (98.5%).

Environmental data on substrate type, habitat distribution, bathymetry, and benthic flora and fauna were assessed. Within the proposed zone the substrate was characterized by silty sand. On the western boundary three small areas of reef substrate (1.5 % of the total survey area) were identified running out from the shoreline and into the zone assessment area. The bathymetry ranged from 31.2 m to 51.2 m and was characterized by a gradual slope from the shoreline to the east.

The silty sand substrate was brown to yellow in colour with shell grit, evidence of active bioturbation; live and dead New Zealand screwshells (*Maoriculpus roseus*), brittle stars, squat lobsters (*Munida haswelli*) and polychaete worms were common across the whole site. The video surveys on the western reef showed the presence of Zoanthids (*Parazoanthus* sp.), Butterfly perch (*Caesioperca lepidoptera*), Bullseye (*Pempheris multiradiata*) and Rosy Wrasse (*Pseudolabrus rubicundus*). Flora included cup sponges, branched sponges, feather stars (*Cenolia tasmaniae*) and red algae (*Phyllospora comosa*).

Lucieer V, Pender A (2014) **An environmental assessment of a proposed marine farming zone extension at Flathead Bay, in the D'Entrecasteaux Channel**. Institute for Marine and Antarctic Studies, Hobart, Tasmania

An environmental assessment of a proposed marine farming zone extension at Flathead Bay, in the D'Entrecasteaux Channel, was conducted in February 2014. The site covered 3.24 km<sup>2</sup> (324 ha) and was orientated in a northerly direction perpendicular to the coastline. The benthic habitats within the zone assessment area comprised of silt (69.5%) and silty

sand (30.5%). Environmental data on substrate type, habitat distribution, bathymetry, and benthic flora and fauna were assessed. Within the proposed zone the substrate was characterized by silt and silty sand. The bathymetry ranged from -17.1 m to -45.9 m and was characterized by a gradual slope from the shoreline to the east with two shallower regions, one in the north east and one on the southern boundary. The silty sand substrate was brown to yellow in colour with shell grit, evidence of active bioturbation; live and dead New Zealand screwshells (*Maoriculpus roseus*), brittle stars, squat lobsters (*Munida haswelli*) and polychaete worms were common across the whole site. Other features on the substrate included drift algae (*Ecklonia radiata*) and (*Phyllospora comosa*), Northern Pacific sea stars (*Asterias amurensis*), gobys (potentially *Nesogobius* sp.), zoanthids (potentially *Parazoanthus* sp.) and a Tasmanian numbfish (*Narcine tasmaniensis*). One notable feature that was identified from the hill-shaded bathymetric image was "pock marks" occurring in the soft sediment on the western boundary of the zone assessment area. The six pock marks that appear on the eastern boundary, inside the zone assessment area were around 20 m in diameter and 1- 2 m deep and occurred in the basins and not on the terraces.

Lucieer V, Pender A (2014) **An environmental assessment of a proposed marine farming zone extension at Lippies Point, in the D'Entrecasteaux Channel**. Institute for Marine and Antarctic Studies, Hobart, Tasmania

An environmental assessment of a proposed marine farming zone extension at Lippies Point, in the D'Entrecasteaux Channel, was conducted in February 2014. Following discussions between the Industry and the Marine Farm Planning division, an amendment to the initial proposal was considered. The amendment is detailed in this report. The area of the site covered 4.11 km<sup>2</sup> (411 ha) and was orientated in a north easterly direction perpendicular to the coastline. The benthic habitats within the zone assessment area comprised of sand (60.8 %) and silty sand (39.2 %). Environmental data on substrate type, habitat distribution, bathymetry, and benthic flora and fauna were assessed. Within the proposed zone the substrate was characterized by sand and silty sand. The bathymetry ranged from 34 m to 49 m and was characterized by a channel to the northern end of the site. The resolution of the bathymetry in this report varies due to the amended section of the proposed region to the north being mapped with single beam acoustics and the southern end being mapped with multibeam acoustics. The amended zone to the north of the site was dominated by silt. Live and dead New Zealand screwshells (*Maoriculpus roseus*) were found across the whole survey site. Brittle stars (*Amphiura* sp.), a heart urchin (*Echinocardium cordatum*) and Polychaete worms were common in all the sediment samples. The native screwshell, *Gazameda gunii*, was not observed in any of the sediment grabs. All of the sediment samples were odourless. The video surveys reported on in this report are for the amended region to the north of the proposed region. For details on the substrate in the southern end of the region, please refer to the report by Lucieer and Pender 2014. The majority of the amended site was characterised by rippled silty sand with high degrees of bioturbation, New Zealand screwshells (*Maoriculpus roseus*) sponge fragments and shell grit. The video data highlighted a large number of sites hosting squat lobsters, both live and dead New Zealand screwshells (*Maoriculpus roseus*) and drift algae (*Ecklonia radiata* and *Phyllospora comosa*). On video transect 5 a heart urchin (potentially *Echinocardium cordatum*) was noted.

Lucieer V, Pender A (2014) **An environmental assessment of a proposed marine farming zone extension at Trumpeter Bay, South Eastern Tasmania.** Institute for Marine and Antarctic Studies, Hobart, Tasmania

An environmental assessment of a proposed marine farming zone extension at Trumpeter Bay on the eastern side of northern Bruny Island was conducted in February and March 2014. The site covered 19.61 km<sup>2</sup> (1960 ha) and demonstrated a depth strata from 30 – 50 m and was orientated in a north south direction. The benthic habitats within the zone assessment area comprised of sand 1926.24 hec (98.24%) and gravel 34.5 hec (1.76%). Environmental data on substrate type, habitat distribution, bathymetry, and benthic flora and fauna were assessed. The dominant sand substrate was characterised by flat regions with high levels of bioturbation, ripples, drift sponge and shell grit. The video data highlighted a large number of sites hosting New Zealand screwshells (*Maoriculpus roseus*). The sediment grabs showed that the sand composition ranged from yellow-brown sand to orange-brown sand with pockets of black silt to gravel. Live and dead New Zealand screwshells (*Maoriculpus roseus*) were found across the whole survey site. Hermit crabs (*Paguristes* sp.), a squat lobster (*Munida haswelli*), Polychaetes, one bivalve (*Venerupis* sp.), live auger shells (*Hastula brazieri*), heart urchins (*Echinocardium cordatum*) were identified from the grab samples. The native screwshell, *Gazameda gunii*, was not observed in any of the sediment grabs. All of the grab samples were odourless. An interesting feature within the site was the presence of localised depressions and channels in the sediment on both the eastern and western boundaries. These depressions and channels were commonly around 50 – 90 m in with and were on average only 1 m lower than the surrounding seafloor. The sediment composition differed to the surrounding sand as these pockets and gutters were comprised of gravel and coarse sand with shell grit.

Lucieer V, Pender A (2014) **An environmental assessment of a proposed marine farming zone west of Wedge Island, South Eastern Tasmania.** Institute for Marine and Antarctic Studies, Hobart, Tasmania

An environmental assessment of a proposed marine farming zone extension west of Wedge Island was conducted between March and June 2014. The site covered 17.22 km<sup>2</sup> (1722 ha), demonstrated a depth strata from 37 – 55 m and was orientated in a north south direction. The benthic habitats within the zone assessment area comprised of sand – 1715.83 ha (99.59 %) and gravel- 7.00 ha (0.41%). Environmental data on substrate type, habitat distribution, bathymetry, and benthic flora and fauna were assessed. The dominant sand substrate was characterised by a dark orange brown colour and exhibited large flat regions with ripples, drift algae and shell grit. The video data highlighted the presence of rippled sand with shell grit. Three video transects captured the presence of New Zealand screwshells (*Maoriculpus roseus*), with one additional video transect identifying *Gazameda* sp. or *Colpospira* sp. Three video transects highlighted polychaete tubes extending above the sediment. The sediment grabs captured samples of a pebble crab (*Dittosa undecimspinosa*), Heart urchins (*Echinocardium cordatum*), bivalves (*Diplodonta tasmanica*) and (*Glycymeris striatularis*), Brittle stars (*Ophiura kinbergi*), Hermit crabs (*Paguristes* sp.), a wavy volute shell (*Amoria undulata*) and a live Peanut Shell (*Adamnestia arachis*). All of the grab samples were odourless. The initial assessment survey captured twenty sediment grab samples across the zone. In this initial survey one sample contained a live specimen of the native screwshell, *Gazameda gunii*. This was positively identified by the Tasmanian Museum and Art Gallery Zoology Department and therefore, following the Marine Farm Assessment protocol, a further twenty grab samples were collected across the site. *Gazameda gunii* was

identified in 3 of the additional 20 samples.

An interesting feature within the site was the presence of localised depressions in the sediment on the eastern boundary. These depressions were found at 47 m depth and were on average only 1 m lower than the surrounding seafloor. The sediment composition differed to the surrounding sand as these pockets were comprised of gravel and coarse sand with shell grit.

Macleod C, Eriksen R, Simpson S, Davey A, Ross DJ (2014) **Assessment of the environmental impacts and sediment remediation potential associated with copper contamination from antifouling paint (and associated recommendations for management)**. FRDC 2011/041. Fisheries Research & Development Corporation, Hobart, Tasmania

This study was undertaken as a collaboration between researchers at the Institute for Marine and Antarctic Studies (University of Tasmania) and CSIRO, and with the cooperation of the Tasmanian Salmon farming industry to determine whether copper concentrations in sediments under salmon farms in the Huon and D'Entrecasteaux Channel have any major or long-term impacts on the local ecology or sediment function and to identify the remediation potential of these sediments and what, if any, management strategies could be used to enhance recovery. Noting that in this instance recovery was assessed as either i) a marked decline in copper over time (recovery in progress) or ii) return to background/ baseline copper concentrations (total recovery).

Conditions were assessed over the short-term (12 months, this study) at sites selected for high copper loads, as well as over the longer-term (> 5 years, incorporating the results of previous farm-based assessments) at sites where the copper concentration history was well-known. Changes in background concentrations were assessed by reviewing copper data from both the farm assessments and a range of previous studies in the region, and integrating broader environmental data on prevailing conditions and exposure. Finally, targeted sedimentation studies provided data on deposition and accumulation rates that could be used to provide longer-term projections for recovery.

A specific concern at the start of this study was that ongoing farming, even without the use of antifoulant nets, could increase the risk of toxicity in sediments where copper concentrations were elevated; the results of this study clearly identify that this is not the case. The results indicate that the risk of serious adverse impacts on sediment processes from current copper contamination levels is relatively low; largely because most of the copper occurs as paint flakes and can't be easily taken up by benthic organisms.

Copper can exist in a variety of forms in the sediments, with some being more toxic than others. The concentrations of relevant forms of copper were assessed, and the associated sediment conditions determined. Whilst antifoulant usage was shown to be the primary source of elevated copper concentrations within farms, local environmental conditions and certain farming practices can have a significant influence on copper accumulation and impact levels throughout the system. Consequently, it was possible to make operational management recommendations that will reduce the potential for impacts into the future. The study also recommends refined regulatory guidelines that should provide better protection with respect to chronic ecotoxicological impacts.

The report makes some key recommendations for both farm management and monitoring. With respect to farm management it is suggested that the use of copper based antifoulants should be minimised, and farms should look to replace nets where there is evidence of metal accumulation in sediments; where antifoulants are used net manipulations and other

activities with the potential for abrasion should be minimised, but where this cannot be avoided (i.e. fish crowding/ transfer) then farms should look to replace with non-antifouled nets; and finally that farms should look to increase the use of monofilament/ plastic nets wherever possible, but especially at depositional sites - it is suggested that copper based antifoulants should be restricted to sites where sediment organic content is <15%. Recommendations for monitoring suggest that where total recoverable copper concentrations in the sediments exceed the ISQG-High (270 mg/kg) then dilute acid extractable copper concentration should also be assessed to ensure that concentrations are below the TV (65 mg/kg) and that regular monitoring should continue to measure total recoverable copper (as this is necessary for consistency with historical datasets and protocols, and therefore essential for assessment of recovery). Industry has already adopted the management recommendations of this study and copper based antifoulant nets have now largely been replaced.

2013

Crawford C, Buxton C, Main A (2013) **Atlantic Salmon Aquaculture Subprogram: Macroalgal monitoring in Macquarie Harbour, Tasmania.** FRDC 2011/086. Fisheries Research & Development Corporation, Hobart, Tasmania

There was a pressing need to monitor the distribution and abundance of intertidal macroalgae in Macquarie Harbour before the expansion of salmon farming commenced to provide 'baseline' data against which the effects of the expanded salmon production on the Harbour environment could be assessed. As different species of macroalgae occur at different times of the year, surveys in autumn and spring were required. The distribution and percentage cover of common macroalgal species at 40 sites throughout the Harbour in autumn and spring 2012 were documented and photographs of each site and the algal species have been collated and stored on CD. This has established a baseline against which the effects of proposed increases in salmon production on macroalgal composition and abundance can be assessed.

Objectives:

1. To conduct preliminary baseline monitoring for macroalgae in Macquarie Harbour in autumn
2. Develop a macroalgal monitoring program for Macquarie Harbour
3. Test monitoring program and conduct seasonal (spring) baseline monitoring

Hindell M, Lea MA (2013) **A risk assessment of fur seals interactions with aquaculture in Tasmania.** Tassal Group Pty Ltd, Hobart, Tasmania

Salmon aquaculture in Tasmania is a well established and growing part of the State's primary industry. Farming Atlantic salmon involves holding very large numbers of fish in large pens suspended in the sea, typically in groups of 10 or more pens known as a farm. The farms are all coastal and located around Tasmania in areas that meet particular requirements of coastal and bathymetric topography and current flow. This high concentration of fish overlaps with the normal range of fur seals, and inevitably results in seals being attracted to pens in an attempt to access the fish. The first harvest of salmon from aquaculture in Tasmania occurred in 1987 (53 tonnes), so seals have been interacting with fish farms for more than 25 years. As the industry has grown (now 40,000 tonnes annually, (TSGA 2013)), the number of interactions has also increased.

Keeley N (2013) **Quantifying and Predicting Benthic Enrichment: Lessons Learnt from Southern Temperate Aquaculture Systems**. Doctor of Philosophy. University of Tasmania, Hobart, Tasmania

This integrated study has a number of important implications for the management of organic enrichment in general but is especially pertinent for fish farming. In particular, recommendations are made regarding the i) adequacy of chemical and biological benthic indicators and their performance in typical non-dispersive and atypical dispersive sites; ii) use and applicability of depositional models in the same environments with emphasis on the role of resuspension, and iii) timing and approach for reintroduction of impacts, with respect to monitoring and management of rotational fallowing strategies to ensure on-going sustainability.

Keeley NB, Cromey CJ, Goodwin EO, Gibbs MT, Macleod CM (2013) **Predictive depositional modelling (DEPOMOD) of the interactive effect of current flow and resuspension on ecological impacts beneath salmon farms**. *Aquaculture Environment Interactions* 3:275-291

Sediment resuspension is an important factor in controlling the impact of any localised point source impacts such as salmon farms; at high-flow (dispersive) sites, resuspension can significantly reduce potential effects. Depositional modelling (DEPOMOD) is widely used to predict localised seabed impacts and includes an optional flow-related resuspension module. This study examined the observed impacts at 5 farms with contrasting flow regimes to evaluate the role of modelled resuspension dynamics in determining impacts. When resuspension was included in the model, net particle export (i.e. no significant net downward flux of organic material) was predicted at the most dispersive sites. However, significant seabed effects were observed, suggesting that although the model outputs were theoretically plausible, they were inconsistent with the observational data. When the model was run without resuspension, the results were consistent with the field survey data. This retrospective validation allows a more realistic estimation of the depositional flux required, suggesting that approximately twice the flux was needed to induce an effect level at the dispersive sites equivalent to that at the non-dispersive sites. Moderate enrichment was associated with a flux of similar to 0.4 and similar to 1 kg m<sup>(-2)</sup> yr<sup>(-1)</sup>, whilst highly enriched conditions occurred in response to 6 and 13 kg m<sup>(-2)</sup> yr<sup>(-1)</sup>, for low and dispersive sites, respectively. This study shows that the association between current flow, sediment resuspension and ecological impacts is more complex than presently encapsulated within DEPOMOD. Consequently, where depositional models are employed at dispersive sites, validation data should be obtained to ensure that the impacts are accurately predicted.

Keeley NB, Forrest BM, Macleod CK (2013) **Novel observations of benthic enrichment in contrasting flow regimes with implications for marine farm monitoring and management**. *Mar Pollut Bull* 66:105-116

We examine macrofaunal and physico-chemical responses to organic enrichment beneath salmon farms in contrasting flow environments, and reveal pronounced flow-related differences in the magnitude and spatial extent of effects. Total macrofaunal abundances at high flow sites were nearly an order of magnitude greater than at comparable low flow sites, representing a significant benthic biomass. These very high abundances occurred in conjunction with moderate-to-high species richness, and were evident in the absence of appreciable organic matter accumulation. Biological responses to increasing sulfide were variable; however a significant biological threshold was evident at 1500 µM. Macrofaunal

responses at high flow sites differed substantially from the Pearson-Rosenberg model. The atypical ecological conditions were attributed to (i) limited accumulation of fine sediments, (ii) maintenance of aerobic conditions in near-surface sediments, and (iii) an abundant food supply. Thus, enhanced resilience to organic waste at well-flushed sites appears related to both biological and physical processes.

Lucieer V, Pender A (2013) **An environmental assessment of a proposed marine farm zone extension at Butlers Point in Great Taylor Bay, South Eastern Tasmania**. Institute for Marine and Antarctic Studies, Hobart, Tasmania

An environmental assessment of a proposed marine farm extension at the current Butlers lease in Great Taylor Bay was conducted in November 2013. Environmental data on substrate type, habitat distribution, bathymetry, benthic flora and fauna was assessed. The survey area covered 2.081 km<sup>2</sup> where 1.989 km<sup>2</sup> (95.58%) was comprised of sand habitat with a fringing hard substrate reef covering - 0.09 km<sup>2</sup> (4.42 %) to the inshore side of the zone. The bathymetric profile of the site ranged from 1.6 m on the shoreward side of the lease (to the South West) to 20 m to the North West. The sandy substrate had a range of particle sizes from fine to coarse sands which correlated with depth. The sand substrate was characterised by screwshells (*Maoricolpus roseus*) both live and dead and numerous small burrows. The sediment grabs showed the presence of both live and dead New Zealand screwshells (*Maoricolpus roseus*), polychaete tubes, ghost shrimp (*Callinassidae*), *Corbula gibba* bivalves, *Caulerpa scalpelliformis*, *Caulerpa trifaria*, hermit crabs (*Paguristes* sp.), crabs (*Hexapus granuliferus*), dead scallop shell (*Pecten fumatus*) and squat lobsters (*Munida haswelli*). The fringing reef habitat was characterised by *Caulerpa trifaria*, *Caulerpa longifolia*, red algae, *Macrocystis pyrifera*, *Sargassum* sp. (which was covered with green filamentous algae), *Phyllospora comosa*, *Ecklonia radiata*, *Caulerpa longifolia*, *Caulerpa trifaria*, plate sponges, numerous Southern Hulafish (*Trachinops caudimaculatus*) and Blue-throat Wrasse (*Notolabrus tetricus*). These findings are consistent with the habitats described by the SeaMap Tasmania surveys conducted in 2000 at this site (Barrett et. al. 2001). Apart from *Macrocystis pyrifera*, no threatened, endangered or protected species were recorded or observed from the video or grab samples.

Lucieer V, Pender A (2013) **An environmental assessment of a proposed marine farm zone extension at Creeses Mistake, Nubeena Tasmania**. Institute for Marine and Antarctic Studies, Hobart, Tasmania

An environmental assessment of a proposed marine farm extension at the current Creeses Mistake lease at Nubeena was conducted in June 2013. Environmental data on substrate type, habitat distribution, bathymetry, benthic flora and fauna was assessed. Within the proposed zone extension, the substrate was 100% sand which was dominated (80% cover) by partially buried screwshells (*Maoricolpus roseus*). The sand also hosted patchy macroalgae growing from shells including red algae, encrusting coralline algae and *Caulerpa* sp. The key species that could be readily identified from the macroalgae assemblage in reef area comprised of *Macrocystis pyrifera*, *Ecklonia radiata*, *Carpoglossum confluens*, *Phyllospora comosa*, *Caulerpa* sp., *Cystophora* sp., red algae and encrusting coralline algae. The substrate within the entire survey area consisted of a large sandy area (99%) with one small area of reef in the north east corner of the survey region (< 1%). The bathymetric profile of the site ranged from 5 m on the north eastern corner to 30 m in the south western corner with a gradual decrease from the east to the west. Apart from

*Macrocystis pyrifera*, no threatened, endangered or protected species were recorded or observed from the video or grab samples.

Macleod C, Eriksen RS, Davey A, Kelly B, Ross DJ (2013) **Long-term Recovery - Review of sediment condition at Marine Farm lease No. 76 (Gunpowder Jetty), North West Bay.** IMAS Technical Report. Institute for Marine and Antarctic Studies, Hobart, Tasmania  
This study was undertaken to assess the Gunpowder Jetty site (Marine Farm lease No.76) 10 years after it was finally vacated to determine whether the benthic, visual and physical chemical conditions were consistent with background conditions and to what extent the system has recovered. The results suggest that, based on the selected environmental condition variables assessed, the sediments around the Gunpowder lease have largely recovered. Whilst some parameters still showed small differences between locations these do not appear to be having a major effect on the ecology or nutrient mineralisation processes and consequently there is no evidence that the farming activity has had any permanent impact on the ecology in this area.

Ross J, Macleod C (2013) **Evaluation of Broadscale Environmental Monitoring Program (BEMP) data from 2009-2012.** Book Technical Report. Institute for Marine and Antarctic Studies, Hobart, Tasmania

There has also been a significant body of research undertaken through IMAS (and as Tasmanian Aquaculture and Fisheries Institute) which has been targeted at characterising environmental conditions in our coastal waterways more broadly, and this information provides the baseline by which waterway health can be more broadly assessed.

The BEMP provides a comprehensive assessment of ecological condition in the D'Entrecasteaux Channel and Huon Estuary. The program commenced in March 2009, and is currently undertaken by all marine finfish farm licence holders in the D'Entrecasteaux Channel and Huon River/Port Esperance MFDP areas. Fifteen sites within the MFDP areas are monitored throughout the year to assess spatial and temporal patterns of water and sediment quality; this includes a broad suite of parameters capturing the physical, chemical and biological characteristics of the system. This dataset provides a significant body of information that can be used by regulators, industry and other stakeholders to assess ecological condition and to support adaptive management strategies.

This report represents a comprehensive summary of both the water and sediment quality data collected as part of the BEMP from 2009-2012. It also provides an evaluation of the data in the context of the major system drivers, previous environmental data sets and broader ecosystem performance measures. Collectively the information in this report has improved our understanding of the key processes and interactions, forms a comprehensive reference point for future assessment of the Huon and Channel system and provides an assessment of ecological functioning at the whole of ecosystem level (i.e. a "state of the system").

2012

Eriksen RS, MacLeod C, Ross DJ (2012) **Evaluation of Approaches to Improve Sediment Remediation (Rate & function) Under Salmonid Fish Cages.** Institute for Marine and Antarctic Studies, Hobart, Tasmania

Salmonid cage farming in sheltered coastal areas can result in localised, spatially predictable and temporal accumulation of organic material around cages, and an associated decline in sediment quality. In Tasmania, following the seabed under cages for a period of



time is a regulatory requirement to allow sediments to recover from the effects of organic enrichment. The following period required varies depending on the prevailing environmental conditions and the intensity and history of operations. Currently following relies on passive remediation, however, if the natural processes can be fine-tuned it may be possible to increase the rate or extent of sediment recovery. Enhancing the recovery process could have significant benefits for both farm production and the environment; enabling farmers to manage the timing of their production at each given site more precisely and to have a means by which to remediate the sediments should conditions become sub-optimal.

This project comprised two parts, firstly a review of options for sediment remediation and ranking of potential options with respect to Tasmanian conditions, and secondly an experimental study to test the viability of selected options. The review identified three main approaches (physical, biological and chemical) to accelerating the natural recovery processes. An assessment matrix for the various options was developed and two methods were identified as having potential under Tasmanian conditions: harrowing and direct introduction of oxygen to the sediments. The viability of harrowing as a remediation tool was tested using a combination of field and laboratory studies. Performance was assessed using two key measures of sediment condition: nutrient fluxes and oxygen penetration depth. Highly degraded farm sediments were collected for the study as these were the most appropriate conditions for testing the efficacy of approaches to improve sediment remediation. The sediments selected had been subject to significant build-up of organic matter and consequently were extremely anoxic and exhibited high rates of ammonia and phosphate efflux. Ammonia fluxes reduced considerably over the period of the trial (14 weeks), consistent with a reduction in the organic content of the sediments and recovery of the nitrification process (conversion of ammonia to nitrate) in the sediments. Oxygen penetration depth of organically enriched sediments increased from 0 to 2mm in both the harrowed and controls sites over 14 weeks. Although the results did not show a significant difference overall between harrowing and passive recovery over the trial duration, there were clear indications of a reduction in variability in sediment quality when undertaken on the most degraded sediments, and some evidence of a trend suggesting that harrowing may improve the process over a longer timeframe. These evaluations were conducted under controlled laboratory conditions, and it is possible that the greater potential for oxygen exchange under field conditions might improve the outcome and enhance the observed trend.

Hindell M, Lea MA (2012) **Status and trends of Australian (*Arctocephalus pusillus doriferus*) and New Zealand fur seals (*A. forsteri*) in Australia and New Zealand.** Tassal Pty Ltd

No summary available

Keeley NB, Forrest BM, Crawford C, Macleod CK (2012) **Exploiting salmon farm benthic enrichment gradients to evaluate the regional performance of biotic indices and environmental indicators.** *Ecol Indicators* 23:453-466

This study evaluates five benthic indicators (total abundance, number of taxa, redox potential, total free sulfides, total organic matter) and ten biotic indices (Margalef's d, Peilou's J', Shannon H', AMBI, M-AMBI, MEDOCC, BENTIX, BOPA, ITI, BQI), to identify those that best define organic enrichment gradients under different flow regimes. Performance was measured against Enrichment Stage (ES), a continuous variable characterising the full range of sediment conditions (natural to azoic). None of the 15 metrics were able to consistently discriminate over the full enrichment gradient for both flow

environments. The most versatile indices were BQI > M-AMBI > AMBI > Log(N) > BENTIX. Of these, M-AMBI best catered for different flow environments, while the BQI was the most effective under highly enriched conditions. Under strong enrichment, i.e. when macrofauna abundance is in decline, changes in redox, sulfides, number of taxa and abundance were reasonably clear. However, the more complex biotic indices were relatively insensitive at this level, highlighting a limited applicability beyond the 'peak of opportunists' (PO). Conversely, in high flow regimes, some of the biological indicators were relatively sensitive to low-to-moderate levels of enrichment that were not well discerned by the physico-chemical variables. A useful subset of variables for assessing enrichment status is recommended, comprising two of the best performing biotic indices that are based on alternative/independent classification schemes (i.e. EG's and ES500.05), total abundance, to aid in discerning PO, and a geochemical variable (redox or S<sub>2</sub><sup>-</sup>). Inconsistencies between metrics were found to be more significant than the variability surrounding the predictive capacity of individual indicators, and as a result there is a risk of ES misclassification where only a single index is used. Whilst there is a recognised need to use combinations of indicators, this study also stresses the importance of focusing on a few regionally validated measures and down-weighting the importance placed on any that are not. Additionally, although using a combination of different indicators may produce a 'safe' average result, it may be inefficient, and the averaging effect has the potential to mask extreme conditions. Hence, there remains a need for expert judgement to select and appropriately weight indicator variables, to identify any erroneous results, and to reliably assess ecological quality status.

Keeley NB, Macleod CK, Forrest BM (2012) **Combining best professional judgement and quantile regression splines to improve characterisation of macrofaunal responses to enrichment.** *Ecol Indicators* 12:154-166

Many benthic quality indices rely on categorising impacts by assigning species to ecological-groups (EGs) that reflect their tolerance to pollution. This is usually based on best professional judgement (BPJ) by experts with access to relevant ecological and taxonomic information. However, international applicability of such indices is restricted in areas where the species taxonomy, biology and response to pollution are poorly understood. In this study we describe an approach that enables objective allocation of EGs in situations where species information is limited. This approach utilised BPJ to categorise the environmental condition of benthic habitats around fish farms in New Zealand in relation to defined enrichment stages (ESs). Quantile regression was then used to model distributions of select taxa. The experts assigned ES scores from 1 to 7, for stations that ranged from relatively natural to excessively enriched (i.e. near-azoic), respectively, with judgements based on a suite of quantitative and qualitative indicators of enrichment, but without reference to detailed species information. The individual BPJ estimates were highly correlated, with minimal bias, indicating good agreement among the experts. Forty key indicator taxa were identified and quantile regression models based on ES (derived as a continuous explanatory variable) were fitted for 34. Abundances of the same taxa were also modelled in response to a more traditional enrichment indicator (organic content, %OM) for comparison with the BPJ technique. The regression approach characterised enrichment responses and objectively identified both the upper and lower tolerance limits of a range of taxa according to their ES and %OM. The models discriminated a number of key indicator taxa, including several that were responsive to low-level changes in ES, but not necessarily %OM. There was reasonable agreement (59%) between EGs derived using the regression approach and

those defined using the AMBI database (one of the most commonly applied benthic quality indices). Moreover, the regression method allowed the classification of 10 additional taxa for which our ecological understanding was limited. A key outcome of this study was the acknowledgement that EG characterisations for species need to be regionally validated, no matter how well defined they might appear to be. The combined BPJ/regression analysis approach described provides a valid means of both assigning and validating EG classifications, which will be particularly useful in situations where the taxa are poorly defined, and will enable existing biotic indices to be more broadly applied and interpreted.

Lucieer VL, Macleod C, Pender AJ, Ross DJ (2012) **Environmental assessment of potential marine farming areas in Macquarie Harbour Tasmania**. Institute for Marine and Antarctic Studies, Hobart, Tasmania

No summary available

2011

Abrantes KG, Lyle JM, Nichols PD, Semmens JM (2011) **Do exotic salmonids feed on native fauna after escaping from aquaculture cages in Tasmania, Australia**. *Can J Fish Aquat Sci* 68:1539-1551

Atlantic salmon, *Salmo salar*, and rainbow trout, *Oncorhynchus mykiss*, are farmed in Tasmania, Australia, where fish sometimes escape into the natural environment. If escapees are able to survive and feed on native fauna, it is likely that they will have ecosystem impacts. Stomach content, body condition (muscle lipid content and Fulton' K), stable isotope, and fatty acid analysis were used to determine if escaped salmonids feed on native fauna. Results indicate that, in general, escaped salmonids do not feed on native fauna. Salmonids loose condition after escaping, and escapee stomachs were mostly empty or contained non-nutritious material or feed pellets. Nevertheless, almost a quarter of rainbow trout stomachs contained native fauna. The majority of escapees had biochemical composition similar to caged animals, indicating that these fish had not switched to feed on local food sources. However, a small fraction of escapees conclusively showed changes in biochemical parameters indicative of a shift to feeding on native fauna. Given the numbers and frequency of escapes, this can have an important impact on native species and on the ecology of Macquarie Harbour.

Eriksen R, Macleod C (2011) **Antifoulants in Salmonid Aquaculture: Environmental Considerations for Marine Cage Farming**. In: Andrews GL, Vexton LA (eds) *Fish Farms Management, Disease Control and the Environment*. Nova Science Publishers, New York. Antifoulant chemicals are commonly used in aquaculture to counteract biofouling, which may be a particularly significant problem to finfish culture operations. Biofouling reduces water flow and oxygen supply, and in turn leads to increased stress levels and disease-susceptibility in the cultured fish. Copper-based net coatings, the main antifoulant used in marine salmonid operations, act as a biocide thereby preventing the attachment of fouling organisms to the cage. There are several environmental concerns associated with the use of copper antifoulants and their persistence in the marine environment, the most significant of which are i) toxic effects on local ecology and ecosystem processes, especially in sediments and ii) potential for bioaccumulation. Many marine invertebrates have been shown to be sensitive to metal toxicity, with juvenile (embryo and larval) stages often being particularly susceptible. As a result where copper is released from aquaculture operations, vulnerable species can be eliminated changing the ecological balance. Other species may be more

robust, with a greater capacity to regulate or sequester metals, and these species may appear unaffected or even thrive in response to the increased metal loads. However, where an animal is able to survive through tolerance or accumulation of metals this can provide an avenue for bioaccumulation and biomagnification as a result of predation from higher trophic level species. Metals in antifoulants can become available to the broader environment either by dissolution from the active paint surface or as a result of ablation or physical damage of the painted surface. Sediments under fish farms using copper-based antifoulants typically show increased total metal loadings, however, it is important to note that for metals in the sediments to have any effect on local ecology or sediment processes they must be "bioavailable", which in turn is dependant on the form (speciation) of the metal. Metal speciation in sediments is complex and strongly related to the geochemical status of the sediments (i.e. redox, pH, oxic status, organic content), which in turn will be influenced by the extent of other processes such as bioturbation and resuspension. In anoxic sediments, metals are generally thought to be less bioavailable than in oxic sediments, as they are often tightly bound as insoluble sulphides. As metal speciation may be influenced by the level of organic enrichment, metal toxicity may fluctuate with variations in organic inputs associated with the farming cycle, and this should be taken into account in any monitoring or evaluation of impact. Until such time as there is a reliable replacement for copper-based antifouling products, sustainable management practices need to ensure that procedures minimise the environmental impact of currently used products and include the development of appropriate monitoring strategies. In this chapter we review possible environmental impacts associated with copper based antifoulant use, the mechanisms and processes that affect metal bioavailability, the ecological consequences that might be associated with particular metal loadings and monitoring and management strategies. Data from research conducted by the Tasmanian salmonid industry (Australia) are discussed.

Macleod C, Eriksen RS (2011) **Antibiotics in Salmonid Aquaculture: Environmental Considerations for Marine Cage Farming**. In: Andrews GL, Vexton LA (eds) *Fish Farms Management, Disease Control and the Environment*. Nova Science Publishers, New York

Antibiotics are typically used in aquaculture operations as a direct response to infectious diseases to ensure the health and well-being of farmed stock. However, there are several environmental issues associated with antibiotic usage that farmers need to understand and address when developing disease management strategies. Some of these can have direct negative implications for farming operations while others relate to effects on the broader environment. Negative outcomes for farming operations include the potential for the development of resistance in the target pathogens. However, the main concerns associated with antibiotic usage relate to effects on non-target organisms, environmental persistence and development of broader resistance. In salmonid aquaculture, antibiotics are generally administered in feed and as such these concerns are primarily related to the presence of waste feed and fish excreta in the sediments and water column. Ensuring good farm management and husbandry practices to maximise the health of fish stocks is the best way to reduce the need for antibiotics and as a result any associated impacts. Rapid and accurate diagnosis of pathogens and development of targeted disease management strategies and alternative therapies, in particular vaccines, is also an important means to reduce and manage overall antibiotic usage. However, where antibiotics are necessary then careful feed management, monitoring feed input and limiting feed wastage, will minimise any impacts. In addition an understanding of the various environmental issues associated with specific antibiotics allows farm managers and veterinarians to make informed choices with

respect to treatment regimes. Finally, establishing an effective monitoring approach for evaluation of potential ecological effects is a key factor in assessing the impact of antibiotics. Development of resistance and accumulation in the sediments are significant environmental concerns, consequently it is important to monitor the incidence of resistance in the environment and in fish bacteria. Accumulation in the sediments may affect natural sedimentary processes, such as biogeochemistry, and it would be prudent to confirm this, and to determine threshold effect levels. Even where assessment of the available data does not suggest that major environmental changes have occurred, it is still important to identify and monitor suitable indicator species to ensure ongoing sustainability. In this chapter we review various antibiotics used in salmonid aquaculture globally, provide a summary of key environmental issues and compare various monitoring and assessment approaches.

2010

Edgar GJ, Davey A, Shepherd C (2010) **Application of biotic and abiotic indicators for detecting benthic impacts of marine salmonid farming among coastal regions of Tasmania.** *Aquaculture* 307:212-218

Analysis of sediment and macrofaunal samples collected during the Tasmanian marine farming finfish monitoring program – a six-year partnership between industry, management and researchers – revealed several univariate indicators to be useful for detecting effects of aquaculture on the benthic environment. Comparisons with reference sites revealed a significant decline in sediment redox potential to at least 4 cm depth at farm sites, and increased proportional abundance of capitellids and decreased bivalve/total mollusc ratio. At compliance sites located 35 m out from lease boundaries, sediment redox potential and faunal assemblage composition were intermediate between patterns found at farm and reference sites. Redox potential at the sediment surface declined on average by 178 eV at reference sites converted to farm sites, with this indicator proving the most sensitive for detecting regional impacts of farming activity. Fish farm effects that extended to regional scales could not be adequately assessed within the project because reference regions without fish farms were not monitored; however, a significant decrease through time at reference and compliance sites in surface redox potential, and increases in sediment organic matter and total macrofaunal abundance, were suggestive that organic enrichment may have extended at low levels across regional scales. Given the implications to biodiversity conservation of region-wide impacts and a need to distinguish fish farm effects from unrelated long-term environmental change, monitoring of reference sites in regions lacking fish farms is urgently needed.

Lawler MM, Lucieer V, Pender A, Macleod C (2010) **Part B: Environmental assessment of a proposed marine farming zone extension at Soldiers Point (SE) in the D'Entrecasteaux Channel.** *Tasmanian Aquaculture and Fisheries*, Hobart, Tasmania  
No summary available

Paxton H, Davey A (2010) **A new species of Ophryotrocha (Annelida: Dorvilleidae) associated with fish farming at Macquarie Harbour, Tasmania, Australia.** *Zootaxa* 2509:53-61

*Ophryotrocha shieldsi*, sp. nov. is described from Macquarie Harbour, Tasmania, Australia, where it occurs in high densities beneath the sea cages of fish farms. SCUBA and ROV underwater observations revealed closely spaced mounds of aggregations of the new species. It is closely related to *O. lobifera* Oug, a species reported from fish farms and

whale-falls in the North Sea, from which it can be distinguished by its ovate rather than triangular dorsal lateral lobes, palps with small globular rather than longer digitate palpostyles, and additional jaw differences.

2009

Bissett A, Cook PLM, Macleod C, Bowman JP, Burke C (2009) **Effects of organic perturbation on marine sediment betaproteobacterial ammonia oxidizers and on benthic nitrogen biogeochemistry**. *Mar Ecol Prog Ser* 392:17-32

The failure of denitrification to remove nitrogen build-up from aquatic systems is often attributed to sediment chemical conditions inhibiting nitrification and therefore the supply of suitable substrates to be denitrified. We investigated the effects of organic fish farm pollution on nitrogen-cycle dynamics and betaproteobacterial ammonia-oxidizing bacteria (beta-AOB) community structure to elucidate the potential role of the nitrifier community on nitrogen biogeochemistry in marine sediments. Porewater nitrogen concentrations, denitrification rates, (beta-AOB 16S rDNA gene quantification, denaturing gradient gel electrophoresis (DGGE) community fingerprints and infaunal counts were determined in samples collected from beneath fish cages and at adjacent, non-impacted control sites. The study was conducted over 2 full, 1 yr production cycles. Although nitrogen cycling was significantly altered beneath cages, changes appeared to result from a reduction in the proportion of ammonia nitrified rather than from inhibition of nitrification per se. DGGE revealed beta-AOB communities shifted rapidly and remained diverse at both cage and reference sites. Quantitative PCR (qPCR) showed beta-AOB numbers did not decline in absolute terms but did decline as a proportion of the total bacterial community at cage sites and at the end of the stocking periods. Sediment infaunal community analysis showed significant effects of organic loading and indicated more bioirrigation at impacted sites. Despite the induction of conditions thought to be detrimental to nitrification and to beta-AOB (low oxygen, reduced sediments, low pH, and high sulphide concentrations), these communities remained diverse and apparently viable, perhaps a result of heavy sediment bioirrigation. However, despite the increase in denitrification, nitrogen left the sediment predominantly as ammonia, thus producing potential point sources of eutrophication.

Crawford C, Macleod C (2009) **Predicting and assessing the environmental impact of aquaculture**. In: Burnell G, Allan G (eds) *New technologies in aquaculture: Improving production efficiency, quality and environmental management*. Woodhead, Cambridge, UK

Aquaculture can affect the environment in a number of ways, including organic enrichment around the farm, increased dissolved nutrients or chemicals in the growing area, escapees affecting native species, loss of habitat and loss of amenity. Likewise, the environment can affect aquaculture, through poor water quality (e.g. pollutants, high particulate matter), predators and nuisances species such as jellyfish or seals, or changing climate regimes. These impacts can be reduced by careful site selection, to provide the appropriate conditions for the species being cultured, and good farm management. New technologies are assisting in minimising these environmental impacts. Recent developments in remote sensing and seabed mapping have improved site selection. Monitoring and assessment of the environment around aquaculture farms is becoming more common, and this is also being supported by new technology. A range of probes is now available that can be used in the field to provide an immediate measure or placed in situ to continuously monitor environmental conditions. Other advances include the use of visual techniques with digital cameras and remotely operated or autonomous underwater vehicles. Computing capability

and mathematical models are also becoming increasingly important to management, both for determining carrying capacity of growing areas and for prediction of impacts. These models are becoming increasingly sophisticated and are capable of linking across different temporal and spatial scales and across trophic levels and incorporating social and economic parameters.

Crawford C, Swadling K, Thompson P, Clementson L, Schroeder T, Wild-Allen K (2009) **Nutrient and Phytoplankton Data from Storm Bay to Support Sustainable Resource Planning**. FRDC 2009/067. Institute for Marine and Antarctic Studies, Hobart, Tasmania  
This project has established a baseline assessment program for water quality and productivity in Storm Bay, and collected environmental data monthly for 12 months. These data are important towards understanding climate variability and assessing the longer term impacts of climate change in the region. They will underpin more informed management of marine resources.

Edgar G, Davey A, Sheperd C (2009) **Broad-scale effects of marine salmonid aquaculture and introduced pests on macrobenthos and the sediment environment in Tasmania between 1998 and 2003**. Internal Report. Tasmanian Aquaculture and Fisheries Institute

This study was undertaken in conjunction with the salmon industry and DPIPW and expands the scope of the earlier investigation of broad-scale effects of salmonid aquaculture in southeastern Tasmania outlined above (Edgar et al. 2005) to include analysis of temporal change over the six year period from 1998-2003. The study also investigated potential interactions between salmon farming and marine pest species using the marine farm monitoring data set to assess impacts of introduced species on the benthic environment. There were clear regional differences in the benthic macrofauna around Tasmania, with Macquarie Harbour fauna being particularly differentiated as depauperate in species and animal numbers. Between 1998-2003 the inshore Tasmanian marine environment appeared to change, with evidence of generalised organic enrichment in several sediment condition measures

Significant effects on the sediment biogeochemistry could be identified on/ near leases in all farming regions other than Macquarie Harbour, where no effect of fish farm activity was detected. Minor effects were detected beyond the lease boundaries in some areas; however, there was no indication of negative impacts on the biota. The study identified several positive and negative indicators of farm impacts, which were consistent with previous studies however, it was noted that those species which were negatively- associated with fish farm impact tended to be localised in distribution and were not significant in the statewide analyses.

Whilst it was not possible to specifically assess the effects of fish farming at the regional scale (the monitoring program did not include reference regions without fish farms), many of the factors measured suggested low levels of organic enrichment within farming regions and that macrofaunal density was also increasing at these scales. However, it is important to note that the scale of fish farm impacts was substantially less than the scale of natural regional variation.

Introduced species were consistently present in the study (although there were fewer in Macquarie Harbour), and increased in proportional abundance by 2-3% per annum.

However, there was no evidence of them displacing native populations. The most abundant and widespread species were: bivalves *Theora lubrica* and *Corbula gibba*, and the screwshell *Maoricolpus roseus*; the bivalve *Raeta pulchella* and fanworm *Euchone limnicola* were also locally abundant. Populations of *M. roseus* were stable through time and largely unaffected by fish farm activity. *C. gibba* flourished between 1998 and 2001 and whilst positively associated with high organic loading and fine sediments it was not related to fish farms and numbers declined markedly in the latter part of the study. *T. lubrica* did respond to fish farm activity, being present at farm and compliance sites but not at reference sites. The findings note that the Tasmanian salmonid farm monitoring program provides an invaluable statewide baseline for assessing environmental impacts not only of salmon farming but also more broadly, and with that in mind recommends that it be expanded to include monitoring of reference sites in regions without fish farms.

Macleod C, Eriksen R (2009) **A review of the ecological impacts of selected antibiotics and antifoulants currently used in the Tasmanian Salmonid Farming industry.** FRDC 2007/246. Tasmanian Aquaculture & Fisheries Institute, Hobart, Tasmania

This study provides a review of the international literature and current research to identify the existing state of knowledge regarding the environmental effects of antibiotics and antifoulants currently used or likely to be used in the Tasmanian salmonid farming industry (marine production phase). It also provides an analysis of local datasets on currently used antibiotics and antifoulants, collected in compliance with current licensing requirements, and makes some recommendations regarding what is needed to appropriately evaluate the environmental impact of current management practices.

The ongoing sustainability of the aquaculture industry requires some use of antibiotics and antifoulants to ensure the health of farmed stocks. However, it is important to ensure that any associated environmental impacts are known and minimised. There is a considerable body of international literature in relation to the environmental effects of both antifoulants and antibiotics. Understanding the main environmental concerns regarding the current usage of selected antibiotics and antifoulants allows for the evaluation of the key ecological risk areas and this can then be used to determine where research or monitoring is most needed. The aim of this review being to assist aquaculture and resource managers appropriately focus future research and monitoring efforts. This study found that although a proportion of this information can be related directly to the local Tasmanian conditions, there are instances where it may be necessary to verify assumptions locally. There are also many synergies between the areas of environmental concern associated with the use of both antibiotics and copper based antifoulants. In both instances the key issues are environmental fate/ persistence and effects on non-target organisms and ecosystem processes (i.e. ecotoxicity and bioaccumulation). Given that neither antibiotics nor antifoulants are used in isolation, there may be synergistic/ antagonistic effects, and consequently the report recommends combining research efforts wherever possible. Fate of residues was one area where a clear need for further understanding was identified; this included further determination of the effects of antibiotics and antifoulants on sediment processes and vice versa and the development of modelling tools to evaluate environmental pathways/ consequences and help to develop appropriate monitoring/ management strategies.

The analysis of the industry based monitoring were encouraging, suggesting limited bioavailability of metals under current conditions. However, there were several areas of concern not covered in the current monitoring, and some of the results were inconclusive, consequently the report indicated a need for additional research to better understand the



local situation, to develop targeted and appropriate monitoring and management strategies and to ensure environmental sustainability, and suggested some specific areas for further investigation. This study was followed up with a workshop at which government and industry stakeholders and relevant experts discussed the project outcomes and proposed future research.

The project led directly to an FRDC research project looking specifically at the fate of copper-based antifoulants in sediments and how that might impact sediment recovery processes.

The findings of this research have been published in:

- Macleod, C and Eriksen, R, (2011). Antibiotics in Salmonid Aquaculture: Environmental Considerations for Marine Cage Farming
- Eriksen, R and Macleod, C (2011). Antifoulants in Salmonid Aquaculture: Environmental Considerations for Marine Cage Farming In "Fish Farms: Management, Disease Control and the Environment". Andrews, G.L. and Vexton, L.A. (Eds), Nova Publishing Ltd. ISBN: 978-1-61209-538-7

Oh E (2009) **Macroalgal assemblages as indicators of the broad-scale impacts of fish farms on temperate reef habitats**. Honours, University of Tasmania, Hobart, Tasmania

Intensive fish culture in open sea pens can deliver large amounts of nutrients to coastal ecosystems. Sheltered areas with high water quality are predominately chosen for this type of mariculture, and these systems may be adversely affected by the presence of the farms. Since macroalgal community composition has been shown to be a good indicator of environmental disturbance on reef, the present study investigated the effect of salmon farms on macroalgae in a semi-enclosed coastal waterway in southern Tasmania. Data on the macroalgal community were collected from two depths at 44 sites of varying distance from twelve active fish farm leases. This included reference sites at distances of 5 km or more. The sites were widely distributed throughout the study area, and varied in their exposure to wave action. The macroalgal community composition differed significantly between sites at 100 m from fish farms and sites at 5 km or more. Sites at 400 m varied in their response to farms, with some sites showing characteristics similar to 100 m sites. Impacts varied between swell exposed sites and sites only subjected to wind-generated waves. *Chaetomorpha* spp. and *Ulva* spp. were abundant near fish farms at exposed sites, whereas the abundance of filamentous green algae increased at sites near fish farms in sheltered sites. The percentage cover of indicator groups such as epiphytes and opportunistic algae in total provided the best indicators of fish farm impacts on a broad scale. The percent cover of canopy forming perennial algae did not decrease near fish farms indicating that their growth and recruitment has not been greatly affected by high levels of sedimentation from fish farms or prolonged fouling by opportunistic algal epiphytes to the present, however further study is needed to examine this in more detail.

The above analysis utilised photographic quadrats to quantify community composition. Most other broad-scale sampling methods used to measure macroalgal composition require expertise to identify species in situ. However, this reduces the capacity of monitoring programs to collect large amounts of data. Using data collected from a subset of 36 sites, the photographic method was compared with a manual quadrat sampling method. The two methods produced similar multivariate results but manual quadrats had a slightly greater capacity to detect the impacts of the fish farms. This indicates that photographic quadrats are likely to be conservative in quantifying effects of fish farms, but still deliver appropriate resolution to detect major changes in dominant macroalgal cover and composition. Some

adjustments to the photographic methods used will allow better resolution for algae obstructed by canopy or epiphytic overgrowth.

Volkman JK, Thompson P, Herzfeld M, Wild-Allen K, Blackburn S, Macleod C, Swadling K, Foster S, Bonham P, Holdsworth D, Clementson L, Skerratt U, Rosebrock U, Andrewartha J, Revill A (2009) **A whole-of-ecosystem assessment of environmental issues for salmonid aquaculture**. Aquafin CRC Project 42(2)/ FRDC 2004/074. CSIRO Marine and Atmospheric Research, Hobart, Tasmania

This project has established a detailed set of data and provides a good picture of the environmental conditions in the Huon Estuary and D'Entrecasteaux Channel. The findings suggest that environmental conditions are generally good with occasional periods of high phytoplankton abundance and low DO. These data have been used to calibrate and validate a suite of sophisticated 3-dimensional hydrodynamic and biogeochemical models of the region that capture the main physical and biological processes. Specific process studies were carried out to illuminate particular aspects of the nutrient-phytoplankton-zooplankton relationships and sediment biogeochemistry. Technical reports providing the details of these studies are provided separately and are listed below.

A major goal of this research was to design a monitoring program with the capacity to detect the effects of those processes judged to be most threatening to the Huon and D'Entrecasteaux ecosystem at the whole-of-ecosystem level. The monitoring program proposed is designed to provide knowledge of how well the ecosystem is functioning with an increased nutrient load and to allow any significant temporal trend(s) in ecological indicators to be detected. A major challenge was converting indicators of ecological condition into recommended quantitative performance measures that can be used in a regulatory manner to adaptively manage the ecosystem. These recommendations have been extensively discussed with industry and DPIW representatives and refined through an iterative process. The measurements and modelling results obtained indicated that the salmonid industry is a significant contributor of nutrients to this region and that these have led to measurable increases in phytoplankton abundance. As a result, the industry is reaching a size where significant further expansion in the same region could lead to deleterious environmental effects.

The information generated as part of this project has been used by the marine farming planning review board to impose a limit on the salmonid industry in the Huon and D'Entrecasteaux based on the industry's projections for salmon production in 2009. This decision was informed in part by the scenario of likely effects on phytoplankton populations produced by our project using the environmental modelling suite. If the industry is to expand further it will either have to demonstrate that it is not having serious environmental impacts (which can only be achieved by a comprehensive monitoring program of the type suggested by the project), or place new production outside of the Huon Estuary and D'Entrecasteaux Channel or find ways to reduce or ameliorate nutrient inputs (e.g. by polyculture, although this study has suggested that this would not assimilate all the nutrients produced by the salmonid industry).

In summary, this project has generated considerable knowledge of the possible environmental effects of salmonid aquaculture at an ecosystem level and led to the development of the monitoring and modelling tools that can be applied to other fish species or to other regions. The research has been materially assisted by the industry's support for this project and their demonstrated commitment to ensuring the environmental sustainability of their operations. Also, the involvement of staff from the Tasmanian Department and

Primary Industries and Environment (DPIWE, now DPIW) Marine Farming Branch was essential to ensure that our research was relevant to the effective regulation of salmonid farming in Tasmania.

The detailed technical reports that were produced in conjunction with this project are listed below:

- Herzfeld, M. Parslow, J., Sakov, P. & Andrewartha, J. Numerical Hydrodynamic Modelling of the D'Entrecasteaux Channel and Huon Estuary - July 2005
- Thompson, P. & Bonham, P. Effects of grazing by microzooplankton on phytoplankton in the Huon Estuary. CSIRO Marine and Atmospheric Research - July 2005
- Thompson, P., Bonham, P., Willcox, S., Crawford, C. Baseline monitoring in D'Entrecasteaux Channel. CSIRO Marine and Atmospheric Research & Tasmanian Aquaculture and Fisheries Institute, University of Tasmania - July 2005
- Thomson, D., Volkman, J., Burke, C., & Purser, J. Sediment Biogeochemistry of the Huon Estuary – July 2005.
- Wild-Allen, K., Parslow, J., Herzfeld, M., Sakov, P., Andrewartha, J. & Rosebrock, U. Biogeochemical Modelling of the D'Entrecasteaux Channel and Huon Estuary - July 2005
- Volkman, J., Parslow, J., Thompson, P., Herzfeld, M., Wild-Allen, K., Blackburn, S., Crawford, C., Bonham, P., Holdsworth, D., Sakov, P., Andrewartha, J. & Revill, A. System-wide environmental issues for sustainable salmonid aquaculture -January 2006
- Bonham, P., Rousseaux, C. & Thompson, P. Effects of grazing by microzooplankton on phytoplankton in the Huon Estuary and D'Entrecasteaux Channel - June 2008
- Clementson, L., Blackburn, S., Berry, K. & Bonham, P. Temporal and spatial variability in phytoplankton community composition in the mouth of the Huon River Estuary - June 2008
- Clementson, L., Blackburn, S., Thompson, P., Berry, K. & Bonham, P. Phytoplankton community composition during spring blooms in North West Bay and Port Esperance, Tasmania - June 2008
- Herzfeld, M. Numerical Hydrodynamic Modelling of the D'Entrecasteaux Channel and Huon Estuary: Phase II - April 2008
- Holdsworth, D., Revill, A., Volkman, J. & Swadling, K. Lipid biomarkers in sediment traps and sediments from North West Bay, Tasmania - June 2008
- Macleod, C., Revill, A., Volkman, J. & Holdsworth, D. Characterisation of the benthic environment of the D'Entrecasteaux Channel and Huon Estuary - July 2008
- Macleod, C. & Foster, S. Evaluation of selected alternative approaches for monitoring nutrient enrichment associated with caged marine salmonid aquaculture in Tasmania - July 2008
- Revill, A., Holdsworth, D., & Volkman, J. Fluxes of organic matter and lipids to sediments in the Huon estuary - June 2008
- Swadling, K., Macleod, C., Foster, S. & Slotwinski, A. Zooplankton in the Huon Estuary and D'Entrecasteaux Channel: community structure, trophic relationships and role in biogeochemical cycling - July 2008
- Thompson, P., Wild-Allen, K., Macleod, C., Swadling, K., Blackburn, S., Skerratt, J. & Volkman, J. Monitoring the Huon Estuary and D'Entrecasteaux Channel for the Environmental Effects of Finfish Aquaculture - June 2008
- Wild-Allen, K. Huon Estuary and D'Entrecasteaux Channel Biogeochemical Model Scenario Simulations for 2002 and 2009: Farm Impacts on Seasonal Pelagic Biogeochemistry - July 2008

2008

Holdsworth D, Reville AT, Volkman JK, Swadling KM (2008) **Lipid biomarkers in sediment traps and sediments from North West Bay, Tasmania.** Aquafin CRC Project 42(2)/FRDC 2004/074. CSIRO Marine and Atmospheric Research, Hobart, Tasmania

As part of the FRDC project A whole-of-ecosystem assessment of environmental issues for salmonid aquaculture (FRDC number 2004/074; Aquafin CRC project number 4.2(2)), a short-term process study of the ecosystem within North West Bay in the D'Entrecasteaux Channel, Tasmania, was carried out in October 2006. Our primary aim was to investigate the composition of organic material present in the water column during this time and to compare this with the sediments at the same sites in order to identify the main sources of organic matter in this ecosystem. In addition, we wanted to determine if any organic matter of obvious fish-farm origin was detectable and to establish a baseline for comparison with any later studies.

Macleod C, Foster S (2008) **Evaluation of selected alternative approaches for monitoring nutrient enrichment associated with caged marine salmonid aquaculture in Tasmania.** Aquafin CRC Project 42(2)/FRDC 2004/074. Tasmanian Aquaculture and Fisheries Institute, Hobart, Tasmania

Increased nutrient input to a system can affect all aspects of the ecology; but is of particular concern for aquatic plants where it can result in phytoplankton blooms and increased macroalgal growth. Fish farming has the potential to add a significant amount of nutrients to surrounding waters, potentially increasing the susceptibility of the local environment to eutrophication. In south-eastern Tasmania production levels of Atlantic salmon have reached approximately 26,000 tonnes. Assuming that approximately 70% nutrients added as fish feed are released to the environment as metabolic wastes and uneaten feed and that 80% of total nutrient losses are dissolved this equates to approximately 55 kg of dissolved nitrogen per tonne of fish produced (Wu, 1995; Black, 2001). Estimates of nutrient outputs based on mass balance modelling suggest slightly lower outputs of between 35–45 kg dissolved nitrogen per tonne of fish (Sanderson et al., 2008). This suggests that, at current production levels, 900–1,400 tonnes of dissolved nitrogen would be released into the environment and would therefore be readily available to macroalgae, microalgae and other plants such as seagrasses.

Eutrophication may occur if the nutrient assimilation capacity of an area is exceeded, so it is important to understand and be able to monitor the nutrient status of the ecosystem. In Phase 1 of this project, seasonal monitoring of intertidal macroalgal composition suggested that macroalgal communities responded to changes in nutrient regimes and could provide an integrated assessment of nutrient inputs, but that seasonal variation in abundance and community composition of natural populations would limit their potential to be used as a simple monitoring tools for eutrophication. Monitoring natural populations is problematic as growth rates and accumulation levels may be confounded by previous exposures.

The primary aim of this component of the project was to identify and evaluate simple approaches for monitoring nutrients in the water column. Direct measurement of water column nutrients is time consuming and expensive and only provides information about the condition at the point in time that the sampling was undertaken rather than its biological consequences. Increases in algal growth can be a useful indicator of the integrated effect of changing nutrient loads over any given time period. Settlement plates were assessed as a simple option to evaluate differences in algal as well as settlement communities, as they also address the inherent variability associated with natural communities by providing a standardized starting point for monitoring and assessment. Settlement plates have been

used successfully to evaluate species recruitment (Walters and Wethey, 1996; Marshall et al., 2003), to monitor species introductions (Hewitt and Martin, 2001) and to evaluate environmental impacts (Glasby, 1998). Plates seeded with algae can provide a common benchmark from which to monitor change in algal growth rates, whilst evaluation of the entire community settling on clear plates will provide information on the differences in species tolerances and growth rates associated with different impacts and nutrient loading regimes.

Macleod C, Reville A, Volkman J, Holdsworth D (2008) **Characterisation of the benthic environment of the D'Entrecasteaux Channel and Huon Estuary**. Aquafin CRC Project 42(2)/ FRDC 2004/074

This study sought to examine the spatial variability in sediment characteristics within the D'Entrecasteaux Channel, North West Bay and Huon Estuary and to describe how this might affect nutrient assimilation rates. This component of the study sampled sediments from throughout the D'Entrecasteaux Channel and combined these data with information already available on the Huon Estuary to characterise the physical properties of the sediment as well as the infaunal communities. The study then used mesocosms to examine differences in nutrient assimilation rates associated with the main benthic ecosystems. Mesocosms allow the study of dynamic properties of communities that could take decades to study in the field (Daehler and Strong, 1996). In mesocosms it is possible to control environmental variables thus eliminating or greatly reducing confounding factors. Furthermore, mesocosms enable better replication than is typically possible in field studies. This information could then be used to inform the management decisions made by industry and regulators to ensure that aquaculture operations are carried out in the most appropriate areas.

Macleod CK, Moltschaniwskyj NA, Crawford CM (2008) **Ecological and functional changes associated with long-term recovery from organic enrichment**. Mar Ecol Prog Ser 365:17-24

The recovery of a soft sediment benthic invertebrate community from high levels of organic enrichment was evaluated after removal of farmed fish at an Atlantic salmon *Salmo salar* culture site in southeast Tasmania. Although the pattern of recovery followed established successional principles, after 36 months neither communities under or at the edge of cages, nor communities 10 m from the edge of the cage, had attained a structure equivalent to that of the reference communities. In the first few months there was little evidence of recovery and the community was dominated by species characteristic of impacted conditions. After 12 months the system had markedly improved, containing a diverse range of species with functional roles similar to those of the reference sites. Once the ecological function of the sediment was restored, subsequent changes in the community structure were relatively minor, simply reflecting the addition of rarer species with longer reproductive cycles and/or larval stages with a greater sensitivity to the negative effects of organic enrichment. These species may be significant where specific community changes, loss of diversity or the possibility of species extinction are the critical issues. However, restoration of system function may be a more useful indicator of generalized recovery from organic enrichment than of community equivalence.

Reville AT, Holdsworth DG, Volkman JK, Swadling K (2008) **Fluxes of organic matter and lipids to sediments in the Huon estuary**. Aquafin CRC Project 42(2)/ FRDC 2004/074. CSIRO, Hobart, Tasmania

As part of the Huon estuary process studies carried out in April and September 2005 for the FRDC project 2004/074 (Aquafin CRC project number 4.2(2)) entitled "A whole-of ecosystem

assessment of environmental issues for salmonid aquaculture”, we deployed sediment traps at two sites in the Huon Estuary (Fig. 1; Table 1). The primary aim was to investigate the composition of material being deposited from the water column to the sediments at these times and to compare the composition of this organic matter with that found in the sediments at the same sites. In particular, we wished to see whether organic matter of fish-farm origin could be detected and whether there were major changes in seasonal fluxes of algal and terrestrial organic matter.

Swadling KM, Macleod CK, Foster S, Slotwinski AS (2008) **Zooplankton in the Huon Estuary and D'Entrecasteaux Channel: community structure, trophic relationships and role in biogeochemical cycling**. Aquafin CRC Project 42(2)/ FRDC 2004/074

As part of the FRDC project entitled A whole-of-ecosystem assessment of environmental issues for salmonid aquaculture (FRDC number 2004/074; Aquafin CRC project number 4.2(2)), studies of mesozooplankton were initiated in 2004 when it was recognised that they play a critical role in nutrient cycling within the pelagic environment. The inclusion of zooplankton into the Aquafin CRC study began in the second phase of the project with the commencement of regular sampling for mesozooplankton in October 2004 and grazing studies in September 2005.

Mesozooplankton are organisms within the range of 200 µm to 2 mm (Sieburth, 1978), and typically include small crustaceans and larval stages of pelagic and benthic animals such as molluscs and polychaetes, along with specialist groups including arrow worms and jellyfish. In this report we outline the dynamics of this zooplankton community over an annual cycle and highlight specific experimental studies designed to determine their grazing impacts. We also include information on the role that mesozooplankton play in carbon flux, determined via material collected in sediment traps.

Thompson P, Wild-Allen K, Macleod C, Swadling K, Blackburn S, Skerratt J, Volkman J (2008) **Monitoring the Huon Estuary and D'Entrecasteaux Channel for the Environmental Effects of Finfish Aquaculture**. Aquafin CRC Project 42(2)/ FRDC 2004/074. Tasmanian Aquaculture and Fisheries, Hobart, Tasmania

The D'Entrecasteaux Channel is naturally oligotrophic with strong trophic coupling and nutrient recycling processes that result in very few algal blooms. In contrast, the Huon Estuary is currently mesotrophic with increased primary production and some uncoupling between trophic levels resulting in increased frequency and density of algal blooms. The most significant threats to the ecological functioning of the D'Entrecasteaux Channel and the Huon Estuary ecosystem are further increases of organic matter and nutrient inputs (eutrophication). The most common symptoms of eutrophication include increased nutrient concentrations, trophic decoupling, increased algal blooms, hypoxia, changes in nutrient cycling, reductions in water clarity, eventual loss of benthic macroflora, changes in food web structure and organic enrichment of the bottom waters and sediments, and/or changes in benthic fauna and microbial processes. The parameters that might be used to monitor for these symptoms or effects of eutrophication were assessed for their suitability using criteria that define a 'good' indicator (Maher et al., 1994). Initially a very comprehensive list of parameters was considered but many were discarded as unsuitable. The final list of parameters is compatible with similar monitoring programs in Australia (e.g. ANZECC, 2000) and worldwide. The reduced list of parameters was subjected to risk assessment (after Gavine and McKinnon, 2002; Fletcher et al., 2004). The risk assessment considers both the likelihood (almost certain to none) and consequence (insignificant to catastrophic) of a

change in ecosystem functioning, with the potential for harm used to estimate risk. Of all the parameters considered low dissolved oxygen (DO) and changes in trophic structure were identified as 'high risk' and most in need of monitoring. Based on recent measurements in the Huon Estuary low DO concentrations are 'likely' ('the event will probably occur approximately once per year') and the consequences of low DO were considered 'major' (recovery to take many years). Therefore low dissolved oxygen is a high risk with three identifiable components: concentration, spatial extent and temporal extent. Changes in trophic coupling, manifested as algal blooms and changes in phytoplankton community composition, were also rated as a high risk and consequently they are an important component of the proposed ecological monitoring. It is likely that existing or proposed ecosystem uses will have both positive and negative impacts on the local populations of various plants and animals. Many of these impacts would be difficult to quantify given the natural variability of these populations over time and in space (low signal:noise ratio). Our major goal was to design a monitoring program with the capacity to detect the effects of those processes judged to be most threatening to Huon and D'Entrecasteaux ecosystem at the whole of ecosystem level. The monitoring program proposed here is designed to provide knowledge of how well the ecosystem is functioning with an increased nutrient load and to allow any significant temporal trend(s) to be detected. We believe that a major improvement in our proposed program, relative to more conventional programs, is the application of modelling to focus monitoring on the areas that are most susceptible to eutrophication. Within any monitoring program there is the potential for some localized impacts on ecosystem functioning or populations to be undetected. Very localized impacts are classified as 'minor' (Fletcher et al., 2004) and are not likely to cause a significant change to ecosystem functioning. One notable exception is the initial colonization by new invasive species. New arrivals such as *Gymnodinium catenatum*, *Noctiluca scintillans*, Northern Pacific sea star (*Asterias amurensis*), wakame (*Undaria pinnatifida*), and New Zealand screw shell (*Maoriculpus roseus*), have all had significant impacts on this ecosystem. Therefore any use of the ecosystem that facilitates colonization by pest species, particularly introduced pests, should be minimized. Very localized reductions in populations of many marine organisms are not considered a major risk to the ecology because of their broadcast spawning, high fecundity and lack of strongly differentiated genotypes at small spatial scales (Elliott et al., 2002).

Along with localized changes there are two other types of environmental impacts not well monitored by the proposed plan. One of these is the possible ecological impacts of aquaculture on rare species. Users of any natural resource have an obligation to ensure their activities do not adversely impact on populations of plants or animals known to be endangered, threatened or protected by Local, State, Commonwealth or international law, treaty or convention. It is recommended that a separate review of possible impacts on endangered, threatened or protected species be undertaken. Second, the proposed plan does not monitor for impacts possibly caused by some other users of the marine environment including contaminant disposal (for example: CO<sub>2</sub>, sewage, pesticides, herbicides, solid wastes, litter) and the relevant authorities may wish to consider expanding this or other monitoring programs to include these indicators. A major challenge was the development of baselines and trigger values for the selected parameters; in order to convert the science into a usable management tool. A thorough review of existing data and high resolution biogeochemical modelling was used to estimate baselines and trigger values. The modelling was also used to optimise site selection reducing the cost of monitoring and increasing its sensitivity. The trigger values are performance measures that the ecosystem

must meet. There are a series of trigger values designed around the proposed monitoring that incorporate the 3 dimensions of risk (intensity, duration and spatial extent). If the monitoring indicates the ecosystem does not meet a particular trigger level then a series of management actions are recommended. Extensive consultation with stakeholders in the regulatory and industrial sectors indicates uptake is very likely with implementation possible in 2008/9. We believe the major improvements in our proposed monitoring program include lower cost and more robust monitoring with an explicit incorporation of risk and direct links to recommended management actions.

2007

Bissett A, Burke C, Cook PL, Bowman JP (2007) **Bacterial community shifts in organically perturbed sediments.** *Environ Microbiol* 9:46-60

Bacterial abundance, diversity and sediment function were investigated in organically perturbed sediments under Tasmanian salmon (*Salmo salar*) farms and adjacent reference sites. Bacterial numbers increased as farming and organic loading progressed through the farm stocking cycle and declined during the fallow period, although not to prestocking levels. Bacterial numbers ranged between approximately  $2 \times 10^8$  and  $3 \times 10^9$  cells per gram of sediment and were higher at cage sites than reference sites. Microelectrode and respiration data also demonstrated a clear effect of organic loading on sediments. Denaturing gradient gel electrophoresis (DGGE) showed that bacterial communities shifted both in response to farm loading and its cessation. A seasonal effect on microbial communities was also evident. Although bacterial communities did shift again during the fallowing period, this shift was not necessarily a return to preloading communities. The complexity of community shifts may be affected by the vast functional redundancy of bacterial groups. All bacterial communities, including those at reference sites, were highly dynamic. Respiration studies of amended sediments indicated that fish farm sediments were at least as resilient and diverse as reference site communities. The results of this study indicate that the functional redundancy of highly complex bacterial communities contributes to their robustness. The relationship between diversity and stability in bacterial communities remains unclear and requires further investigation before an understanding of bacterial response to perturbation is possible.

Macleod CK, Moltschaniwskyj NA, Crawford CM, Forbes SE (2007) **Biological recovery from organic enrichment: some systems cope better than others.** *Mar Ecol Prog Ser* 342:41-53

This study examined the short-term recovery response at 2 salmon farms in southern Tasmania where organic loads were equivalent, but where background environmental conditions differed markedly. Although benthic communities at each of the farm locations showed good recovery over the 3 mo fallow period, community structure did not fully return to that observed under reference conditions at either location. At the Stringers Cove site the primary ecological functions of the background community were restored, but this was not the case at Creeses Mistake. These differences in recovery response were a direct reflection of background environmental conditions. Stringers Cove sediments had naturally high organic carbon content and as a result there was greater similarity in the ecological function of the unimpacted and impacted conditions at this location than at Creeses Mistake, where, under unimpacted conditions, the sediments had a very low organic content. In addition, the background fauna at Stringers Cove contained several species with reproductive strategies suited to rapid recruitment and well adapted for early recolonisation in organically enriched sediments. In contrast, the background fauna at Creeses Mistake not only changed more



with the impacts of organic enrichment, but was less able to re-establish populations directly by immigration, needing to rely to a greater extent on remediation of the sediments by transitional species before being able to colonise. This has important implications for environmental management, as it suggests that the sediments in some areas have greater resilience to organic inputs.

2006

Bissett A, Bowman J, Burke C (2006) **Bacterial diversity in organically-enriched fish farm sediments.** FEMS Microbiol Ecol 55:48-56

The bacterial diversity and community structure within both organically enriched and adjacent, unimpacted, near-shore marine sediments at two fish farms in southern Tasmania, Australia, was examined using 16S rRNA gene clone library construction and analysis. Sediments at both caged and reference sites at both farms showed a very high level of microbial diversity. Over 900 clones were analysed and grouped into 631 unique phylotypes. Reference sites were dominated by Delta- and Gammaproteobacteria and the Cytophaga-Flavobacteria-Bacteroides group. Cage site sediments were also dominated by these phylotypes, as well as members of the Alpha- and Epsilonproteobacteria. Diversity and coverage indices indicated that the actual diversity of the sediments was much greater than that detected, despite a large sampling effort. All libraries were shown to be statistically different from one another (Po0.05). Many phylotypes did not group with cultured bacteria, but grouped with other environmental clones from a wide array of marine benthic environments. Diversity and evenness indices suggested that although both parameters changed after farming, diverse communities were present in all sediments. The response of the microbial community to organic load suggested that random, rather than predictable, succession events determine community composition and diversity, and that sediment type may influence bacterial community and sediment response to organic perturbation.

Crawford C, Thompson P, Jordan A, Foster S, Mitchell I, Bonham P, Willcox S (2006) **Development of broad scale environmental monitoring and baseline surveys in relation to sustainable salmon aquaculture in the D'Entrecasteaux Channel region.**

Aquafin CRC Project 441. Tasmanian Aquaculture and Fisheries Institute, Hobart, Tasmania

This study aimed to:

- (i) conduct interim monitoring in the Huon estuary,
- (ii) to collect relevant biological data for the characterisation and modelling of the D'Entrecasteaux Channel, and
- (iii) to investigate cost effective and scientifically rigorous methods for broad-scale monitoring of the environmental effects of salmon farming in the Huon Estuary and D'Entrecasteaux Channel.

Interim monitoring in the Huon Estuary using a suite of environmental variables (temperature, salinity, dissolved oxygen, turbidity, nutrients, chlorophyll a and microalgal species composition and abundance) indicated no deterioration in the health of the estuary since the Huon Estuary Study (HES) in 1997-98. Nevertheless, lower dissolved oxygen levels and increased ammonium concentrations observed in the deeper basin of the lower estuary and Port Cygnet in the summer-autumn period of 2002-03, similar to measurements taken in 1996-97 during the HES, suggest that this region of the estuary has the highest risk of degradation. Although average values for dissolved oxygen and ammonium over twelve months were within acceptable limits, it is the rare extreme events that have the potential to markedly impact on the system. As a consequence, it is recommended that the salmon

industry monitor the dissolved oxygen levels in bottom waters of the lower estuary weekly during summer and autumn.

The baseline monitoring conducted in the D'Entrecasteaux Channel over 16 months has provided important baseline data on the health of this region. The environmental variables monitored (temperature, salinity, dissolved oxygen, turbidity, nutrients, chlorophyll a and microalgal species composition and abundance) did not show any signs that could be considered indicative of eutrophication or human-induced nutrient inputs. For example, chlorophyll a values were all <2, which is low by international standards for chlorophyll a. These data collected during the baseline monitoring have been/are important to the development and calibration of a hydrodynamic model for the Channel. They suggest that the Channel waters are naturally nutrient poor and thus susceptible to ecological change if the nutrient concentrations increase. Sites with low flushing rates, such as North West Bay and Barnes Bay, are the most vulnerable to additional nutrient loading.

An investigation of suitable biological indicators for monitoring the broad-scale effects of salmon aquaculture also showed that there were no obvious impacts of salmon farming in the region. This research has shown that monitoring intertidal macroalgae, especially a few dominant species, is considered to be a simple, cost effective method of investigating nutrient impact. It is also recommended that the macroalgal data collected as part of the ecological assessment of two marine protected areas in the D'Entrecasteaux Channel are assessed every 2-5 years in relation to potential increase in nutrients from salmon farms. (The collection of these data is funded through other research programs). However, monitoring seagrass is not considered to be an informative, cost effective monitoring method because of the limited areas of seagrass in the region and the natural fluctuation in abundance of the dominant seagrass species.

Macleod C (2006) **The Recovery of Benthic Communities Following Organic Enrichment: Examples from Caged Finfish Aquaculture**. Doctor of Philosophy, University of Tasmania, Hobart, Tasmania

Together the results of the present study have increased our understanding of the recovery processes associated with organic enrichment in southern temperate regions and indicate that, since recovery response differs depending on the background environmental conditions, establishment of baseline conditions and local benchmarks is essential in evaluation of impact and recovery, for establishment of a regulatory framework and for ongoing environmental management. However, these baselines and the subsequent management protocols must be established at a spatial scale relevant to the community (ecological) changes.

Macleod CK, Moltschaniwskyj NA, Crawford CM (2006) **Evaluation of short-term fallowing as a strategy for the management of recurring organic enrichment under salmon cages**. Mar Pollut Bull 52:1458-1466

Rotation of cages within fish farm leases and the subsequent fallowing of areas of seabed is commonly used to allow recovery of infaunal communities following periods of organic enrichment. To investigate the effect of different background environmental conditions on recovery response, two Atlantic salmon (*Salmo salar*) fish farm sites in southeast Tasmania were sampled over two commercial fallowing cycles. Despite similar stocking levels and feed input there were significant differences in the way in which sediment at each farm responded to the cessation of fish stocking. Sediments at both farms showed some improvement in the community structure over a three month fallow period, but the community structure only

recovered to that present before stocking not to that at the reference sites. The similarity of the impact sites to the reference sites increased from ca. 25% to 31% at one site and 11% to 27% at the other after fallowing. Rate and extent of recovery were affected by farm location, initial impact of the sediments, and length of fallow period. Initial recovery was faster at the more sheltered site than at the more exposed site, possibly reflecting differences in environmental resilience with the more sheltered location better able to assimilate organic inputs. Accordingly general fallowing management protocols may need to be adapted to reflect differences between sites. The findings of this study suggest that the recovery response of benthic communities can be predicted once baseline conditions are understood.

Volkman JK, Parslow J, Thompson P, Herzfeld M, Wild-Allen K, Blackburn S, Crawford C, Bonham P, Holdsworth D, Sakov P, Andrewartha JR, Reville A (2006) **System-wide environmental issues for sustainable salmonid aquaculture**. Aquafin CRC Project 42/FRDC 2001/097. CSIRO Marine and Atmospheric Research, Hobart, Tasmania  
 This report covers the work of project CRC 4.2 (FRDC 2001-097). This is part of a 7 year program which will be completed through project CRC 4.2(2) (FRDC 2004-074) by mid-2008. By agreement with Aquafin CRC and FRDC, the current project is presented as an Interim Report, comprising an overview of results and discussion, together with a non-technical summary. Six technical reports have also been prepared, providing detailed methods and results as well as animations of some of the modelling results which are included on the accompanying CD. The program will be reported as a whole, including benefits, adoption and outcomes, at the end of the follow on project in 2008.

2005

Edgar G, Macleod C, Mawbey RB, Shields D (2005) **Broad Scale Effects of Salmonid Aquaculture on Macrobenthos and the Sediment Environment in southeastern Tasmania**. J Exp Mar Biol Ecol 372:70-90

This study investigated several key biotic and abiotic metrics recommended in previous studies for assessing environmental impacts of fish farm waste to determine those most effective for discriminating fish farm impacts at different distances from cages, with emphasis on those able to distinguish more distant effects. The study compared sediments and associated macrobenthos at sites sampled within 20 fish farm leases distributed across southeastern Tasmania and identified major natural changes along a regional cline. Introduced taxa were strongly represented in the fauna, comprising 45% of total macrofaunal biomass. Large differences were evident between sites affected by different levels of organic farm waste. Sites located adjacent (<10 m) to farm cages had significantly depressed sediment redox levels, a dominance of opportunistic (capitellid and dorvilleid) polychaetes, and low macrofaunal species richness. Subtle impacts extended across farm lease areas in the form of depressed redox potential at 40 mm depth and consistent changes to the macrobenthic community. Minor farm effects were also evident at sites sampled 35 m outside farm lease boundaries, most notably as elevated population numbers of the polychaete *Terrebellides* sp., bivalve *Mysella donaciformis* and heart urchin *Echinocardium cordatum*. Amongst the univariate metrics examined, redox potential at 40 mm depth and the ratio of bivalves to total molluscs provided the most sensitive indicators of farm impacts, with the latter metric relatively insensitive to spatial variation between locations within the region studied.

Macleod C, Helidoniotis F (2005) **Ecological status of the Derwent and Huon Estuaries.** NHT/NAP Project No 46928, Tasmanian Aquaculture and Fisheries Institute, Hobart, Tasmania

This study was undertaken to provide baseline information on the ecology of the two main estuaries in southern Tasmania with the intention of assisting in the ongoing management of these systems. The Derwent and Huon estuaries are similar in their biogeographical, climatic and physical characteristics but differ markedly in their levels of industrialisation and impacts. The Derwent Estuary is highly impacted with several major industrial and urban contamination sources throughout its catchment. Heavy metals in particular, represent a considerable threat to the system. In contrast the Huon Estuary is largely unimpacted, finfish aquaculture being the only major industry affecting the system. Both the Derwent and the Huon estuaries are strongly depositional with the majority of the estuaries composed of soft sediments. Information on the benthic ecology is an essential pre-requisite to any effective management strategy. Accordingly, baseline information on the current biological condition of these systems was collected.

This study found similarities in the pattern of community distribution along the estuaries. In neither system were there any areas where fauna was completely absent, in fact diversity was high throughout most of the sample sites. The faunal community in both estuaries was most strongly related to the natural geomorphology and salinity gradient of the estuaries, and in turn to the depositional character of the system and the organic content. Changes throughout the estuaries were gradual but several discrete communities were identified within each estuary and the species which most strongly characterised these communities are described. The community distribution in the Derwent was slightly more complex than the Huon, as in addition to the natural gradient there were also multiple anthropogenic gradients. Prior to this study it was anticipated that the extremely high concentrations of metals throughout the Derwent would be the most significant structuring influence on the ecology of the system. However, the results show that metal contamination was not the overriding determinant of benthic infaunal community composition, although both organic enrichment and metal contamination had a significant influence on the community structure in localised areas.

The biological information provides an important resource for researchers and managers. It represents a baseline ecological reference for any future assessment of sediment condition and ecological status. The study defines species indicative of communities characterising particular regions within the system. These biological zonation patterns will enable environmental managers to locate biologically relevant monitoring sites and to evaluate improvements and deterioration in the estuarine condition both spatially and temporally. This study mapped the current status of invasive species in both estuaries providing a point of reference to monitor and manage their spread and distribution. Examination of the environmental preferences of these species suggests that in general their environmental tolerances are broad and that range extension is more likely to result from opportunity and reduction in competitive pressure rather than any particular environmental preference. This has significant implications for the management of these ecologically and potentially economically important species.

Although this study was specifically focussed on the Derwent and Huon estuaries, the findings can be applied in a much broader spatial context. Comparison of the faunal information for the lower estuary regions with other local studies suggests that the community characterisation would be applicable to other estuaries in south-eastern Tasmania. The general response of the main species and infaunal community groups to

metal contamination could potentially be applicable throughout southern temperate Australia, whilst the functional community response and invasive species information has even wider application.

Thompson D, Volkman J, Burke C, Purser J (2005) **Sediment Biogeochemistry of the Huon Estuary**. Aquafin CRC Project 42/ FRDC 2001/097. CSIRO Marine and Atmospheric Research, Hobart, Tasmania

Sediments play a vital role in the ecological functioning of an estuary by retaining much of the organic matter and minerals supplied naturally by rivers, catchment run-off and inputs from the overlying water column. Concentrations of metals and organic compounds are often several orders of magnitude higher in the sediments than in the overlying water column, thus simplifying their analysis. Surface sediments provide an integrated picture of inputs over relatively short time frames of a few years. Hence, they can give an indication of local inputs in the context of an estuary-wide baseline and provide a better view of longer term average inputs, in contrast with the snapshots revealed by water column studies. Sediment cores provide a record of estuarine conditions over years to decades and longer depending on the sedimentation rate. Gradients of nutrients and oxygen in porewaters with depth can provide estimates of the fluxes of solutes into or out of the sediment.

The organic matter in sediments is remineralised by the microbial and faunal populations present thus liberating nutrients and consuming oxygen. Sediment organic matter shows varying degrees of resistance to degradation. It is common practice to define a refractory component (i.e. that part of the organic matter that is not degradable over a defined time frame usually months to years) and a labile component that is degraded over hours to days. The quality of organic matter (as roughly reflected in the C:N ratio) is a prime determinant of the rates and recycling pathways of carbon and nitrogen (Herbert 1999) in sediments. Where organic matter undergoing decomposition has a high C:N ratio (such as that from terrestrial plant sources), much of the nitrogen remineralised may be reassimilated into microbial biomass (Schlesinger 1997). Organic matter that is more labile and has a lower C:N ratio (such as that derived from algal material) will stimulate rapid remineralisation rates and a release of nitrogen from the sediment (Hansen and Blackburn 1992). Identifying these sources of organic matter in sediments provides an insight as to how the organic matter is likely to be processed. A C:N ratio close to that of the Redfield ratio (6.7) is indicative of organic matter derived from marine microalgae while organic matter derived from terrestrial sources can have a C:N ratio of 20 or more (Bordovskiy 1965). This approach is, however, very approximate as degradation may significantly alter these ratios (Thornton and McManus 1994).

Ratios of  $^{12}\text{C}/^{13}\text{C}$  isotopes provide a good estimate of the relative contribution of terrestrial and marine sources to sedimentary OM (Fry and Sherr, 1984). Terrestrial OM (largely of higher plant origin) will generally have a  $\delta^{13}\text{C}$  value of -26 to -30‰ and organic matter with a marine origin will generally have a  $\delta^{13}\text{C}$  of -19‰ to -23‰ depending on the particular organisms present (Heip et al., 1995). The relative proportion of marine and terrestrial carbon in a sample can then be estimated by linear additions of these end-members. While this approach is relatively simple and gives an integrated estimate of sources for the total carbon in the sample, it will only provide useful information when there are two well-defined end-members. Furthermore, this technique gives little information about the type of marine or terrestrial organic matter in question.

Various proxies have been used to estimate the sources of organic matter in sediments and from this inferences about the amount of labile organic matter present can be made. For example, biochemicals such as carbohydrates, proteins and lipids are rapidly degraded in sediments and so measures of their abundance provide an estimate of the labile organic matter present (e.g. Pusceddu et al, 1999). Alternatively, lipid and pigment biomarkers can allow the various sources of various sub-fractions of organic matter to be identified. Fatty acids provide a range of useful markers for microalgae, macroalgae, bacteria, seagrasses and terrestrial plants (Volkman et al. 1980, Meziane et al. 1997, Volkman et al. 1998, Kharlamenko et al. 2001). Sterols have also been used to identify sources of organic matter including that derived from faeces, diatoms and terrestrial sources (Volkman 1986, Barrett et al. 1995). Triterpenoid alcohols such as  $\alpha$  and  $\beta$ -amyrins, lupeol, taraxasterol, betulin etc. are widely used as markers for higher plants (e.g. Volkman et al. 1987, 2000), even though some of these have additional minor sources (Volkman 2005). Hopanoid alcohols are excellent markers for cyanobacteria and other prokaryotes (Summons et al. 1999). While these methods are useful, they are often time consuming and they still only provide an indirect measure of labile organic matter. Accordingly, we attempted to develop a simple procedure based on the biochemical oxygen demand (BOD) incubation technique (see later) for determining labile organic matter in sediments.

Sediment can also be remobilised by tidal currents or during high energy events such as floods thus changing the benthic characteristics of a particular region. Cycles of resuspension and deposition can lead to enhanced remineralisation as the particle surfaces are repeatedly exposed to oxidising conditions (e.g. Abril et al., 1999).

Thompson P, Bonham P, Willcox S, Crawford C (2005) **Baseline monitoring in D'Entrecasteaux Channel**. Aquafin CRC Project 42/ FRDC 2001/097. CSIRO Marine and Atmospheric Research, Hobart, Tasmania

None of the stations sampled in D'Entrecasteaux Channel during the sixteen months from January 2002 to March 2003 showed any significant signs that would be considered indications of serious eutrophication or anthropogenic nutrient inputs. The commonly accepted primary symptoms of eutrophication include hypoxia, anoxia, excessive phytoplankton blooms and high ambient nutrient concentrations (Gray 1992). For example, all of the sites sampled had mean annual chlorophyll a concentrations less than the 2  $\mu\text{g L}^{-1}$  that is an internationally accepted criterion for oligotrophic waters. Some sites, however, were better or worse than others. The site judged to be in the best environmental health was Little Taylors Bay with a relatively short flushing time, high dissolved oxygen, low ammonium and low chlorophyll a. In contrast both NW Bay and Barnes Bay had greater  $\text{NH}_4$  and chlorophyll a concentrations than most other sites. NW Bay is showing modest but early symptoms of eutrophication and seems to maintain a reasonably good health primarily due to its short flushing time. Barnes Bay is also reasonably healthy but its relatively slow flushing time suggests this Bay would be more susceptible to eutrophication than most other embayments in D'Entrecasteaux Channel.

2004

Macleod C, Bissett A, Burke C, Forbes S, Holdsworth D, Nichols P, Reville A, Volkman J (2004) **Development of Novel Methods for the Assessment of Sediment Condition and determination of management protocols for sustainable finfish cage aquaculture operations**. Aquafin CRC Project 41/ FRDC 2000/164. Tasmanian Aquaculture and Fisheries Institute, Hobart, Tasmania

Organic enrichment of the sediments is one of the most significant impacts from caged fish farming. However, the effect that differing farming practices, such as rotational farming/fallowing, have on the level of impact, or the effect that different background environmental conditions may have on overall impact was less clearly understood. This project was initiated to assess the rate of recovery associated with fallowing practices, to determine if current farming practices were sustainable and to identify indicators of sediment degradation and recovery that could be used by farmers to gauge the environmental status of the sediments within their lease and make appropriate management decisions.

This research showed that although finfish aquaculture significantly affected the sediments under the cages, it was possible to manage sediment conditions to ensure that ongoing farming can be sustained. The research found a clear relationship between farm management practices and level of impact, and identified 9 distinct stages of sediment condition that could be used to enable farmers to easily classify sediment condition.

Several established environmental monitoring approaches were found to be poor indicators of sediment recovery, although useful measures of sediment degradation. Semi-quantitative video assessment was determined to be the most effective approach for simple farm-based assessment of sediment condition. When linked with farm data, the condition of a lease can be reviewed in a management context and informed management actions undertaken.

Furthermore, when video footage is assessed with farm data it is possible to categorise the sediment condition to a particular stage and also predict the likely future classification on the basis of the proposed farming schedule. If there is any uncertainty as to the classification resulting from the visual assessment, the findings could be validated with infaunal grabs and subsequent evaluation of key species. Other approaches (e.g. redox/sulphide, signature lipid analysis, microbial status) can be undertaken if a greater sensitivity or understanding of the system processes is required. It is important to note that the proposed protocols were developed specifically in relation to on-farm monitoring, and were not intended for regulatory or compliance purposes although the findings have informed ongoing monitoring.

A number of publications are associated with this project:

- Macleod, C.K., Moltshaniwskyj, N.A., Crawford, C.M. and Forbes, S.E. (2007) Biological Recovery from Organic Enrichment associated with Finfish Cage Aquaculture: Some Systems Cope Better than Others. *Marine Ecology Progress Series* 342: 41-53.
- Macleod, C.K., Moltshaniwskyj, N.A. and Crawford, C.M. (2006) Evaluation of short-term fallowing as a strategy for management of recurring organic enrichment under salmon cages. *Marine Pollution Bulletin* 52. 1458-1466.
- Bissett, A., Cook, P. L. M., Macleod, C., Bowman, J. P., Burke, C. (2009) Effects of organic perturbation on marine sediment betaproteobacterial ammonia oxidizers and on benthic nitrogen biogeochemistry. *Marine Ecology Progress Series* 392. 17-32

Macleod C, Forbes S (2004) **Guide to the assessment of sediment condition at marine finfish farms in Tasmania**. Aquafin CRC Project 41 (Extension). Tasmanian Aquaculture and Fisheries Institute, Hobart, Tasmania

The application of the recommended techniques is fully described in an interactive farm field guide that was developed as an extension of this project. The field guide & associated training fully explains the sampling procedures and analytical techniques underpinning the visual assessments (Macleod et al., 2004). The research undertaken as part of the Aquafin CRC has significantly informed the understanding of environmental interactions with salmon farming.

Macleod CK, Crawford CM, Moltschaniwskyj NA (2004) **Assessment of long term change in sediment condition after organic enrichment: defining recovery.** Mar Pollut Bull 49:79-88

Sediment condition at an Atlantic salmon (*Salmo salar*) culture site in S.E. Tasmania, Australia was evaluated to determine the rate and extent of recovery after removal of farmed fish. By local standards the cage sediment at the start of this survey was markedly degraded but comparison with results from impact studies in Scotland, Canada and Norway suggests that the sediments were considerably less impacted than in northern temperate areas. The impact at the cages diminished rapidly with both time and distance; after only 2 months conditions were markedly improved. The macrobenthos indicated a slower recovery than chemical measures, after 36 months the benthic faunal community structure under the cages still differed from reference conditions even though other sediment measures had recovered. This study highlighted two other key issues in relation to monitoring and management of sediment recovery. First, techniques used to determine impact may not be appropriate for evaluation of recovery. Second, establishment of local baseline standards is extremely important to ensure appropriate evaluation of both impact and recovery.

2003

Crawford C (2003) **Environmental management of marine aquaculture in Tasmania, Australia.** Aquaculture 226:129-138

Marine farming is an important rural industry in coastal bays and estuaries of Tasmania. The two main species cultured are the introduced Pacific oyster, *Crassostrea gigas*, and Atlantic salmon, *Salmo salar*. Legislation has been introduced to assist the development of aquaculture, and this includes requirements for environmental management, such as baseline assessments and routine monitoring of leases. Local impacts on the seabed around salmon farms are monitored using video footage, analysis of benthic invertebrate infauna, and chemical measures (redox and organic matter). Monitoring of shellfish farms is minimal because our research has shown that shellfish culture is having little impact on the environment. Research related to management of aquaculture wastes is ongoing. Studies include investigating appropriate inexpensive measures for an industry-wide long-term monitoring program. Mitigation measures against excessive loadings of organic matter from fish farms, mainly by fallowing, i.e. rotating the position of fish pens around a lease, are currently being researched. Rates of recovery of a heavily impacted salmon lease area after the removal of fish have also been studied. A new project is investigating system-wide effects of salmon farming on the environment, in particular, increased release of nutrients into waterways. This includes monitoring dissolved oxygen, nutrients and phytoplankton, modelling the system, and investigating ecological indicators of eutrophication.

Wildish DJ, Hargrave BT, MacLeod C, Crawford C (2003) **Detection of organic enrichment near finfish net-pens by sediment profile imaging at SCUBA-accessible depths.** J Exp Mar Biol Ecol 285:403-413

In 2002 Dr David Wildish, an internationally renowned environmental researcher from Canada with particular expertise in the impacts of mariculture visited Tasmania. He collaborated on a project to assess the viability of using sediment profile imagery as a means to monitor salmon farm impacts. The findings indicated that, although SPI was very effective at detecting organic enrichment, the application was limited to soft sediments and there were technical difficulties that would need to be resolved before it could be generally applied in the Tasmanian context



2002

Crawford C, Macleod C, Mitchell I (2002) **Evaluation of techniques for environmental monitoring of salmon farms in Tasmania**. Technical Report Series. Tasmanian Aquaculture & Fisheries Institute, Hobart, Tasmania

This study was commissioned by the Tasmanian government (DPIW) to assess several environmental variables/techniques for their suitability (i.e. are practicable, inexpensive, and scientifically credible) as indicators of organic enrichment from salmon farms and for inclusion in an industry wide monitoring program.

The general conclusion was that no one variable was sufficiently reliable as an indicator of environmental condition, and that several variables should be routinely monitored. Also, the monitoring program should be regularly assessed and improved as more data become available. Of the physical/chemical variables investigated, only redox was considered to be suitable. Organic matter, as measured by Loss on Ignition, was found to be highly correlated with sediment particle size but not with the level of organic input, and %C and %N were suitable indicators of organic matter only at very high concentrations. Similarly, stable isotopes of nitrogen and carbon in fish food were effective indicators only at high levels of organic enrichment. The community structure of the macrobenthic invertebrate fauna was found to be a sensitive and reliable measure of sediment condition. Multivariate analysis of the data was able to separate the fauna into major, moderate, and minimal impact levels. In degraded conditions, the ubiquitous polychaete, *Capitella capitata* sp. complex, occurred at very high densities and may be suitable as an indicator species.

Identification of organisms to family level was found to be sufficient to show levels of organic enrichment; however, identification to species level provided more subtle information on the condition of the sediment. The number of benthic infaunal samples required to reliably assess an impact was suggested to include monitoring at fixed sites, at sites that have been determined to have had relatively high levels of impact and at several reference sites.

Video recordings were found to be suitable for a monitoring program because they provide a relatively inexpensive, instant, permanent record of sediment conditions that is readily interpreted by stakeholders. Degraded conditions were clearly evident in the video footage, in particular from the presence of *Beggiatoa* bacterial mats, black sediments, waste food and faeces, and from the decline in macroalgal cover at specific locations. Video recordings identified severe impacts similar to the macrofauna, but moderate levels of impact were not so obvious.

The findings of this report were integral to the development of the current Marine Farm Monitoring program.

Macleod C, Mitchell I, Crawford C, Connell RD (2002) **Evaluation of sediment recovery after removal of finfish cages from Marine Farm Lease No. 76 (Gunpowder jetty), North West Bay**. Tasmanian Aquaculture and Fisheries Institute, Hobart, Tasmania

This study was a joint initiative of the aquaculture industry (Aquatass Tasmania Australia Pty Ltd) and the Department of Primary Industries, Water and Environment (DPIWE) with the aim of assessing the rate of sediment recovery and identifying biological/ biogeochemical indicators of improvement over time after a farm was vacated.

The results indicate that although the cage sediment was highly impacted at the time of cage removal the extent of impact diminished rapidly with both time and distance from the cages.

The influence of the cages was not generally detectable beyond 35m and after 2 months conditions could be classed as transitional. At the end of the study (24 months), although the

sediment biogeochemistry appeared to have recovered, the benthic community structure within the lease still differed significantly from that of reference stations.

The findings of this research have been published in:

- Macleod C.K., Crawford, C.M. and Moltschaniwskyj, N.M. (2004a) Assessment of long term change in sediment condition after organic enrichment: defining recovery (2004) *Marine Pollution Bulletin*. 49: 79-88.
- Macleod, C.K., Moltschaniwskyj, N.A. and Crawford, C.M. (2008) Ecological and Functional Changes Associated with Long-Term Recovery from Organic Enrichment. *Marine Ecology Progress Series*. 365: 17-24.

2001

Crawford C, Mitchell IM, Macleod CKA (2001) **Video assessment of environmental impacts of salmon farms**. *ICES J Mar Sci* 58:445-452

Video recordings have become a common method for monitoring the benthic environment around salmon farms, but generally they are only assessed qualitatively. We made a quantitative assessment of video recordings and compared the results with benthic invertebrate faunal data from the same sites. Transects around two Atlantic salmon (*Salmo salar* L.) farms were videoed, with environmental variables that clearly showed change with levels of organic enrichment ranked according to their degree of occurrence. These variables included *Beggiatoa* cover, pellets and faeces, sediment colour, and abundance of flora and fauna. Analysis of the data by multivariate statistics indicated that quantitative data from video recordings can clearly detect major organic enrichment, but that they are not as sensitive as benthic infaunal data to lower levels of disturbance. This assessment technique will need to be tailored to different environmental conditions, but shows promise for long-term monitoring programs.

2000

Macleod C (2000) **Techniques for farm-based assessment of sediment health associated with the commercial culture of Atlantic salmon (*Salmo salar* L.) in Tasmania**. Master of Science, University of Tasmania, Hobart, Tasmania

Many studies have been carried out to evaluate the effects of organic enrichment on the marine environment, several of which specifically investigated the environmental impacts of cage fish farming. These studies have generally been conducted from a government or regulatory standpoint and to date, none have been undertaken from a farm-based perspective. Thus, there have been no studies aimed at improving the self-assessment capability of farms or developing farm management protocols to ensure environmental sustainability. The current study was undertaken with both these objectives in mind. Initially, the project reviewed techniques routinely employed for monitoring of aquaculture operations as well as methods that have been used to evaluate other sources of organic enrichment in the marine environment. These techniques were then assessed according to three basic criteria; simplicity, reliability and robustness, to identify those that could be considered applicable for farm-based use. The methods thus selected included macrofaunal assessment, evaluation of sedimentation rates, determination of organic matter content, and measurement of sediment redox potential. These techniques were then evaluated at two fish farm leases to determine how they would respond to a) the spatial variability at each site, and b) the temporal effects of operational variability at the two sites over the production cycle. The performance of each technique was judged against species level evaluation of

the macrofaunal community structure as an indicator of the sediment condition. The results suggested that both sedimentation rate and organic matter were unsuitable as farm-based measurements. Measurement of redox potential was found to be a simple and reliable indicator of sediment condition, accurately reflecting the benthic condition. However, the redox results should be interpreted with caution, particularly when taken in isolation. Time series redox measurement showing a clear pattern of effect is preferred. However, isolated redox measurements can be used when viewed in conjunction with some other substantiating evidence. Further examination of the macrofaunal results suggested that *Capitella capitata* complex abundance could also be a good indicator of sediment condition. However, once again, evaluation of the significance of this species complex is most useful when the interpretation incorporates a time series of observations. The macrofaunal results also indicated that assessment of annelids to family level alone may be sufficient to determine site condition, an outcome that could markedly reduce the costs of benthic assessment to farmers. Finally, the results from other major faunal groups, showed some very interesting patterns which could prove useful in evaluating sediment condition. The abundance of echinoderms appeared to be directly related to environmental conditions; total absence indicating highly enriched conditions, dominance by *Echinocardium cordatum* suggesting moderately impacted conditions whilst a more diverse echinoderm fauna seemed to be indicative of unimpacted conditions. In addition, the molluscan community structure at each of the study sites exhibited a shift from bivalve to gastropod domination. This change was reflected at all sample stations and consequently suggests either that the reference locations for each of the sites were influenced by the farm or that the final gastropod species, an introduced species, may itself have induced the change.

McGhie TK, Crawford CM, Mitchell IM, O'brien D (2000) **Degradation of fish-cage waste in sediments during fallowing**. *Aquaculture* 187:351-366

The composition and rate of degradation of organic waste deposited in sediment underneath and adjacent to fish cages in the Huon Estuary, Tasmania, Australia, were investigated. Sediment samples from two near-adjacent sites, but with different sediment types and depths, were analysed for total organic matter, lipids (fatty acids and sterols), %C,  $\delta^{13}\text{C}$ , %N,  $\delta^{15}\text{N}$ , and redox potential during a 12-month fallowing period. Additionally, representative samples of fish food and fish faeces were analysed. It was found that most of the accumulation of organic matter was confined to an area directly underneath the fish cages, but at 30m from the center of the cage, indicators of fish cage waste (faeces and fish food) were still elevated compared with reference sites. As both fish food and faeces have distinctive fatty acid profiles, the relative proportion of food and/or faeces deposited on the sediment could be determined. After 12 months fallowing, fish-farm-derived organic matter in surface sediment at the center of the cage remained greater than at 30m distance, even though redox potentials indicated that normal oxic conditions had returned.

1999

Mitchell I, Crawford C, Brown A (1999) **Environmental Assessment of Proposed Marine Farm Zones in Blackmans Bay**. Tasmanian Aquaculture and Fisheries Institute, Hobart, Tasmania

No summary available

1997

Hodson SL, Lewis TE, Burke CM (1997) **Biofouling of fish-cage netting: Efficacy and problems of in situ cleaning.** *Aquaculture* 152:77-90

The rapid biofouling of fish-cage netting in Tasmania, Australia, necessitates the frequent changing of nets for on-shore cleaning. To reduce the cost associated with this capital- and labour-intensive process, a prototype underwater net cleaner was designed and constructed for the Tasmanian Atlantic salmon industry. This study describes trials aimed to determine the efficacy of this prototype, and to identify areas for improvement. The reduction in open area of netting mesh due to fouling growth, together with subsequent increases in open area after cleaning, were monitored by image analysis of underwater photographs. The preferential removal of larger fouling species and the occurrence of resistant species were documented. The design of the cleaning head and displacement of netting away from the cleaner reduced the severity of scrubbing, limiting fouling removal. However, with increased contact between the brushes and netting the cleaner prevented significant fouling development over a 10-week period. Scanning electron microscopy was used to identify the extent of residual fouling after cleaning, and the difficulty of removing fouling from crevices in the netting surface and from the sides of netting bars. The system can be highly effective, but the current design for presentation of the brushes greatly limits cleaning efficacy. The research has identified areas for design improvement, the problems associated with residual fouling and regrowth and the requirements for effective in situ net cleaning.

1995

Hodson SL, Burke CM, Lewis TE (1995) **In situ quantification of fish-cage fouling by underwater photography and image analysis.** *Biofouling* 9:145-&

Close-up underwater photography and image analysis were used to quantify mesh occlusion by biofouling of salmon-cage netting. This technique allows fast, non-destructive sampling of cages in situ for the determination of temporal and spatial changes in fouling. The area of net blockage can be easily determined, allowing rapid evaluation of cleaning or antifouling performance.

1994

Hodson SL, Burke C (1994) **Microfouling of Salmon-Cage Netting - a Preliminary Investigation.** *Biofouling* 8:93-105

Microfouling development on salmon-cage netting in Tasmania, Australia, was studied by scanning electron microscopy (SEM). Temporal changes and distribution of the fouling microorganisms were documented for the horizontal netting bars in winter and spring. Diatoms were dominant on the upper surfaces of the netting bars and protista on the lower surfaces. This distribution is considered to be a consequence of shading of the lower surfaces from light and the abundance of protista is attributed to high nutrient levels associated with the dense fish population. With increasing immersion time, there was a progression in diatom communities from prostrate forms to large erect and stalked forms in winter, and tube-dwelling forms in spring. Both the rates and the depth of fouling varied between seasons. Copper-based and silicone-based antifoulants effectively delayed fouling development. The former also selected for diatom species known to be copper-tolerant. In contrast, the silicone-treated netting was colonised mostly by the same species as the untreated netting.

1991

Ye L, Ritz DA, Fenton GE, Lewis ME (1991) **Tracing the influence on sediments of organic waste from a salmonid farm using stable isotope analysis.** J Exp Mar Biol Ecol 145:161-174

Stable C isotope measurement in combination with analysis of benthic macrofaunal communities were used to trace the dispersion and effects of solid organic waste derived from a fish cage on marine sediments. The proportion of cage-derived organic C to total organic C in the sediment decreased with increasing distance from a fish cage. A highly organically enriched zone under the cage was characterized by > 75% fish cage-derived organic C in the sediments, a semi-enriched zone (> 10 m from the cage) was characterized by 60% and a lightly enriched zone (> 60 m) by ~40%. Measurable effects of farm organic waste on organisms extended from under the cage to between 10 and 30 m from it. Macrobenthic communities under the cage and 10 m from the cage showed signs of moderate disturbance whereas those from 30 to 150 m appeared undisturbed.

## **Future Directions**

Completed publications:

2019

Carter C, Macleod C, Ross J, Gardner C, Fudge M, Bannister J (2019) **Report from the Global Salmon Conference, Hobart 2017**. Hobart, Tasmania

This report comes from the Tasmanian Global Salmon Symposium partnership and reflects the international conference, Planning for a Blue Future, that was held in Hobart in December 2017. It contains information about the conference, provides a record of the knowledge shared at workshops and provides a summary discussion on each of the three themes of the conference: Future Farming, Biosecurity, and Environment.

2018

Bush F (2018) **Land-based salmon aquaculture. Emerging issues**. Institute for Marine and Antarctic Studies, Hobart, Tasmania [Internal review]

The coastal zone already has many uses and users and consequently growing the salmon farming industry within this environment often faces opposition. As a result, alternative ways of farming salmon are continually being explored; one of which is the development of land-based farming. Within this short paper the benefits and challenges associated with the land-based farming of salmon will be reviewed, particularly with reference to their on-growing. The locations where interest and development in land-based salmon farming is occurring will be explored and finally future direction will be considered.

Bush F (2018) **Offshore Aquaculture. Emerging issues**. Institute for Marine and Antarctic Studies, Hobart, Tasmania [Internal review]

Within this short paper, the benefits and challenges associated with offshore aquaculture will be reviewed. While the main objective of this overview is to provide information on offshore salmon aquaculture, examples using other species will also be considered where relevant. The locations where interest and development in offshore aquaculture is occurring will be reviewed and finally future direction for the industry will be considered.

Bush F, Macleod C, Ross J (2018) **How do you farm salmonids**. Topical Reviews. IMAS, Hobart, Tasmania [Magazine article]

This short review provides a brief overview of the current options available for farming salmon in different environments.

King AS, Elliott NG, Macleod CK, James MA, Dambacher JM (2018) **Making better decisions: Utilizing qualitative signed digraphs modeling to enhance aquaculture production technology selection**. Mar Policy 91:22-33

Understanding causal relationships within complex business environments represents an essential component in a decision-maker's toolset when evaluating alternative aquaculture production technologies. This article assesses the utility of employing signed digraph qualitative modeling to support technology selection decision-making through evaluating the adoption of three alternative production expansion strategies (offshore production, IMTA, or land-based RAS) by the Atlantic salmon industry. Results underlined the benefits of strategically understanding the dynamics of demand growth, emphasized the requirement to address societal concerns early; and indicated that levels of ambiguity are lowest with

expansion offshore and highest with land-based RAS growout. The research suggests that signed digraph modeling can provide an objective perspective on the levels of uncertainty and causal linkages within a business environment when exploring aquaculture adoption technology scenarios.

2016

King AS, Elliott NG, James MA, MacLeod CK, Bjorndal T (2016) **Technology selection—the impact of economic risk on decision making**. *Aquacult Econ Manage* 22:383-409  
Developing an understanding of economic variance (risk) is critical when evaluating alternative aquaculture production technologies. This article assesses the efficacy of employing a quantitative stochastic analysis technique to support technology selection decision making by undertaking a case study investment assessment of three alternative production expansion strategies (offshore sea-pens, land-based RAS growout and larger post-smolt) for the Tasmanian salmon industry. Results demonstrate that salmon aquaculture is undertaken with considerable underlying levels of economic risk, expansion offshore probably represents the lowest initial capital investment and greatest economic return, and that levels of financial uncertainty increase with land-based RAS production. The study highlights stochastic modeling provides significant “added value” over single-point deterministic analysis and that developing an appreciation of the input variability is a key component in critically evaluating alternative production technologies.

2008

Battaglione S, Carter C, Hobday A, Lyne V, Nowak B (2008) **Scoping Study into Adaptation of the Tasmanian Salmonid Aquaculture Industry to Potential Impacts of Climate Change**. Tasmanian Aquaculture and Fisheries Institute, Hobart, Tasmania  
This scoping study was conducted under the Australian Government’s National Agriculture & Climate Change Action Plan: Implementation Programme<sup>1</sup> which identifies and supports initiatives for coordinated action on climate change. The objectives of the study were to:  
a) Identify and review key climate change information needs as they relate to the Tasmanian salmonid aquaculture industry;  
b) Scope the likely impacts of climate change as they relate to the Tasmanian salmonid aquaculture industry; and  
c) Scope possible solutions for adaptation and identify viable industry development opportunities.  
The project is presented in three parts: Part A Predictive modelling is a review of models that can inform understanding of future environmental conditions, identification of key environmental variables, and a summary of potential delivery mechanisms; Part B Salmon performance at higher temperatures is a review of impacts on salmonid health and nutrition; Part C Alternate species and solutions discusses adaptation strategies, attributes of new species, biosecurity and other challenges.

## **Other Relevant Research**

2019

Alexander KA (2019) **Conflicts over Marine and Coastal Common Resources: Causes, Governance, and Prevention**. Routledge, London

This book explores the types of conflicts that occur over marine and coastal resources, the underlying causes, and attempts to prevent them. Despite the emergence of various marine and coastal governance approaches to address the effects of human activities within the marine environment, conflict continues. In this book, the author outlines the reasons conflicts can, and do, arise in the marine and coastal environment. Drawing on case studies from both the northern and southern hemispheres, the book takes a broad view of how we interact with our environment, of how and why conflict is perpetuated as a political and cultural phenomenon, and how this varies or remains constant across space and place. The case studies explore not only the underlying perceptions and needs of those involved in marine and coastal conflict and the types of conflicts that arise in oceanic and coastal areas, but also the underpinning reasons for these conflicts. Marine and coastal resource conflicts have the potential to derail conservation efforts and blue growth policies, as well as the United Nations Sustainable Development Goals. Thus, it is imperative we understand the drivers and exacerbating factors of marine and coastal conflict. Arguing that there is an urgent need for renewed thinking and focus on conflict prevention, the author develops a theory of marine and coastal conflict which allows us to understand those factors and the means to help prevent such conflicts arising in the first place. This book will be of interest to students and researchers of coastal and marine science and environmental management as well as those working in the field of marine resource management, including coastal zone managers and fisheries managers.

Alexander KA, Abernethy KE (2019) **Determinants of socially-supported wild-catch fisheries and aquaculture in Australia**. FRDC Project No 2017-158. FRDC, Hobart, Tasmania

This project identified and tested the determinates of socially-supported wild-catch fisheries and aquaculture in Australia. This project was developed collaboratively with the Human Dimensions Research Subgroup and relevant industry stakeholders and extends previous FRDC projects by examining differential definitions and assessments of social acceptability. It investigates determining factors beyond the social values and perceptions associated with 'sustainability' and seafood production practices, to factors and processes such as those associated with culture, capacity, relationships, participation, and trust, and whether/how these determinates contribute to societal support.

Alexander KA, Hobday AJ, Cvitanovic C, Ogier E, Nash KL, Cottrell RS, Fleming A, Fudge M, Fulton EA, Frusher S, Kelly R, MacLeod CK, Pecl GT, van Putten I, Vince J, Watson RA (2019) **Progress in integrating natural and social science in marine ecosystem-based management research**. Marine and Freshwater Research 70:71-83

Climate change, in combination with population growth, is placing increasing pressure on the world's oceans and their resources. This is threatening sustainability and societal wellbeing. Responding to these complex and synergistic challenges requires holistic management arrangements. To this end, ecosystem-based management (EBM) promises much by recognising the need to manage the ecosystem in its entirety, including the human dimensions. However, operationalisation of EBM in the marine environment has been slow.



One reason may be a lack of the inter-disciplinary science required to address complex social–ecological marine systems. In the present paper, we synthesise the collective experience of the authors to explore progress in integrating natural and social sciences in marine EBM research, illustrating actual and potential contributions. We identify informal barriers to and incentives for this type of research. We find that the integration of natural and social science has progressed at most stages of the marine EBM cycle; however, practitioners do not yet have the capacity to address all of the problems that have led to the call for inter-disciplinary research. In addition, we assess how we can support the next generation of researchers to undertake the effective inter-disciplinary research required to assist with operationalising marine EBM, particularly in a changing climate.

Cullen-Knox C, Fleming A, Lester L, Ogier E (2019) **Publicised scrutiny and mediatised environmental conflict: The case of Tasmanian salmon aquaculture.** *Mar Policy* 100:307-315

This paper analyses mediatised environmental conflict over the Tasmanian salmon aquaculture industry's performance. It compares the Senate Inquiry into the "Regulation of the fin-fish aquaculture industry in Tasmania", the influential Four Corners investigative journalism television program 'Big Fish' and news media coverage following each of these mediatised public investigations. The concept of "mediatised environmental conflict" is applied to reveal how these different modes of investigation influence public debate. Both the Senate Inquiry and the Four Corners program allowed previously invisible actors and networks to be made visible, while rendering others largely silent, particularly scientists despite strong references to science within the debate. Also, the traditional role of ENGOs in holding industries and Governments to account has shifted in this case to an industry player. Considerable differences in the discourses was observed, raising further questions concerning accountability and transparency in public-policy decision-making in relation to management of marine resources.

Lyle J (2019) **Fishing for Atlantic salmon following a major escape event: inferences about dispersal, survival and ecological impact.** Institute for Marine and Antarctic Studies, Hobart, Tasmania

A major storm event in southern Tasmania during May 2018 resulted in substantial damage to salmonid farming infrastructure located off the east coast of Bruny Island and the escape of an estimated 120,000 Atlantic salmon. The escape attracted significant interest from recreational fishers as well as raising concerns about the potential ecological impacts of such a large loss of fish. In order to better understand the dynamics of dispersal, survival and impacts of the escaped Atlantic salmon, an on-line survey of recreational fishers was implemented. Over 120 fishers participated in the survey. Dispersal from the farm site was rapid but appeared to be largely restricted to south-eastern Tasmania and within the general Storm Bay region, including associated embayments and tributaries. During the first 4-6 weeks there were several reports of escapees being schooled up in various locations throughout the region, often associated with areas of freshwater or tidal inflows or within rivers and creeks. In such situations they were readily captured by gillnet and line fishing methods. Based on capture dates, and assuming that most if not all were from the May escape event, at least some Atlantic salmon had survived at liberty for almost four months. This does not necessarily mean that these fish were thriving, in fact there was only limited evidence to suggest active feeding on native fauna. Most recreational fishers fishing for the escapees did so to take advantage of a windfall opportunity and/or to capture a premium

table fish. A proportion of fishers did, however, express concerns about the ecological impacts of escapees and were motivated to contribute to the removal or fish down of the introduced species.

Pecl GT, Ogier E, Jennings S, van Putten I, Crawford C, Fogarty H, Frusher S, Hobday AJ, Keane J, Lee E, MacLeod C, Mundy C, Stuart-Smith J, Tracey S (2019) **Autonomous adaptation to climate-driven change in marine biodiversity in a global marine hotspot.** *Ambio*

While governments and natural resource managers grapple with how to respond to climatic changes, many marine-dependent individuals, organisations and user-groups in fast-changing regions of the world are already adjusting their behaviour to accommodate these. However, we have little information on the nature of these autonomous adaptations that are being initiated by resource user-groups. The east coast of Tasmania, Australia, is one of the world's fastest warming marine regions with extensive climate-driven changes in biodiversity already observed. We present and compare examples of autonomous adaptations from marine users of the region – including shellfish and salmonid aquaculture industries - to provide insights into factors that may have constrained or facilitated the available range of autonomous adaptation options and discuss potential interactions with governmental planned adaptations. We aim to support effective adaptation by identifying the suite of changes that marine users are making largely without government or management intervention, i.e. autonomous adaptations, to better understand these and their potential interactions with formal adaptation strategies.

Stephenson RL, Hobday AJ, Cvitanovic C, Alexander KA, Begg GA, Bustamante RH, Dunstan PK, Frusher S, Fudge M, Fulton EA, Haward M, Macleod C, McDonald J, Nash KL, Ogier E, Pecl G, Plaganyi EE, van Putten I, Smith T, Ward TM (2019) **A practical framework for implementing and evaluating integrated management of marine activities.** *Ocean Coast Manage* 177:127-138

Despite frequent calls for Integrated Management (IM) of coastal and marine activities, there is no consensus on the 'recipe' for successful adoption and implementation, and there has been insufficient evaluation of successes and failures of IM to date. The primary rationale for IM is to overcome four major deficiencies of sector-based management: a) management of diverse activities by different agencies using different approaches, b) management generally focused on a subset of primarily ecological objectives that do not properly articulate or evaluate social, cultural, economic and institutional objectives, c) no mechanisms to evaluate or advise on tradeoffs among objectives of activities in relation to objectives and d) no mechanisms to evaluate the cumulative effects of all managed activities. To help overcome this gap in knowledge, here we draw on our collective experiences working in Australia and Canada to develop and articulate a framework to help guide the practical implementation and evaluation of IM, which we define as: 'An approach that links (integrates) planning, decision-making and management arrangements across sectors in a unified framework, to enable a more comprehensive view of sustainability and the consideration of cumulative effects and trade-offs.'

We argue that IM will be most easily and effectively achieved by linking and modifying existing sector-based plans in an overarching IM initiative that has nine key features: 1) Recognition of need for IM, 2) A shared vision by stakeholders and decision-makers for IM, 3) Appropriate legal and institutional frameworks for coordinated decision-making, 4) Sufficient and effective processes for stakeholder engagement and participation, 5) A

common and comprehensive set of operational objectives, 6) Explicit consideration of trade-offs and cumulative impacts, 7) Flexibility to adapt to changing conditions, 8) Processes for ongoing review and refinement, and 9) Effective resourcing, capacity, leadership and tools. Drawing on these features we then articulate a process for the implementation and evaluation of IM that recognises five phases: i) Preconditions and drivers of change, ii) Intentional design and institutional rearrangement, iii) Enablers and disablers iv) An implemented IM process, and v) Review of IM performance and modification. Combination of the nine features of IM with the five phases in IM development provides a framework for implementation and a lens for evaluation of IM processes. We suggest that this framework provides a guide to the appropriate design of practical IM, which will assist in overcoming the current management deficiencies and improve the sustainability of marine resources in the face of change.

2017

Cullen-Knox C, Haward M, Jabour J, Ogier E, Tracey SR (2017) **The social licence to operate and its role in marine governance: Insights from Australia**. Mar Policy 79:70-77 Traditionally, the ‘social licence to operate’ (SLO) refers to the societal expectations imposed on corporate and commercial activities, often displayed by the willingness for corporations to go beyond the requirements of formal regulations. Alternatively, this paper investigates the emerging influence of the SLO in shaping government decisions regarding the use and impact of the marine environment and its resources. Using expert interviews, text analysis and case study analysis, this research delineated the contemporary SLO as it has manifested in Australian marine governance, with the results indicating that this is potentially occurring at a pace faster than can be systematically reacted to within the current political decision-making processes. Under these emerging conditions, the risk has been identified that traditional government decision-making and stakeholder consultation processes are lagging in their capacity to adapt to ensure that public policy processes can support and engage in this shifting dialogue and ensure the influence of information is appropriately weighted. This research highlights an emerging adjustment of community presence in marine governance and the immediate complexities and challenges this creates for government decision-making. In particular, it begins to explore the interaction of differing information, how this information is carried through communication channels, stakeholder behaviour, approaches to withholding or granting a SLO and the responsibility this carries.

2015

Macleod C, Ross DJ, Carter C, Gardner C, Haward M, Ogier E, Crawford C, Lyle J, Semmens J, Battaglione S, Frusher S, Johnson C (2015) **Submission to the Senate Environment and Communications References Committee Inquiry into Fin-fish Aquaculture in Tas**. Institute for Marine and Antarctic Studies, Hobart, Tasmania [Advice note]

This submission comprehensively reviews research undertaken by the University of Tasmania’s Institute for Marine and Antarctic Studies (IMAS) and its predecessors, commonly in collaboration with other organisations, over the last 20 years. This research has significantly contributed to knowledge of the environmental impacts and interactions of finfish aquaculture in Tasmania, and provides independent advice and understanding to support decisions regarding the management and regulation of the salmonid farming industry. To

date IMAS researchers have played key roles in both identifying and responding to “knowledge gaps” and will continue to do so in the future.

2014

Leith P, Ogier E, Haward M (2014) **Science and Social License: Defining Environmental Sustainability of Atlantic Salmon Aquaculture in South-Eastern Tasmania, Australia.**

Soc Epistemol 28:277-296

Social license reflects environmental and social change, and sees community as an important stakeholder and partner. Science, scientists, and science policy have a key role in the processes that generate social license. In this paper, we focus on the interaction between science and social license in salmon aquaculture in south-eastern Tasmania. This research suggests that social license will be supported by distributed and credible knowledge co-production. Drawing on qualitative, interpretive social research we argue that targeted science, instilled by appropriate science policy, can underpin social license by supporting emerging, distributed, and pluralistic knowledge production. Where social license is important and environmental contexts are complex, such knowledge production might support environmental governance, and so improve outcomes in coastal zone management and beyond.

2013

Ogier E, Macleod C (2013) **Your Marine Values - Public Report 2013 online version.**

IMAS Technical Report. Institute for Marine and Antarctic Studies, Hobart, Tasmania

The Your Marine Values study commenced in 2012 and is aimed at identifying what is important about the local marine environment for those different communities which have a direct interest in the marine system. The study included a series of workshops and a survey to engage people and to record their views (see Appendix C and D for more information). People who contributed came from a range of communities, marine industries, managing agencies. Seventeen key marine values have been identified through the Your Marine Values study. These include regionally relevant environmental values and those values (social, economic and ecological) that affect and are likely to be affected by aquaculture (see Appendices C and D for the extended set of values identified).

2012

Purser G, Forteath G (2012) **Salmonids.** In: Lucas JS, Southgate PC (eds) Aquaculture.

Wiley-Blackwell, United Kingdom

No summary available

2010

Buxton C, Jungalwalla P (2010) **Salmon Aquaculture Subprogram: Facilitation and Administration.** FRDC 2007/229. Tasmanian Aquaculture and Fisheries Institute, Hobart, Tasmania

This project implemented the agreed Strategic R&D Plan for the salmon industry for the period 2007-2008. Building on FRDC project 2003/200 it continued to provide a cost effective and economical administrative framework and process which:

- engaged stakeholders, specifically the salmon farming industry and the State managers responsible for its development, in the process of identifying and prioritising research needs

and monitoring the projects designed to meet these needs;

- enabled research groups to shape their programs to the needs of industry, government and other stakeholders; and
- transferred of useful information from research projects to end-users.

As a consequence, the projects undertaken in the SAS research portfolio were focussed on outcomes that were strongly supported by end-users, there was a high level of active collaboration between researchers and industry in carrying out these projects, and the adoption of successful research results has been rapid. The impact of these research outcomes has already been substantial.

Jobling M, Arnesen AM, Benfey T, Carter CG, Hardy R, Le Francois NR, O'Keefe R, Koskela J, Lamarre SG (2010) **The Salmonids (Family: Salmonidae)**. In: Le F, N. R., Jobling M, Carter CG, Blier PU (eds) *Finfish Aquaculture Diversification*. CABI, Wallingford, UK  
This chapter combines sections on whitefishes, charrs, Atlantic salmon and Pacific salmon and trouts, with a view to detailing key aspects of their biology and culture.

2006

Buxton CD, Jungalwalla P (2006) **Atlantic Salmon Aquaculture Subprogram: Facilitation and administration**

No summary available

2003

Battaglione SC, Cobcroft JM (2003) **Aquaculture Subprogram: Facilitation, Administration and Promotion**. Aquafin CRC Project 5B2 & FRDC 200/223

No summary available

Cheshuk BW, Purser GJ, Quintana R (2003) **Integrated open-water mussel (*Mytilus planulatus*) and Atlantic salmon (*Salmo salar*) culture in Tasmania, Australia**.

*Aquaculture* 218:357-378

In a field experiment, Tasmanian blue mussels (*Mytilus planulatus*) were cultured within an Atlantic salmon (*Salmo salar*) farm in North West Bay (NWB), Tasmania to test if enhanced mussel performance and reduced organic enrichment resulted from integrated mussel-salmon culture. The performance of mussels grown within the fish farm lease (70 and 100 m from the fish cages) was not appreciably different from that of mussels grown distant to the farm (500 and 1200 m from the fish cages). After culturing mussels for 14 months, no difference among culture sites was indicated for any measured parameter except shell length ( $P < 0.0001$ ) and condition index ( $P < 0.01$ ). However, these differences among culture sites were minor, with final mean shell lengths and condition index being within 2.0 mm, and 11 parts per thousand, respectively. Growth of mussels cultured within the fish farm was not enhanced due to several contributing factors: (a) solid wastes (feed particles and faeces) from the farm did not significantly increase particulate food concentrations above ambient levels, (b) phytoplankton production within the farm was not enhanced, (c) mussels were cultured too distant to intercept settling particulate wastes emanating from the fish cages, and (d) ambient seston concentrations were consistently above the pseudofaeces threshold concentration, thereby limiting ingestion of fish farm particulate wastes.

Purser GJ, Forteath G (2003) **Salmonids**. In: Lucas J, Southgate P (eds) *Aquaculture Farming Aquatic Animals and Plants*. Blackwell Publishing Asia Pty Ltd, Carlton  
No summary available

2001

Bransden MP, Carter CG, Nowak BF (2001) **Alternative methods for nutrition research on the southern bluefin tuna, *Thunnus maccoyii* (Castelnau): evaluation of Atlantic salmon, *Salmo salar* L., to screen experimental feeds**. *Aquacult Res* 32:174-181  
Development of a formulated pellet to replace 'bait' fish in farmed southern bluefin tuna (SBT), *Thunnus maccoyii* (Castelnau), feeds is in progress: however, inherent difficulties have been encountered in conducting large-scale growth trials to evaluate experimental pellet quality. Consequently, alternative methods are being investigated to develop a suitable feed more rapidly. This work explored whether Atlantic salmon, *Salmo salar* L., could be used as a 'surrogate' species for nutrition research on SBT by screening experimental SBT feeds. Atlantic salmon (initial mean weight 161.4 g) were fed a commercial salmon feed (Com.Sal), a commercial northern bluefin tuna feed (Com.NBT), one of three experimental SBT feeds (CRC-A, -B, -C) or dry pelleted pilchards for 42 days, at which time growth was assessed. In vivo and in vitro protein digestibility of the feeds was also evaluated. Weight gain of Atlantic salmon fed Com.Sal (99.8 g) and Com.NBT (93.5 g) were found to be significantly ( $P < 0.01$ ) greater compared to those fed CRC-C (68.2 g) and pilchard (56.1 g), Weight gain of salmon fed the CRC-A (81.6 g) and CRC-B (85.3 g) were also significantly different from the pilchard-fed group, but not from CRC-C. No significant correlation was found between salmon and SBT growth, although difficulties with conducting growth trials on SBT may have limited the ability to compare data. In vitro ingredient digestibilities, however, were used to predict whole feed digestibility, which was found to significantly correlate to weight gain in SBT. Although the use of digestibility techniques appears useful in formulating SBT feeds, the use of a 'surrogate' species such as Atlantic salmon to screen SBT feeds needs to be more fully investigated.

2000

Hodson SL, Burke CM, Bissett AP (2000) **Biofouling of fish-cage netting: the efficacy of a silicone coating and the effect of netting colour**. *Aquaculture* 184:277-290  
The efficacy of a silicone coating (Veridian 2000) to reduce fouling on salmon-cage netting was examined at a salmon farm in Tasmania, Australia. Significantly less fouling occurred on the white silicone-coated netting (1.9 kg/m<sup>2</sup>) compared to uncoated white (7.8 kg/m<sup>2</sup>) and black (8.5 kg/m<sup>2</sup>) netting after 163 days immersion. On silicone-coated netting the green alga *Ulva rigida* dominated the fouling mass, with smaller amounts of solitary ascidians. In contrast, solitary ascidians dominated the uncoated black and white netting and accounted for more than 75% of the fouling mass. Netting colour significantly affected the growth and composition of algal fouling, but had no effect on invertebrate fouling. Cleaning experiments demonstrated that fouling organisms were poorly adhered to the silicone coating and that relatively little effort was required for their removal. Silicone coatings may provide an effective non-toxic solution to reduce fouling on sea-cages and to increase the ease of fouling removal.

**Zone Assessment Reports:**

**Baseline Environmental Data for Proposed Marine Farm Zones in the D'Entrecasteaux Channel & Huon River (1996)**

Mitchell IM, Crawford CM, Brown A

**Baseline Environmental Data for Proposed Farm Zones in the Furneaux Group (1997)**

Mitchell IM, Brown AI, Crawford CM

**Baseline Environmental Data for Proposed Marine Farm Zones in Great Oyster Bay (1997)**

Mitchell IM, Brown AI, Crawford CM

**Environmental Assessment of the Proposed Marine Farm Zone at Long Reach, Tamar River (1999)**

Mitchell I

**Environmental Assessment of Proposed Marine Farm Zones in Blackman Bay (1999)**

Mitchell I, Crawford C, Brown A

**Environmental Assessment of Proposed Marine Farming Regions in the D'Entrecasteaux Channel (2001)**

Mitchell I

**Environmental Assessment of Revised and Proposed Marine Farming Areas in the Huon River and Port Esperance Region (2001)**

Mitchell I

**Environmental Assessment of Proposed Marine Farming Region at Electrona Wharf, North West Bay (2002)**

Mitchell I, Lawler M, Jordan A

**Environmental Assessment of Proposed Marine Farming Zone Extension Off Soldiers Point, D'Entrecasteaux Channel (2005)**

Lawler M

**Environmental Assessment of Proposed Marine Farming Zones in Norfolk Bay and The Tasman Peninsula (2005)**

Lawler M

**Environmental Assessment of Proposed Marine Farming Zone in Macquarie Harbour (2005)**

Lawler M

**Environmental Assessment of an Extension to the Marine Farming Zone Near Zuidpool, in the D'Entrecasteaux Channel (2007)**

Lawler M

**Environmental Assessment of a Proposed Marine Farming Zone at Deep Bay, in Port Cygnet (2008)**

Lawler M

**Environmental Assessment of a Proposed Marine Farming Zone in the Tamar River (2008)**

Lawler M

**Environmental Assessment of a Proposed Marine Farming Zone Extension at Soldiers Point, in the D'Entrecasteaux Channel (2009)**

Lawler M

**Environmental Assessment of a Proposed Marine Farming Zone Extension at Lippies Point, in the D'Entrecasteaux Channel (2009)**

Lawler M

**Part B: Environmental Assessment of a Proposed Marine Farming Zone Extension at Soldiers Point (SE) in the D'Entrecasteaux Channel (2010)**

Lawler MM, Lucieer V, Pender A, Macleod C

**An Environmental Assessment of a Proposed Marine Farm Zone Extension at Creeses Mistake, Nubeena Tasmania (2013)**

Lucieer V, Pender A

**An Environmental Assessment of a Proposed Marine Farm Zone Extension at Butlers Point in Great Taylor Bay, South Eastern Tasmania (2013)**

Lucieer V, Pender A

**An Environmental Assessment of a Proposed Amendment to the Marine Farming Zone at Zuidpool Rock in the D'Entrecasteaux Channel (Assessment 1) (2014)**

Lucieer V, Pender A

**An Environmental Assessment of a Proposed Amendment to the Marine Farming Zone at Zuidpool Rock in the D'Entrecasteaux Channel (Assessment 2) (2014)**

Lucieer V, Pender A

**An Environmental Assessment of a Proposed Marine Farming Zone Extension at Browns Point, in the D'Entrecasteaux Channel (2014)**

Lucieer V, Pender A

**An Environmental Assessment of a Proposed Marine Farming Zone Extension at Flathead Bay, in the D'Entrecasteaux Channel (2014)**

Lucieer V, Pender A

**An Environmental Assessment of a Proposed Marine Farming Zone Extension at Lippies Point, in the D'Entrecasteaux Channel (2014)**

Lucieer V, Pender A

**An Environmental Assessment of a Proposed Marine Farming Zone Extension at Trumpeter Bay, South Eastern Tasmania (2014)**

Lucieer V, Pender A

**An Environmental Assessment of a Proposed Marine Farming Zone West of Wedge Island, South Eastern Tasmania (2014)**

Lucieer V, Pender A



## **Appendix 2 - IMAS Externally Funded Projects Listing Relevant to Salmon Research**

### **Salmon Health and Nutrition**

2019

#### **Optimising dietary phosphorus inclusion to reduce skeletal deformity in triploid Atlantic salmon (2019)**

Adams, M.B.; Adams, L.R.; Carter, C.G.

Skeletal deformities in cultured fish species are a recurrent production issue requiring further understanding to implement practical mitigation approaches. Skeletal deformities are a significant welfare concern given their disabling effect on affected fish which can lead to impaired growth and mortality. Product downgrades are associated with deformity where inefficient processing lead to reduced yield, and reduced saleability. Skeletal deformities (inclusive of spinal and jaw deformities), can affect up to 30% of farmed triploid Atlantic salmon populations. It was previously reported (FRDC project 2014-248) skeletal deformity of Atlantic salmon has a significant impact for the Tasmanian industry for the higher volume of triploid populations grown by Petuna Seafoods in Macquarie Harbour. It is critical that high quality triploid stocks can be grown rapidly and reliably to avoid significant economic losses imparted by poor performance, removal of deformed fish and product downgrades. It is equally essential that fish welfare is enhanced and maintained for these stocks. Triploid salmon have different requirements for dietary phosphorus (P) relative to diploids, during both freshwater and marine phases, and if fed a standard diploid salmon feed, triploids exhibit slower growth and higher occurrence/prevalence of skeletal anomalies. Furthermore, triploids have different gut morphology relative to diploids and it is suggested that triploid digestive efficiency, growth and welfare outcomes vary significantly from diploid salmon. These observations encouraged feed producers to develop new solutions to cope with these dissimilarities and assist Atlantic salmon producers to improve welfare and commercial output particularly in triploid stocks. A tank-based longitudinal study will enable empirical quantification of phosphorus requirements under standardised and hatchery-relevant conditions during the freshwater growth phase of triploid Atlantic salmon. The study will maintain separate groups of fish with different dietary histories from first feeding until pre-smoltification and assess the consequence of diet history upon the rate of growth and prevalence of skeletal deformity prior to seawater readiness. The trial is designed to establish the effect of improved diet design to reduce skeletal deformity and optimise growth performance in Tasmanian stocks of Atlantic salmon. The aims of this project are to:- Determine the effect of different phosphorus inclusion levels on performance and deformity in a Tasmanian population of triploid Atlantic salmon in an experimental RAS.-Establish phosphorus requirements for Tasmanian triploid salmon during the freshwater phase using an inclusion break point design.

2018

#### **Cawthron Collaborative Research for three PhD Operating & TAG Travel Costs (2018-2021);**

Carter CG; Bridle AR; Wilson RR; Nowak BF; Bowman JP

This is a PhD project located at IMAS and funded by Cawthron Institute, NZ from a MBIE grant. This is an exciting opportunity to link in with international research on aquaculture of a salmonid species (chinook salmon) and to gain skills and experience in forward looking technology around molecular biology and recirculation aquaculture systems. The research will focus on understanding the impact of genome (family) and environmental change under controlled conditions on chinook salmon. Environmental variables include temperature,

salinity and pH because these impact on local NZ industry performance and sustainability. Research will advance fundamental knowledge about the chinook salmon proteome and how proteomics can be used to understand drivers of growth and growth efficiency. The project will develop proteomic based methods and relate this to other molecular based approaches. The research will contribute to sustainable aquaculture by understanding what happens to salmon when exposed to various situations that may be encountered in aquaculture. It will equip you with a range of new skills from maintenance of fish to advance molecular analyses.

#### **Understanding gill necrosis in farmed Atlantic salmon (2018 - 2020);**

Nowak BF

Gill health is essential for fish performance. There are a range of gill conditions affecting Atlantic salmon while in grow out cages. One of the less investigated but not uncommon pathologies is gill necrosis. The project will focus on potential causes of gill necrosis including environmental factors (for example hydroids, other biofouling, plankton, temperature), effect of gill health in hatcheries and the presence of pathogens and other microorganisms on the gills at the time of necrotic changes. The outcomes will include improved understanding of the gill necrosis including potential management strategies based on risk factors.

#### **Skin health of New Zealand farmed Chinook salmon (2018 - 2022);**

Nowak, BF; Ghosh, B

This is a PhD project located at IMAS and funded by Cawthron Institute, NZ from MBIE (AquaHealth Program). This is an exciting opportunity to link in with an international team focussed on investigating health of chinook salmon farmed in New Zealand. The research is centred on assessing skin health in chinook salmon farmed in New Zealand. This project will investigate skin lesions and skeletal deformities in chinook salmon through characterising the fish with and without the lesions. It will initially focus on skin lesions. Two approaches will be used: following production fish (effect of smolt quality on performance in grow-out - in particular skin lesions) and intervention experiments, for example:-Vaccines (RLO and T. maritimum)-Anti-inflammatory-Anti-microbials-Diet-Biofouling (presence/absence)Other husbandry based experiments could be included if the industry was interested. Some of the intervention studies may provide a solution or partial solution for the industry. Multidisciplinary approach will be used. For all sampled fish skin lesions and skeletal deformities will be documented. It will equip the student with a range of new skills to advance fish health management and will contribute to sustainable aquaculture.

#### **Performance of Atlantic salmon following simulated thermal delousing with AQUI-S sedation (2018);**

Adams, M. B.

Atlantic salmon (n = 300) will be transferred from a commercial hatchery, on grown in FW until reaching 400-500g and then transferred to 12 x 350L tanks contained within a collective experimental recirculating aquaculture system (RAS) at IMAS - Launceston. Once habituated to systems and acclimated to SW (3 weeks) the baseline food intake will be recorded for 2 weeks, and the fish will be divided into four groups of three tanks with fish in each group treated with either: -1.) Un-sedated transfer to a 35 second bath @ 15C-2.) Sedated transfer to a 35 second bath @ 15C-3.) Un-sedated transfer to a 35 second bath @ 33.4C -4.) Sedated transfer to a 35 second bath @ 33.4C A sample of each tanks population (n = 5) will be captured, anaesthetised and killed immediately before and after treatment. Remaining fish will be monitored for feed intake and growth over a period of 30 days following treatment.

#### **Does sedation of fish during transfer to and treatment with FW influence AGD redevelopment, fish performance and stress in vivo? (2018);**

Adams, M. B.; Bridle, A. R

This project aims to investigate whether sedation will impact upon disease redevelopment, stress physiology and performance subsequent to treatment. The response will be measured by collecting sample for analysis of AGD re-development (histology and PCR), key stress hormones and metabolites as well as documenting feed intake and growth for a period both before and after simulated treatment.

2017

**Development of an amoebic gill disease vaccine to protect Atlantic salmon (2017-2020);**

Bridle, AR; Nowak, BF

Amoebic gill disease (AGD) is the most significant health problem affecting Atlantic salmon aquaculture in Tasmania, which is the largest and most economically important aquaculture industry in the country. In the last decade, AGD has become a legitimate health threat to the multibillion dollar global Atlantic salmon industry. Given the well-documented 10-20% production cost associated with AGD to the Tasmanian industry it is clear a solution is needed before AGD fully establishes itself in the largest Atlantic salmon producing nations. This project aims to identify candidate vaccine antigens and produce an experimental vaccine against AGD that will benefit the Tasmanian and international Atlantic salmon aquaculture industries.

**Gill health assessment of harvestable Atlantic salmon (2017);**

Adams, M. B.

Provide gill health assessments from commercially harvestable Atlantic salmon. Gill samples collected by farm personnel from Atlantic salmon during harvest operations will be sent to IMAS Launceston and analysed qualitatively and quantitatively for signs of disease (principally amoebic gill disease) or other anomalies.

**Commercial treatment of AGD affected Atlantic salmon (2017-2018);**

Adams, M. B.; Knowles, J.; Percival, S.

Provision of gill health assessments from commercially treated Atlantic salmon. Gill samples collected by farm personnel from Atlantic salmon during commercial treatments for AGD will be sent to IMAS Launceston and analysed qualitatively and quantitatively to compare efficacy between different commercially approved and executed treatment regimes.

**Gross scoring of fixed gill samples for EAF/Skretting (2017);**

Adams, M. B.

An AGD challenge conducted in the IMAS Experimental Aquaculture Facility requires that gill samples are excised from euthanized fish (n=660 in total), fixed in specimen jars and sent to IMAS-Launceston on a weekly basis. Upon arrival gill arches are dissected out, photographed and the resultant gill images are scored according to the severity of gross pathological change based on Taylor et al 2009.

**Salmonid Nutrition and Performance (2017)**

Adams L. R.; Adams, M. B.

The project covers consultancy projects conducted for Skretting Australia across fish nutrition, growth and health performance, and ingredient assessment studies.

**Salmonid Nutrition and Performance – Fish & Digestibility Chemical Analysis (2017)**

Adams, L. R.; Adams, M. B.

**Literature Review – chinook salmon (2017);**

Nowak, BF; Crosbie, PBB

2015

**Dietary carotenoid utilisation at high temperature by Atlantic salmon (*Salmo salar*) (2015-2017)**

Adams, L.R.; Adams, M.B.; Carter, C.G.

**Immune gene expression in early amoebic gill disease (2015);**

Bridle, AR

Existing samples stored in RNA later from control Atlantic salmon and salmon infected with *Neoparamoeba perurans* collected at three different timepoints (eight fish in each group at each time) early in the infection will be analysed for gene expression to characterise the Th1/Th2 immune response elicited during amoebic gill disease of Atlantic salmon (*Salmo salar*). This is a collaborative project using existing samples of which results will be published jointly by the investigators.

**AGD Literature Review (2015);**

Nowak, BF

2014

**Ongoing salmon and oyster research in a new Experimental Aquaculture Facility (EAF) (2014-2015);**

Carter, CG; Cunningham, M; Main A

**Optimising a combination treatment for AGD in Atlantic salmon (2014);**

Adams, M. B.

The project aim is to test the efficacy of hydrogen peroxide combined with freshwater to resolve amoebic gill disease at high temperature. Results will indicate whether treatment of AGD with a combination treatment can be applied under high temperatures commonly experienced in situ. Successful outcomes will likely lead to further optimisation trials in vivo and in situ.

**Further optimisation of a combination treatment for AGD (2014);**

Adams, M. B.

This project will test the efficacy of hydrogen peroxide combined with freshwater to resolve amoebic gill disease in vWo at low and high temperature. The synthesis of previous trial findings (undertaken with Huon Aquaculture Group) and the results obtained from this project will determine temperature dependent safety and efficacy guidelines for the combined FW/peroxide treatment of fish in vivo that are moderately affected with experimentally induced AGO.

**The effect of diet on the response of Atlantic Salmon to Amoebic Gill Disease (AGD) 2 (2014);**

Nowak, BF; Leef, MJ

This project will determine if commercially available diets can support Atlantic salmon during an experimental challenge of amoebic gill disease.

**Investigating the impact of nutrition, in-feed bioactive compounds and environment on gut and gill microbial population dynamics and histology in farmed Atlantic salmon (2014 - 2015);**

Bowman, JP; Nowak BF

This project will determine if commercially available diets can support Atlantic salmon during an experimental challenge of amoebic gill disease.

2013

**The effect of diet on the response of Atlantic salmon to amoebic gill disease (2013);**  
Nowak, BF; Leef, MJ

**Analysis of fish samples (2013);**  
Nowak, BF, Leef, MJ

**Combination treatment of Atlantic salmon affected by amoebic gill disease (AGD) (2013);**

Adams, M. B.

This project will combine the use of hydrogen peroxide and freshwater to treat amoebic gill disease in Atlantic salmon. The experiment aims to identify whether a synergistic effect between the two treatment options will occur by comparing three different concentrations of hydrogen peroxide in freshwater over a fixed time duration relative to freshwater and seawater only control groups.

**Detection of Neoparamoeba perurans in water samples (2013-2014);**  
Nowak, BF; Bridle, AR

**Effect of diet on resistance to Amoebic Gill Disease (2013);**  
Nowak, BF; Bridle, AR

**Presence of N. perurans in the environment (2013);**  
Nowak, BF; Bridle, AR

2012

**Assessing the biological value of Tasmanian grown lupins in salmon feeds (2012)**  
Adams, L. R.; Wilkinson, R.

**TSGA IPA: Comparative susceptibility and host responses of endemic fishes and salmonids affected by amoebic gill disease in Tasmania, Project: 2011-070 (2012-2014);**  
Adams, M. B.; Bridle, A. R.; Nowak, B. F.

**Atlantic salmon jaw deformity in culture (2012)**

Cobcroft, J. M.; Battaglione, SC

Skeletal deformities occur in aquaculture fish species and have a negative impact on performance, health and the harvested product. Lower jaw deformity (UD) occurs in Atlantic salmon cultured in Tasmania and is associated with lower survival and fewer premium harvest fish. The Tasmanian salmon industry produced 37,360 tonnes in 2011 (AU\$>400 million), with plans to expand to 45,000 tonnes by 2015. This study will investigate the physiological and molecular basis for UD, providing new knowledge that will contribute to the industry's production of quality fish. Novel techniques will be applied in enzyme activity and gene expression, opening an important industry linkage.

**Supply of amoebae attached to Petri dishes for in vitro trials at Huon Aquaculture to be carried out by Ridley AgriProducts Pty Ltd (2012);**

Nowak, BF; Crosbie, PBB

This project entails the supply of the causative agent of amoebic gill disease, Neoparamoebae perurans, isolated from Atlantic salmon with the disease to the marine farming site of the Huon Aquaculture Company. The amoebae will be isolated from salmon held at the aquaculture facility. Amoebae will be attached to Petri dishes and transported to

Hobart for collection. A total of 100 plates are required and these will be sent in batches of 20 over the course of 5 days.

**Atlantic Salmon Aquaculture Subprogram: Atlantic Salmon Subprogram – Culture and cryopreservation of *Neoparamoeba perurans* (AGD), Project: 2012-048 (2012-2014)**  
Crosbie, PBB; Bridle, AR; Nowak, BF

**Experimental Aquaculture Facility (EAF) (2012-2018)**  
Carter, CG; Nixon, PA; Frappell, P; Buxton, CD

**Atlantic Salmon Aquaculture Subprogram: The effects of AGD on gill function: Use of a perfused gill model, Project: 2011-069 (2012 - 2013);**  
Leef, MJ; Nowak, BF

2011

**Ingredient assessment of locust and grasshopper meal, for inclusion in salmonid feeds (2011-2012);**  
Adams, L. R.

**Alternative treatment strategies for amoebic gill disease; is hydrogen peroxide a potential therapeutic option? (2011);**  
Adams, M. B.

**How do Atlantic salmon recover from hypoxic events? – Impacts of water temperature and genetics (2011);**  
Wilkinson, R; Katersky Barnes, RS; Carter CG

**Tenacibaculum maritimum vaccine trial (2011);**  
Nowak, BF; Crosbie, PBB; Leef, MJ

**The effect of an immunostimulating and immunosupporting diet (Protec) on the response of Atlantic Salmon to amoebic gill disease (AGD) (2011 - 2012);**  
Nowak, BF; Leef, MJ

2010

**Effect of AQUI-S anaesthesia during freshwater treatment on AGD re-development (2010 – 2011);**  
Adams, M. B.

**Atlantic Salmon Subprogram: Hydrogen peroxide treatment of Atlantic salmon affected by AGD, Project: 2010-218 (2010)**  
Nowak, B. F.; Adams, M. B.; Crosbie, P. B. B.

**Atlantic Salmon Aquaculture Subprogram: Oxygen regulation in Tasmanian Atlantic Salmon, 2010-203 (2010-2011);**  
Katersky Barnes, RS; Carter CG

**Understanding Fish-pathogen Interactions in Diseases affecting Australian Mariculture (2010);**  
Nowak, BF

2009

**Skretting #9: Understanding Digestive Tract Function in Atlantic Salmon (2009);**  
Carter, CG; Katersky Barnes, RS

**Fish Meal Replacement in Aquaculture Feeds: Tracing the Use of Plant Based  
Protiens and Lipids in Crustacean Body Tissues (2009);**  
Adams, L. R.

**Skretting Australia Salmon Nutrition Trials (2009)**  
Battaglone, SC; Carter, CG; Beech, A

**Improving our Understanding of Welfare in Farmed Fish through Behavioural  
Investigations and Use of Self Feeders (2009);**  
Purser, GJ

**Comparative study on the impact of Phaffia Rhodozyma yeast & other stimulatory  
substances on the immune response in Atlantic Salmon (2009);**  
Nowak, BF

2008

**Skretting #8: Dietary development for Atlantic salmon at high temperature (2008);**  
Carter, CG; Katersky Barnes, RS

**Atlantic Salmon Aquaculture Subprogram:**  
Rickettsia-like organism vaccine development for the salmonid aquaculture industry, Project:  
2008-222;  
Extension Funding Application- AGD Vaccine Phase III, Project: 2008-218 (2008 - 2012);  
Nowak BF; Cook M; Crosbie PBB; Elliott N

**Aquafin CRC - AGD Vaccine Phase III-Sea-based Trials, Vaccine Refinement and  
Commercialisation (2008 - 2009);**  
Nowak, BF; Crosbie, PBB

**Using the Mucosal Antibody Response to Recombinant Neoparamoeba perurans  
Attachment Proteins to Design an Experimental Vaccine for Amoebic Gill Disease  
(2008 - 2013)**  
Nowak, BF; Cook, M; Crosbie, PBB

**Reducing skeletal malformations in cultured marine fish using gene expression,  
improved nutrition and advanced system operation (2008-2010);**  
Battaglone SC; Nowak BF; Cobcroft JM

**Postgraduate Support – Reovirus detection and epidemiology (2008 – 2010);**  
Nowak, BF

2007

**Skretting #6: Apparent Digestibility of Large Salmon (2007);**  
Carter, CG

**Keeping Track of Fish in Aquaculture: The Application of Video and Radio Frequency  
Identification (RFID) Systems (2007)**  
Purser GJ; Williams RN

**Polyphasic taxonomy analysis of vibronaceae and assessment of pathogenicity to salmonids (2007-2009);**

Carter CG; Nowak BF; Adams MB; Planko DHH

2006

**Skretting Australia Salmon Feed Trials (2006);**

Battaglione, SC

**The effect of high temperature on apparent digestability of Atlantic Salmon (2006);**

Carter, CG

**SBT Aquaculture Subprogram: Improving Fish Husbandry and Performance through Better Understanding of the Relationship of Fish Stress and Health, Project: 2006-225/2008-220 (2006 - 2010);**

Nowak, BF; Porter, MJR

**Genetic Factors Influencing Resistance to Amoebic Gill Disease (AGD) in Atlantic Salmon (2006-2008);**

Nowak, BF

2005

**Apparent Digestibility of poultry products to Atlantic salmon – Tasmania (2005);**

Carter, CG

**Aquaculture Nutrition Subprogram: Assessment of growth performance under limiting environmental conditions, Project: 2004-237 (2005-2007);**

Carter, CG

**Aquafin CRC – Atlantic Salmon Aquaculture Sub-program: environmental control of growth and early maturation in salmonids, Project: 2005-201 (2005-2008);**

Carter, CG

**Aquatic Animal Health Subprogram: Development of National Investigation and Reporting Protocols for Fish Kills in Recreational and Capture Fisheries (2005);**

Nowak BF; Crane M; Jones Brian

2004

**Aquafin CRC - Enhanced hatchery production of Striped Trumpeter, *Latris lineata*, in Tasmania through system design, microbial control and early weaning, Project: 2004-221;**

Battaglione, S

**Aquafin CRC - Atlantic Salmon Aquaculture Subprogram: Establishment of Challenge for AGD, Project: 2004-215 (2004 - 2008);**

Nowak, BF; Crosbie, PBB

**Effects of husbandry on Amoebic Gill Disease, Project: 2004-214 (2004-2007);**

Nowak, B. F.; Adams, M. B.

**Commercial AGD and salmon health project, Project: 2004-213; Nutrient intake and growth performance in Salmon – Project 2 (2004);**



Carter, CG

**Use of Immunomodulation to Improve Fish Performance in Australian Temperate Water Finfish Aquaculture, Project: 2004-210 (2004 - 2007);**

Nowak BF; Morrison RN

**Aquaculture Nutrition Subprogram: Evaluation of Value-added Grain Protein Products for Atlantic Salmon and Black Tiger Prawns (2004 - 2005);**

Glenncross, B; Carter, CG; Smith, D

**Can Feeding Entrainment Improve the Level of Food Intake and Growth of Atlantic Salmon Smolt Transferred to Seawater Grow-out? (2004);**

Purser, GJ

2003

**Nutrient intake and growth performance of Atlantic salmon (2003);**

Carter, CG

**Management and Control of Amoebic Gill Disease in Atlantic Salmon - International Collaboration (2003 - 2004);**

Nowak, BF

**Interdisciplinary Network for Aquatic Animal Health (2003);**

Nowak BF; Powell MD; Bowman JP; Carter CG; Geraghty DP; Carson J

2001

**Effect of Fish Oil Replacement on Atlantic Salmon Performance under Tasmanian Summer Conditions (2001);**

Carter, CG

**Aquafin CRC - Atlantic Salmon Aquaculture Subprogram:**

**Control of precocious sexual maturation in Atlantic salmon, Project: 2001-246;**

**Model development for epidemiology of Amoebic Gill Disease, Project: 2001-245;**

**Treatment and Pathophysiology of Amoebic Gill Disease, Project: 2001-205 (2001 - 2004);**

Powell MD; Nowak BF

**Host-pathogen interactions in Amoebic Gill Disease, Project: 2001-244 (2001 - 2003);**

Nowak BF; Carson J

**Why do Fish Die from Gill Diseases? (2001 - 2003);**

Powell, MD; Nowak, BF

2000

**Why do Atlantic Salmon Die from Amoebic Gill Disease: Cardiac Dysfunction? (2000);**

Nowak BF; Powell MD

**Aquafin CRC - Atlantic Salmon Aquaculture Subprogram: Effective Treatments for the control of Amoebic Gill Disease, Project: 2000-266 (2000);**

Powell MD; Nowak BF

1999

**Aquafin CRC - Atlantic Salmon Aquaculture Subprogram: development of selective enrichment culture-polymerase chain reaction (SEC-PCR) for the detection of bacterial pathogens in covertly infected farmed salmonid fish, Project: 1999-201;**

**Pathophysiology of Amoebic Gill Disease in Atlantic Salmon: a new approach (1999);**  
Nowak BF; Powell MD

**Factors Affecting AGD-related Mortality in Cultured Atlantic Salmon (1999);**  
Powell MD; Nowak BF

1998

**Aquaculture feed development for Atlantic salmon (*Salmo salar*), Project: 1998-322 (1998 - 2000);**  
Carter, CG; Foster, CK

1995

**Effects of environmental factors on the health of Atlantic salmon gills (1995 - 1998);**  
Nowak, BF

1994

**Aquaculture diet development subprogram: fish meal replacement in aquaculture feeds for Atlantic salmon, Project: 1993-120.05 (1994-1996);**  
Carter, CG

**An integrated marine aquaculture system using Atlantic salmon and shellfish (1994 - 1997);**  
Purser GJ

1993

**Optimisation of feed distribution to salmon in sea-cage culture, Project: 1993-233 (1993 - 1994);**  
Purser, GJ

1992

**Nutritional energetics in Atlantic salmon with special emphasis on protein to energy ratio (1992-1995);**  
Carter, CG

## **Environmental Interactions and Management**

2019

### **CRC Project: Seaweed solutions for sustainable aquaculture (2019-2022)**

Macleod, C.; Hurd, C. L.; Alexander, K. A.; Bellgrove, A.; Evans, B.; Sanderson, C.  
This project will develop a sustainable Integrated Multi-Trophic Aquaculture (IMTA) model that supports commercial seaweed production. To do this, the research will i) define the seaweed culture proposition (identify species, growing techniques and products) and ii) develop a regionally relevant IMTA partnership model that brings together salmon, shellfish and seaweed producers to ensure economic, environmental and societal benefits.

### **Derwent Estuary Program Reef Monitoring (2019 - 2020);**

White CA; Kruijnk S; Johnson OJ

This project aims to:

Use the rocky reef algal communities as a biological indicator of nutrient availability

Undertake Rapid Visual Assessment (RVA) surveys at six locations twice per year to assess functionality of algae communities.

Report on how function of algal communities may change over time.

2018

### **Top predators and feeding people from the ocean: natural behaviour, habituation, and the foraging ecology of Australian fur seals in Tasmania (2018);**

Lea, M. A.; Hindell, M. A.; Alderman, R.; Alexander, K. A.; Cummings, C. R.

Questions of how to interact with marine predators in terms of food production have never been more pressing. Healthy top predator populations are essential for regulating the mechanisms that promote biodiversity, ecosystem function and resilience (Rooney, McCann et al. 2006, Estes, Terborgh et al. 2011, Ripple, Estes et al. 2014, Lynam, Llope et al. 2017). However, interactions between predators and human food producers present various challenges (Carter and Linnell 2016, Nyhus 2016). Effects on marine wildlife range from bycatch to resource competition, to direct persecution. Conversely, carnivores can prey directly on livestock or fish corralled by humans, compete for resources, damage gear and infrastructure, and pose risks to human safety. Given that global demand for seafood is projected to increase (Smith, Roheim et al. 2010), anthropogenic climate change will impact fisheries catches (Barange, Merino et al. 2014), and that many global fish stocks are already overfished (Pikitch 2012), these challenges are likely to intensify into the future. The convergence of these factors creates a brewing storm of human wildlife conflict. Developing methods of interacting with wildlife while producing food from the ocean, that promote sustainable coexistence, is paramount to their long-term conservation. Interactions between seals, wild fisheries and aquaculture are a longstanding issue in Tasmania. While social, economic, and political dimensions influence the way society approaches seal-fishery interactions, an understanding of the relevant ecological dynamics of the species in question should be equally influential in this regard. Yet we currently understand very little about the way the Australian fur seal, *Arctocephalus pusillus doriferus*, uses the Tasmanian marine environment. Developing an understanding of the foraging ecology of this species in the region represents an opportunity to inform evidence based, scientific fisheries management policies, as well as to enhance our understanding of the local marine environment. This socioecological project therefore seeks to fill key data gaps in our understanding to the ecology of the species in the region in terms of spatiotemporal habitat use and diet, explore how this information can inform more intelligent management practices, and ask what trade-offs can be agreed upon by multiple and diverse stakeholders to articulate broadly endorsed goals for coexistence.

**Storm Bay Observing System: Assessing the Performance of Aquaculture Development, Project: 2018-131;**

Ross, D. J.; MacLeod, C.; White, C. A.; Hadley, S. A.; Barrett, N. S.; Swadling, K. M.; Lucieer, V. L.

Storm Bay is identified in the Tasmanian Governments Sustainable Industry Growth Plan for the Salmon Industry as a priority area for the possible expansion of salmonid marine farming. The Governments intention is that the proposed developments would be managed under an adaptive management framework with the following pre-conditions: A staged development approach, with an initial limit on feed input that would provide for 30,000 tonnes of production. A comprehensive environmental monitoring program. The development of a biogeochemical model, to help to understand i) the information provided by the environmental monitoring and ii) the effects of any changes to farming operations in the region. The work proposed in this study will support the design and implementation of an effective, efficient and reliable monitoring program, providing expert advice on sampling locations, timelines and strategies and testing this data over 3-4 years to refine and improve the program. This monitoring program will deliver observational data to directly assess environmental performance and to assist the skill assessment of the integrated 3D model being developed by CSIRO. A second key element of the proposal is to develop a lease scale module for predicting near scale effects; this would be nested and relocatable in the CSIRO modelling system. A comprehensive map of seafloor habitats and bathymetry will underpin the monitoring program and modelling efforts.

2017

**Seal relocation risk, effectiveness and natural context – Phase 1 (2017)**

Lea, M. A.; Hindel, M. A.; Alexander, K. A.; Alderman, R.

This research project will investigate the risks, effectiveness, and natural context of the Tasmanian fur seal relocation program using biotelemetry to assess seal movement and foraging behaviour in SE Tasmania.

**Communicating the research, management and performance of Tasmanian marine resource industries by video, Project: 2017-106;**

**Vulnerability of the endangered Maugean Skate population to degraded environmental conditions in Macquarie Harbour, Project: 2016-068 (2017 - 2020);**

Lyle, JM; Semmens, JM; Stehfest, K; Barrett, NS

This project aims to assess the impacts and implications of environmental conditions experienced in a heavily impacted estuarine system (Macquarie Harbour) on the physiology and survival of an endangered species (Maugean skate) with a view to formulating threat abatement and recovery plans for the species.

**Characterisation of successional pattern of *Beggiatoa* at Petuna Site 133 - identifying categories and related production and functional ecology (2017);**

Macleod, C

**Understanding oxygen dynamics and the importance for benthic recovery in Macquarie Harbour, Project: 2016-067 (2017 - 2020);**

Ross DJ; MacLeod C; Semmens JM

Sustainable finfish aquaculture is dependent on a benthic environment that can assimilate and process farm particulate wastes. This project will help inform the likely effectiveness and duration of any given fallowing or remediation strategy, and as such is essential for both operational management of farming activities and the long-term management of the harbour.

This will involve a combination of benthic surveys, a real time observation network and oxygen transport modelling. The central outcome of this research will be better advice on appropriate fallowing and remediation strategies for the conditions currently occurring in Macquarie Harbour. Robust scientific understanding of benthic and water column condition in the Harbour, with a particular focus on dissolved oxygen dynamics in the water column, will provide a much better appreciation of the response of benthic communities to fallowing and remediation strategies.

2016

**Huon/Channel Nutrient Enrichment Assessment - establishing the potential effects of Huon Aquaculture Company P/L (HAC) nitrogen inputs (2016);**

Ross, DJ; Macleod, C

**Characterisation of sediments in Port Esperance (2016);**

Macleod, C; Ross, DJ

This project aims to determine whether salmon aquaculture contributes to sedimentation on reefs in areas where they co-occur.

**Seal habitat use and salmon aquaculture interactions in Storm Bay, Tasmania (2016);**

Lea MA; Hindell MA

Prepare a report corresponding to following research needs: What is known about seal population, movements and colonies in Storm Bay (and wider SE TAS if relevant) What is known about seal and salmon farm interactions in Tasmania Is there a causal link between increase in salmon farms and increase in seal population?

**Effects of the Petuna salmonid farm outfall on invertebrate assemblages at Brumby's Creek (2016);**

Purser, GJ

Sampling of the Invertebrate fauna in Brumby's Creek at control, outfall and downstream sites has been undertaken on three occasions to assist Petuna identify species assemblages as part of the EPA sampling program. Samples were collected and identified.

**Petuna Storm Bay Shark Desk-Top Study (2016);**

Semmens, JM

**Uptake of aquaculture waste by keystone reef species (2016 - 2017);**

White, CA

Aquaculture of Atlantic salmon in Tasmania is currently expanding, with the potential for adverse interactions with reef ecosystems a significant concern. As reef ecosystems in Tasmania have broad conservation, social and economic values, there is a need for better understanding of how these systems may respond. There are many ways intensive farming of fish can impact on the broader environment, with enhancement of nutrients from waste feed and faeces key amongst these. Using a lipid biomarker approach, this project aims to determine whether aquaculture waste may supplement the diet of key mobile invertebrate species (abalone, *Haliotis rubra*; sea urchins, *Heliocidaris erythrogramma*; and benthic shrimp, *Palaemon intermedius*) within reef ecosystems in south-east Tasmania. Results will provide critical information on how these species may use current food resources and whether the addition of aquaculture-derived nutrients and organic carbon influences food web dynamics. This data will help assess whether these keystone species are suitable canaries for assessment of overall reef ecosystem health, and addresses a key knowledge gap regarding the broader ecosystem level interactions of intensive fish farming. This project will make an important contribution to the conservation and management of reef ecosystems, as aquaculture continues to expand into the future.

2015

**Managing ecosystem interactions across differing environments: building flexibility and risk assurance into environmental management strategies, Project: 2015-024 (2015 - 2019);**

Ross DJ; MacLeod C; Barrett NS; Wild-Allen K

This project aims to support sustainable development of the aquaculture industry by providing regionally relevant environmental management advice to both government and industry. Specifically, this project will provide robust monitoring and management advice and strategies that take into account the nuances of local farming environments. This will result in improvements in: RISK ASSESSMENT - Providing an understanding of regional variability in sediment processes/recovery associated and benthic/ pelagic coupling with existing and newly developed farming operations and the resultant implications for any assumptions used in both monitoring and in predictive (biogeochemical and depositional) modelling.

BENCHMARKING - Comparing environmental monitoring and management approaches in existing and newly developed farming areas based on previous impact/recovery understanding and associated monitoring and regulation criteria to establish those response principles that are common to all areas and those that are regionally specific (with specific reference to visual assessments and biological and biogeochemical measures).

MANAGEMENT ADVICE - Providing fit for purpose management advice to government and industry that allows regionally structured optimisation of farm management, along with risk appropriate management and monitoring criteria in different locations. Providing an understanding of the ecological significance of any differences in observed effects across regions and how that information might be used to inform and improve site specific and regional modelling and management approaches.

**TSGA IPA: Understanding Dorvilleid ecology in Macquarie Harbour and their response to organic enrichment, Project: 2014-038 (2015 - 2016);**

Ross, DJ; Macleod, C

**Natural values of the World Heritage waters of Macquarie Harbour (2015);**

Barrett NS; Edgar GJ

The project is based around a ten day survey within the TWWHA component of Macquarie Harbour. The survey will be conducted during summer autumn of 2015 and will focus on benthic communities (including epi-benthic invertebrate fauna and in-fauna), smaller fish communities (particularly at deeper depths) and aspects of the Maugean skate use of this area, such as presence of eggs, that may compliment past and current research on this species. The methodology will follow current practice for sampling each of the faunal groups to be sampled. For in-fauna, sampling will be via sediment cores in to a similar manner to that undertaken in Bathurst Harbour/Port Davey, Tasmanian estuarine biodiversity research, the My Lyell mine remediation study, and in Salmonid marine farm monitoring programs (including Macquarie Harbour). Replicated samples will be collected throughout the TWWHA region and at a range of depths, from the immediate subtidal down to 20 m. Epi-benthic mobile invertebrate fauna will be censused via timed swims with abundance recorded on a log scale as per rapid biodiversity assessment methods described for the Derwent Estuary.

**Improving prediction of rocky reef ecosystem responses to human impacts (2015 - 2019);**

Edgar GJ; Barrett NS; Knott N; Howe SA; Duffy JE

The project aims to add a new functional dimension to the understanding of inshore ecosystems, facilitating improved management of our living marine heritage. Project goals are to (i) extend huge field datasets on the density and distribution of thousands of marine fishes, invertebrates and macro-algae, (ii) combine these data using recent advances in quantitative ecological modelling to describe transfer of biomass between species at hundreds of sites with primary focus on southern Australia, and (iii) provide site-level indices of major food web processes that, when combined with before, after, control, impact data, allow improved prediction of ecological consequences of fishing, climate change, pest outbreaks, and pollution.

**Sense-T Stage 2: Sensing Macquarie Harbour (2015-2016);**

Semmens, JM; Main, A; McCulloch, J; Ross, DJ; Carter, CG; Stehfest, K

This Sense-T project brings together the Tasmanian Salmonid Growers' Association, IMAS and CSIRO to collect and analyse data from salmon farms in Macquarie Harbour. Sensors will be attached to individual 'sentinel' fish in pens in addition to sensors in the environment. Data will be collected on fish behaviour as well as environmental conditions such as water temperature, dissolved oxygen and depth. This will: help to reduce feed wastage by ensuring fish are fed when environmental conditions are suitable; collect new or previously difficult to obtain information about the harbour environment and the impact of salmon farming on variables such as oxygen levels; improve the salmon industry's efficiency and productivity; provide real-time data to support decision-making; and, highlight areas where the industry could improve its environmental practices.

**Reassessment of intertidal macroalgal communities near to and distant from salmon farms and an evaluation of using drones to survey macroalgal distribution, Project: 2014-241 (2015 - 2016);**

Crawford, C; Lucieer, A

This project is a reassessment of intertidal macroalgal communities near to and distant from salmon farms and an evaluation of using drones to survey macroalgal distribution.

2014

**Macquarie Harbour Dissolved Oxygen Assessment (2014-2016);**

Ross, DJ

The main tasks of this project can be summarised as follows: to collate and check EPA and industry monitoring data; to analyse historical time series of bottom water DO, salinity and temperature; to assist with analysis of factors related to bottom water DO including river flow, along- and across harbour wind stress, daily tidal range, fish biomass, water temperature etc. Using existing benthic flux information and carbon inputs from farming estimate the contribution of fish farming and non-aquaculture sources to oxygen consumption in the harbour.

**TSGA IPA: Predicting marine currents, nutrients and plankton in the coastal waters of south eastern Tasmania in response to changing weather patterns, Project: 2014-031;**

**Tasmania's coastal reefs: deep reef habitats and significance for finfish production and biodiversity, Project: 2014-012 (2014-2016)**

Lyle, JM; Lucieer, VL; Hill, NA; Barrett, NS; Buxton, CD

**Population ecology and genetic variability of the endangered Maugean skate in Macquarie Harbour (2014)**

Semmens, JM; Lyle, JM; Buxton, CD; Barnett, A

2013

**Postgrad Research Funds - M. Auluck - Characterising benthic pelagic interactions in Macquarie Harbour - organic matter processing in sediments and the importance for nutrient dynamics (2013 - 2014);**

Ross DJ; MacLeod C; Auluck M

Macquarie Harbour has been supporting salmon farming for the past 10 years however detailed understanding of the harbour is lacking thereby impeding necessary measures to help create an ecologically sustainable fish farming practice. The objective of this project therefore is to examine the relationship between nutrient flux rates, nutrient pathways and also sediment and water variables in Macquarie Harbour in relation to salmon fish farming. Survey works for this project involve grabbing sediment cores for nutrient fluxes will be measures. Other sediment variables would also be obtained such as macrofauna abundance and biomass, microalgae biomass and particle size distribution. The information and data obtained from this project will be used to calibrate existing water column and sediment modelling of the harbour, which Will be used as a reference to develop better management tool to further sustain the environmental health of the harbour.

**Aquaculture Production Innovation Hub: Phase II communication, extension and opportunities (2013 - 2014);**

Cobcroft JM; MacLeod C; Elizur A; Lewis Tom; Li X; Main A; Parkinson S; Robinson N

**Evaluation of practices on salmon farms to mitigate escapes and ecological impacts (2013);**

Lyle, JM

**Movement, habitat utilisation and population status of the endangered Maugean skate and implications for fishing and aquaculture operations in Macquarie Harbour, Project: 2013-008 (2013-2015)**

Lyle, JM; Semmens, JM; Buxton, CD; Barrett, NS; Ross, DJ

2012

**Understanding Behaviour in Farmed Fish Species (2012 - 2014);**

Purser GJ; Wilkinson R

**TSGA IPA: Clarifying the relationship between salmon farm nutrient loads and changes in macroalgal community structure/distribution (Existing student support), Project: 2011-042 (2012 - 2014);**

Macleod, C; Ross, DJ

**Sustainable Marine Research Collaboration Agreement (SMRCA) (2012-2021);**

Carter, CG; Gardner, C; Buxton, CD

**Seafood CRC: An evaluation of the options for expansion of salmonid aquaculture in Tasmanian waters, Project: 2011-735 (2012 - 2015);**

Macleod, C; Elliott, N

**INFORMD Stage 2: Risk-based tools supporting consultation, planning and adaptive management for aquaculture and other multiple-uses of the coastal waters of southern Tasmania (2012 - 2016);**



Condie S; Hepburn Mark; Little Rich; MacLeod C; Proctor W; Ross DJ; Wild-Allen K; Ogier EM

**Atlantic Salmon Aquaculture Subprogram: Characterising benthic pelagic interactions in Macquarie Harbour - organic matter processing in sediments and the importance for nutrient dynamics, Project: 2012-047 (2012 - 2014);**

Ross DJ; Cook PLM; Hartstein N; Lucieer VL; MacLeod C; Valentine JP; Walsh PH

**Atlantic Salmon Aquaculture Subprogram: Macroalgal monitoring in Macquarie Harbour, Project: 2011-086 (2012-2013);**

Crawford, C; Buxton, CD

**Status of fur seals and their interactions with aquaculture in Tasmania (2012);**

Hindell MA; Lea MA

2011

**Atlantic Salmon Aquaculture Subprogram: Assessment of the environmental impacts and sediment remediation potential associated with copper contamination from antifouling paint and associated recommendations for management, Project: 2011-041 (2011 - 2013);**

MacLeod C; Ross DJ; Eriksen RS; Pender AJ

2010

**Tactical Research Fund: Nutrient and Phytoplankton Data from Storm Bay to Support Sustainable Resource Planning, Project: 2009-067 (2010 - 2011);**

Crawford C; Swadling KM; MacLeod C; Frusher SD

**Atlantic Salmon Aquaculture Subprogram: Evaluation of approaches to improve sediment remediation (rate and function) under salmonid fish cages, Project: 2010-063 (2010 - 2011);**

Macleod, C; Ross, DJ

**Effect of Human Activities on the Health of Estuaries - Using C and N Stable Isotopes to Identify the Source and Fate of Organic Matter and Nitrogen (2010);**

Crawford, C; Ross, DJ; Gibson, JAE

2009

**Improving our Understanding of Welfare in Farmed Fish through Behavioural Investigations and Use of Self Feeders (2009);**

2008

**Scoping Study into Adaptation of the Tasmanian Salmonid Aquaculture Industry to Potential Impacts of Climate Change (2008)**

Battaglione, SC; Nowak, BF

**Tactical Research Fund: A review of the ecological impacts of selected antibiotics and antifoulants currently used in the Tasmanian salmonid farming industry and development of a research programme to evaluate the environmental impact of selected treatments, Project: 2007-246 (2008);**

Macleod, C; Volkman, JK

2007

**Anthropogenic Influences on the Source, Transformation and Fate of Carbon and Nitrogen in Coastal Waters: A Case Study of the Derwent Estuary (2007 - 2010);**

Ross, DJ; Keough, MJ; Crawford, C

2004

**Increased Frequency and Duration of Noctiluca 'Red Tides' in Southern Tasmanian Waters: Possible Range Extension and Impacts on Aquaculture (2004);**

Hallegraeff, GM

**A whole of ecosystem assessment of environmental issues for salmonid aquaculture (2004-2008);**

Volkman J; Thompson PA; Revill AT; Blackburn SI; Parslow J; Herzfeld M; MacLeod C; Crawford C

2001

**Ecology of Moon Jellyfish Aurelia sp. In Southern Tasmania in Relation to Atlantic Salmon Farming (2001-2003);**

Crawford, C; Moltschaniwskyj, NA

2000

**Aquafin CRC - Atlantic Salmon Aquaculture Subprogram: Development of Novel Methods for the Assessment of Sediment Condition and Determination of Management Protocols for Sustainable Finfish Cage Aquaculture Operations, Project: 2000-164 (2000 - 2004);**

MacLeod C; Burke CM; Volkman JK

**Changes in Sheltered Marine Habitats off Southeastern Tasmania Caused by Human Activity (2000);**

Edgar, GJ

1996

**Development and field testing of an algal bloom monitoring buoy for the Australian aquaculture industry (1996);**

Hallegraeff, GM

1992

**Development of an Algal Bloom Monitoring Programme for the Tasmanian Fish Farming Industry (1992);**  
Hallegraeff, GM; Jameson, I

## **Other Relevant Research**

2019

### **Aquaculture-Community Futures: North West Tasmania (2019-2021)**

Alexander, K. A.; Ogier, E. M.; Evans, J. D.

In order to secure the future of Australian aquaculture, it is increasingly clear that, alongside effective and responsible production, building and maintaining community support is vital. The Tasmanian salmon industry is acutely aware of the need to garner and maintain societal support. A number of factors contribute towards the achievement of community acceptability (or social license to operate). These include: the perception that a company offers benefits; that it contributes to the well-being of the region, respects the local way of life and acts fairly; that it listens, responds and exhibits reciprocity; and that relations are based on an enduring regard for each others interests. Many of these factors are based on understanding, and contributing towards the achievement of, that which is valued by local and regional communities. This project aims to understand the regional development and well-being futures envisaged by residents of NW Tasmania and how salmonid farming can contribute to meeting these shared values.

### **Social License to operate for aquaculture (SoLic) (2019-2022)**

Osmundsen, T.; Tvetras, R.; Alexander, K. A.

Sustainable growth in the Norwegian aquaculture industry presupposes stronger social anchoring both nationally and locally. Media analysis of the public debate on aquaculture in Norway shows that aquaculture activities are often criticized, and that disapproval is voiced from a mix of different interest groups. Improving social approval for aquaculture is a two-way process where society's knowledge about and understanding of the aquaculture industry need to be strengthened, while the industry must acknowledge its social responsibility and respond to signals from society. While we do know that the industry's social anchorage in Norway is surprisingly weak compared to other types of food production, we have less knowledge of how social approval and trust towards the industry is distributed in the public, how/whether this is linked to misconceptions, lack of knowledge, poor dialogue between the industry and the public, and/or different values. To understand the foundation for social license for aquaculture in Norway, we propose to investigate the characteristics of trust towards aquaculture activities and public regulation on a national (macro) level, distinguishing between society-industry-government, rural-urban, and centre-periphery dimensions. At the meso level, we will investigate mediatized environmental conflicts as these have been enacted in the Norwegian public arena in recent years. Finally, the investigation will include the micro level focusing the analytical lens on specific coastal communities where aquaculture is minor or major part of the industrial structure, and where economic effects are particularly visible. SoLic will focus on the context of Norwegian aquaculture but will also include smaller comparative cases in Australia and Iceland. Social license for Tasmanian aquaculture has been an ongoing research focus over the last two years, through research funded by the Human Dimensions sub-program of the Fisheries Research Development Corporation, amongst others. There is a wealth of information available that is currently not directly or easily transferable. It requires collation and synthesis

to identify the determinants of social license, as well as the mechanisms that strengthens/diminishes a social license, the consequences, and the possible strategies industry applies. This project will draw together knowledge from existing research projects, literature and documentation, and use key informant interviews to address gaps. A case study approach will be used to address the question: How does social license (or a lack of) play out in Tasmanian salmon aquaculture? A single-case study design will be used (to understand social license at the state level), but with multiple embedded units of analysis (i.e. cases at the local level). Two key analytical techniques will be used: explanation building and logic models. These will be validated by expert informants.

2018

**Planning for a Blue Future Salmon – informing R&D, regulation and industry development, Project: 2017-149 (2018);**

Carter, CG; Gardner, C; Macleod, C; Ross, DJ; Eagle, GE

The Tasmanian salmon industry is seeking to grow production safely and sustainably over the next two decades, further increasing the tangible benefits to the Tasmanian community. The aim is to deliver this by being the most environmentally sustainable salmon industry in the world creating an industry that all Tasmanians can be proud of. To achieve this bold vision for a world class salmon industry in Tasmania, it requires a clear focus on development of both sea and land-based farming operations, utilisation of the best available technologies, and collaboration with researchers and aquaculture innovators. To support this development, scientists, industry, regulators and non-government organisations will come together in late-2018 for a Salmon Symposium, with the purpose of drafting an integrated roadmap for the future of the industry. This Symposium will provide a platform to review, analyse and consider pathways to implementation of worlds best practices and future developments in farming systems, environmental management, biosecurity, science and policy. To prepare for the 2018 Symposium, the Institute for Marine and Antarctic Studies (IMAS) is hosting a pre-symposium conference from 6th-8th December 2017. This planning conference will bring together international experts who will work with industry representatives, researchers, regulators and non-government organisations to set a clear direction and build an agenda for the 2018 event. To support the planning and management of these events a steering committee was formed with representatives from UTAS, government and industry. The steering committee is supported by a science committee that has reviewed and advised on international speakers, formulated discussion topics and devised the format for the series of public talks and conference workshops that will form the initial event. The science committee will also oversee the development of a one or more green policy papers on environmental management, biosecurity and future farming operations that will assist the ongoing management of the industry and provide the basis of the 2018 symposium program. The 2018 Symposium will include workshops in order to continue the new knowledge based approach established at the 2017 meeting. The conferences will create new knowledge through the workshops that aim to document, analyse and assess current best practice in order to define World Best Practice and consider implementation at a global level.

**National Fisheries and Aquaculture Industry Contributions Study 2018, Project: 2017-210 (2018 - 2020);**

Ogier EM; Jennings SS; Morison J; McIlgorm A; Curtotti R

Objectives are to:1. Provide an estimate of the economic contribution of wild catch fisheries and aquaculture to the Australian (national) economy, and of the economic contribution of jurisdictionally-based fisheries and aquaculture make to their State/Territory economies2. Provide measures of a range of social and economic contributions made by selected

fisheries/aquaculture sectors at the regional/product scale<sup>3</sup>. Develop a robust and nationally-consistent framework to support data collection and estimation of contributions in the future

**Determinants of socially-supported fisheries and aquaculture, Project: 2017-158 (2018);**

Alexander, K. A.; Abernethy, K.

The fisheries and aquaculture industries are increasingly and acutely aware of the need to garner societal support, but unsure of how to address poor societal support at its root, who needs to be involved to address the problem, and effective pathways to improving societal support. However, there is a wealth of information available to address these gaps, including learnings from international fisheries and aquaculture, and the historical successes and failures within Australia. This project will draw together knowledge from existing literature and key informant interviews, and deliver the findings to industry in an appropriate, practical, and applicable format. The objectives of this research are to: 1. Define societal support; 2. Identify determining factors (internal and external) affecting societal support; Identify means by which to detect, assess and monitor societal support; and 4. Identify successful engagement behaviours and interventions.

2017

**Human Dimensions Research Subprogram Management (2017 - 2020);**

Ogier EM; Jennings SS

The project will conduct meta-analyses, gap analyses, synthesis and evaluation of major findings and decision-support tools from previous social science and economic research of Australian fisheries and aquaculture. It will use these research findings to inform and coordinate future FRDC RD&E investment, as well as produce specific research outputs such as meta-analysis, gap analysis, synthesis and evaluation of major findings and decision-support tools from previous social science and economic research of Australian fisheries and aquaculture. This knowledge will generate findings that will be used to develop new conceptual and analytical frameworks, as well as impact pathways, for understanding and addressing current and anticipated social and economic challenges facing fisheries and aquaculture.

2016

**From global ideals to local realities – the foundations of sustainability (2016);**

Alexander, K.A

The focus of this project is the development of robust indicators for social, economic and environmental aspects of sustainability in aquaculture. A vast number of standards and indicators have been developed worldwide, however little is known about the use of these indicators, nor what aspects are missing/contradictory/overlapping/ inadequate. It is these aspects that this project will address.

2015

**Social Science and Economics Research Coordination Program (2015 - 2017);**

Ogier, E

This project will undertake analysis and evaluation of major findings and tools from previous research aimed at improving the social acceptability and of fisheries and aquaculture, and

the optimum management of aquatic resources, as well as targeted research to meet identified gaps.

2008

**Building economic capability to improve the management of marine resources in Australia, Project: 2008-306;**

**Aquafin CRC – Atlantic Salmon Aquaculture Subprogram: Facilitation and Administration, Project: 2007-229 (2008-2009)**  
Buxton, CD

2006

**Marine Links Extension - Aquaculture (2006);**  
Purser, GJ; Adams, LR

2005

**SSA Core Membership of the Australian Food Safety Centre of Excellence, Project: 2005-620;**

2004

**Tasmanian Fisheries and Aquaculture Research and Development Plan 2003-2005, Project: 2004-313 (2004)**  
Buxton, CD; Laws, E; Ibbott, T

## Summary of IMAS Research Projects (Funded)

### FRDC Funded Projects:

Project Code	Project Title	Funding Amount(Projects)
2018-131	Storm Bay Observing System: Assessing the Performance of Aquaculture Development	\$ 3,645,677.00
2017-210	National fisheries and aquaculture industry social and economic contributions study: Phase 1	\$ 393,218.00
2017-158	Determinates of socially-supported wild-catch and aquaculture fisheries in Australia	\$ 67,832.80
2017-149	Planning for a Blue Future Salmon - informing R&D, regulation and industry development	\$ 300,000.00
2017-106	Communicating the research, management and performance of Tasmanian marine resource industries by video	\$ 84,799.90
2016-068	Vulnerability of the endangered Maugean Skate population to degraded environmental conditions in Macquarie Harbour	\$ 421,129.00
2016-067	Understanding oxygen dynamics and the importance for benthic recovery in Macquarie Harbour	\$ 2,211,646.40
2015-024	Managing ecosystem interactions across differing environments: building flexibility and risk assurance into environmental management strategies	\$ 1,078,729.00

2014-241	Reassessment of intertidal macroalgal communities near to and distant from salmon farms and an evaluation of using drones to survey macroalgal distribution	\$ 67,165.00
2014-038	TSGA IPA: Understanding Dorvilleid ecology in Macquarie Harbour and their response to organic enrichment	\$ 87,135.40
2014-031	TSGA IPA: Predicting marine currents, nutrients and plankton in the coastal waters of south eastern Tasmania in response to changing weather patterns	\$ 339,527.66
2014-012	Tasmania's coastal reefs: deep reef habitats and significance for finfish production and biodiversity	\$ 237,199.00
2013-008	Movement, habitat utilisation and population status of the endangered Maugean skate and implications for fishing and aquaculture operations in Macquarie Harbour	\$ 263,825.00
2012-048	Atlantic Salmon Aquaculture Subprogram: Culture and cryopreservation of Neoparamoeba perurans (AGD)	\$ 142,074.13
2012-047	Atlantic Salmon Aquaculture Subprogram: characterising benthic pelagic interactions in Macquarie Harbour - organic matter processing in sediments and the importance for nutrient dynamics	\$ 209,239.30
2011-735	Seafood CRC: an evaluation of the options for expansion of salmonid aquaculture in Tasmanian waters	\$ 128,268.00
2011-086	Atlantic Salmon Aquaculture Subprogram: macroalgal monitoring in Macquarie Harbour, Tasmania	\$ 26,920.24
2011-070	TSGA IPA: Comparative susceptibility and host responses of endemic fishes and salmonids affected by amoebic gill disease in Tasmania	\$ 227,357.00
2011-069	Atlantic Salmon Aquaculture Subprogram: The effects of AGD on gill function - use of a perfused gill model	\$ 75,439.99



2011-042	TSGA IPA: clarifying the relationship between salmon farm nutrient loads and changes in macroalgal community structure/ distribution (Existing Student Support)	\$ 45,000.00
2011-041	Atlantic Salmon Aquaculture Subprogram: assessment of the environmental impacts & sediment remediation potential associated with copper contamination from antifouling paint and associated recommendations for management	\$ 525,384.00
2010-218	Atlantic Salmon Aquaculture Subprogram: Hydrogen peroxide treatment of Atlantic salmon affected by AGD	\$ 27,252.00
2010-203	Atlantic Salmon Aquaculture Subprogram: oxygen regulation in Tasmanian Atlantic salmon	\$ 142,129.00
2010-063	Atlantic Salmon Aquaculture Subprogram: evaluation of approaches to improve sediment remediation (rate & function) under salmonid fish cages	\$ 74,177.00
2009-070	EI-Nemo SE: risk assessment of impacts of climate change for key species in South Eastern Australia	\$ 140,163.66
2009-067	Tactical Research Fund: Nutrient and phytoplankton data from Storm Bay to support sustainable resource planning	\$ 44,143.00
2008-306	Building economic capability to improve the management of marine resources in Australia	\$ 948,572.00
2008-222	Atlantic Salmon Aquaculture Subprogram: Rickettsia-like organism vaccine development for the salmonid aquaculture industry	\$ 157,000.00
2008-218	Atlantic Salmon Aquaculture Subprogram: extension funding application- AGD Vaccine phase III	\$ 612,000.00

2007-246	Tactical Research Fund: A review of the ecological impacts of selected antibiotics and antifoulants currently used in the Tasmanian salmonid farming industry and development of a research programme to evaluate the environmental impact of selected treatments.	\$ 52,560.00
2007-229	AquaFin CRC - Salmon Aquaculture Subprogram: Facilitation and administration	\$ 46,143.00
2005-620	SSA Core Membership of the Australian Food Safety Centre of Excellence	\$ 38,400.00
2005-201	AquaFin CRC - Atlantic Salmon Aquaculture Subprogram: environmental control of growth and early maturation in salmonids	\$ 333,571.00
2004-237	Aquaculture Nutrition Subprogram: assessment of growth performance under limiting environmental conditions	\$ 466,788.00
2004-221	AquaFin CRC - Enhanced hatchery production of Striped Trumpeter, <i>Latris lineata</i> , in Tasmania through system design, microbial control and early weaning	\$ 1,199,315.03
2004-215	AquaFin CRC - Atlantic Salmon Aquaculture Subprogram: establishment of challenge for AGD	\$ 652,222.00
2004-214	AquaFin CRC - Atlantic Salmon Aquaculture Subprogram: effects of husbandry on AGD	\$ 209,941.00
2004-213	AquaFin CRC - Atlantic Salmon Aquaculture Subprogram: commercial AGD and salmon health project	\$ 499,671.00
2004-210	AquaFin CRC - Atlantic Salmon Aquaculture Subprogram: use of immunomodulation to improve fish performance in Australian temperate water finfish aquaculture	\$ 288,959.00
2003-200	AquaFin CRC - Atlantic Salmon Aquaculture Subprogram: strategic planning, project management and adoption	\$ 211,983.00

2001-246	Aquafin CRC - Atlantic Salmon Aquaculture Subprogram: control of precocious sexual maturation in Atlantic salmon	\$ 521,251.00
2001-245	Aquafin CRC - Atlantic Salmon Aquaculture Subprogram: model development for epidemiology of Amoebic Gill Disease	\$ 322,051.00
2001-244	Aquafin CRC - Atlantic Salmon Aquaculture Subprogram: host-pathogen interactions in Amoebic Gill Disease	\$ 860,814.00
2001-205	Aquafin CRC - Atlantic Salmon Aquaculture Subprogram: treatment and pathophysiology of Amoebic Gill Disease	\$ 659,515.00
2000-266	Atlantic Salmon Aquaculture Subprogram: effective treatments for the control of amoebic gill disease	\$ 86,392.00
2000-223	Aquafin CRC - Atlantic Salmon Aquaculture Subprogram: facilitation, administration and promotion	\$ 210,366.00
2000-164	Aquafin CRC - Atlantic Salmon Aquaculture Subprogram: development of novel methods for the assessment of sediment condition and determination of management protocols for sustainable finfish cage aquaculture operations	\$ 467,269.00
1999-201	Aquafin CRC - Atlantic Salmon Aquaculture Subprogram: development of selective enrichment culture-polymerase chain reaction (SEC-PCR) for the detection of bacterial pathogens in covertly infected farmed salmonid fish	\$ 155,784.00
1998-322	Aquaculture Diet Development Subprogram: feed development for atlantic salmon ( <i>Salmo salar</i> )	\$ 203,109.00
1993-233	Optimisation of feed distribution to salmon in sea-cage culture	\$ 50,314.00

1993-120.05	Aquaculture diet development subprogram: fish meal replacement in aquaculture feeds for Atlantic salmon	\$ 190,768.75
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**Projects Funded Through Other Agencies:**

Start Year	Funding source	Project Title	Total cash funding
2019	BioMar Ltd	Optimising dietary phosphorus inclusion to reduce skeletal deformity in triploid Atlantic salmon	\$136,888
2019	Derwent Estuary Program	Derwent Estuary Program Reef Monitoring	\$31,495
2019	The Research Council of Norway	Social License to operate for aquaculture (SoLic)	\$65,369
2019	Department of Industry, Innovation and Science	CRC Project: Seaweed solutions for sustainable aquaculture	\$2,921,653
2018	Holsworth Wildlife Research Endowment	Top predators and feeding people from the ocean: natural behaviour, habituation, and the foraging ecology of Australian fur seals in Tasmania	\$6,735
2018	Huon Aquaculture	Does sedation of fish during transfer to and treatment with FW influence AGD redevelopment, fish performance and stress in vivo?	\$48,359
2018	Huon Aquaculture	Gill health assessment – Cages 1735 & 1736	\$2,099
2018	AQUI-S New Zealand Ltd	Performance of Atlantic salmon following simulated thermal delousing with AQUI-S sedation	\$33,369
2018	Cawthron Institute New Zealand	Cawthron Collaborative Research for three PhD Operating & TAG Travel Costs	\$89,650
2018	Cawthron Institute New Zealand	Skin health of New Zealand farmed Chinook salmon	\$128,520

2018	Tassal Operations Pty Ltd	Understanding gill necrosis in farmed Atlantic salmon	\$24,000
2017	Tassal Operations Pty Ltd	Seal relocation risk, effectiveness and natural context – Phase 1	\$234,581
2017	New Zealand and Ministry for Primary Industries	Expert review of report on 'Grouping Aquaculture Species by Environmental Effects: Preliminary Assessment'	\$2,000
2017	Petuna Aquaculture Pty Ltd	Characterisation of successional pattern of Beggiatoa at Petuna Site 133 - identifying categories and related production and functional ecology	\$4,282
2017	Huon Aquaculture	Gill health assessment of harvestable Atlantic salmon	\$7,087
2017	Skretting Australia	Gross scoring of fixed gill samples for EAF/Skretting	\$10,928
2017	Skretting Australia	Salmonid Nutrition and Performance	\$41,210
2017	Skretting Australia	Salmonid Nutrition and Performance – Fish & Digestibility Chemical Analysis	\$11,554
2017	Cawthron Institute New Zealand	Literature Review – chinook salmon	\$10,099
2017	Huon Aquaculture	Commercial treatment of AGD affected Atlantic salmon	\$9,909
2017	ARC	Development of an amoebic gill disease vaccine to protect Atlantic salmon	\$375,000
2016	DPIPWE	Huon/Channel Nutrient Enrichment Assessment - establishing the potential effects of Huon Aquaculture Company P/L (HAC) nitrogen inputs	\$264,312
2016	DPIPWE	Environment: Planning	\$156,300
2016	DPIPWE	Environment: Emerging issues	\$234,450
2016	DPIPWE	Environment: Monitoring and Management	\$234,450
2016	DPIPWE	Characterisation of sediments in Port Esperance	\$19,176
2016	New Zealand Ministry for Primary Industries	Expert review of the methodology for benthic site assessments for proposed salmon sites	\$4,720

2016	CBM Sustainable Design	Seal habitat use and salmon aquaculture interactions in Storm Bay, Tasmania	\$8,818
2016	Petuna Aquaculture Pty Ltd	Effects of the Petuna salmonid farm outfall on invertebrate assemblages at Brumby's Creek	\$5,461
2016	Petuna Pty Ltd	Petuna Storm Bay Shark Desk-Top Study	\$6,900
2016	Scottish Association of Marine Science	From global ideals to local realities – the foundations of sustainability	\$30,123
2016	Royal Zoological Society of New South Wales	Uptake of aquaculture waste by keystone reef species	\$7,000
2016	CSIRO-Commonwealth Scientific & Industrial Research Organisation	Macquarie Harbour Dissolved Oxygen Drawdown	\$10,909
2015	DPIPWE	Natural values of the World Heritage waters of Macquarie Harbour	\$52,000
2015	Norwegian University of Life Sciences	Immune gene expression in early amoebic gill disease	\$10,215
2015	BC Salmon Farmers Association	AGD Literature Review	\$3,539
2015	Australian Research Council	Improving prediction of rocky reef ecosystem responses to human impacts	\$1,352,100
2015	TSGA	Sense-T Stage 2: Sensing Macquarie Harbour	\$677,100
2015	DSM Nutritional Products France, Skretting Australia, Van Diemen Aquaculture Pty Ltd	Dietary carotenoid utilisation at high temperature by Atlantic salmon ( <i>Salmo salar</i> )	\$70,000
2014	Skretting Aquaculture Research Centre	The effect of diet on the response of Atlantic Salmon to Amoebic Gill Disease (AGD) 2	\$122,834

2014	Huon Aquaculture	Further Optimisation of a combination treatment for AGD	\$32,661
2014	Huon Aquaculture	Optimising a combination treatment for AGD in Atlantic salmon	\$23,114
2014	Winifred Violet Scott Charitable Trust; Holsworth Wildlife Research Endowment	Population ecology and genetic variability of the endangered Maugean skate in Macquarie Harbour	\$100,000
2014	Huon Aquaculture	Investigating the impact of nutrition, in-feed bioactive compounds and environment on gut and gill microbial population dynamics and histology in farmed Atlantic salmon	\$103,325
2014	TSGA	Macquarie Harbour Dissolved Oxygen Assessment	\$97,946
2014	ASC RC	Ongoing salmon and oyster research in a new Experimental Aquaculture Facility (EAF)	\$500,000
2013	Tassal Operations Pty Ltd	Evaluation of practices on salmon farms to mitigate escapes and ecological impacts	\$10,900
2013	Skretting Aquaculture Research Centre	The effect of diet on the response of Atlantic salmon to amoebic gill disease	\$112,374
2013	BioMar Ltd	Effect of diet on resistance to Amoebic Gill Disease	\$70,633
2013	Advanced Analytical Australia Pty Ltd	Analysis of fish samples	\$16,408
2013	Huon Aquaculture	Combination treatment of Atlantic salmon affected by amoebic gill disease (AGD)	\$17,255
2013	Marine Harvest	Presence of N. Perurans in the environment	\$6,071
2013	Tassal Operations Pty Ltd	Detection of Neoparamoeba perurans in water samples	\$2,065
2013	ASC CRC	Aquaculture Production Innovation Hub: Phase II communication, extension and opportunities	\$297,387
2013	DHI Water and Environment (M) SDN. BHD	Postgrad Research Funds - M. Auluck - Characterising benthic pelagic interactions in Macquarie Harbour - organic matter processing in sediments and the importance for nutrient dynamics	\$25,262

2012	Ridley AgriProducts Pty Ltd	Supply of amoebae attached to Petri dishes for in vitro trials at Huon Aquaculture to be carried out by Ridley AgriProducts Pty Ltd	\$7,223
2012	Univeristy of the Sunshine Coast	Atlantic Salmon jaw deformity in culture	\$20,000
2012	Sketting Pty Ltd	Assessing the biological value of Tasmanian grown lupins in salmon feeds	\$8,756
2012	Tassal Operations Pty Ltd	Status of fur seals and their interactions with aquaculture in Tasmania	\$17,744
2012	Department of Industry and Science, DPIPWE, Huon Aquaculture, Skretting Australia	Experimental Aquaculture Facility (EAF)	\$4,768,000
2012	DPIPWE	Sustainable Marine Research Collaboration Agreement (SMRCA)	\$32,894,834
2012	Ridley Aqua-Feed Pty Ltd	Understanding Behaviour in Farmed Fish Species	\$30,000
2011	CRC	Seafood CRC: an evaluation of the options for expansion of salmonid aquaculture in Tasmanian waters	\$233,488
2011	Pfizer	Tenacibaculum maritimum vaccine trial	\$62,782
2011	University of Tasmania	How do Atlantic salmon recover from hypoxic events? – Impacts of water temperature and genetics	\$15,000
2011	University of Tasmania	Alternative treatment strategies for amoebic gill disease; is hydrogen peroxide a potential therapeutic option?	\$13,771
2011	Skretting Pty Ltd	The effect of an immunostimulating and immunosupporting diet (Protec) on the response of Atlantic Salmon to amoebic gill disease (AGD)	\$87,362
2011	Leigh Atkinson	Ingredient assessment of locust and grasshopper meal, for inclusion in salmonid feeds	\$6,300



2010	Winifred Violet Scott Charitable Trust	Effect of Human Activities on the Health of Estuaries - Using C and N Stable Isotopes to Identify the Source and Fate of Organic Matter and Nitrogen	\$48,000
2010	Australian Academy of Science	Understanding Fish-pathogen Interactions in Diseases affecting Australian Mariculture	\$10,000
2010	AQUI-S New Zealand Ltd	Effect of AQUI-S anaesthesia during freshwater treatment on AGD re-development	\$6,022
2009	Naturxan LLC	Comparative study on the impact of Phaffia Rhodozyma yeast & other stimulatory substances on the immune response in Atlantic Salmon	\$81,177
2009	Australian Academy of Science	Improving our Understanding of Welfare in Farmed Fish through Behavioural Investigations and Use of Self Feeders	\$10,200
2009	Skretting Pty Ltd	Skretting Australia Salmon Nutrition Trials	\$54,025
2009	University of Tasmania	Fish Meal Replacement in Aquaculture Feeds: Tracing the Use of Plant Based Proteins and Lipids in Crustacean Body Tissues	\$9,135
2009	Skretting Pty Ltd	Skretting #9: Understanding Digestive Tract Function in Atlantic Salmon	\$19,815
2008	Skretting Pty Ltd	Skretting #8: Dietary development for Atlantic salmon at high temperature	\$49,879
2008	Natural Heritage Trust	Scoping Study into Adaptation of the Tasmanian Salmonid Aquaculture Industry to Potential Impacts of Climate Change	\$50,000
2008	TSGA	Postgraduate Support – Reovirus detection and epidemiology	\$12,000
2008	ARC	Reducing skeletal malformations in cultured marine fish using gene expression, improved nutrition and advanced system operation	\$614,307
2008	ASC RC	Using the Mucosal Antibody Response to Recombinant Neoparamoeba perurans Attachment Proteins to Design an Experimental Vaccine for Amoebic Gill Disease	\$115,894
2007	University of Tasmania	Keeping Track of Fish in Aquaculture: The Application of Video and Radio Frequency Identification (RFID) Systems	\$8,000
2007	Skretting Pty Ltd	Skretting #6: Apparent Digestibility of Large Salmon	\$19,744

2007	TSGA	Polyphasic taxonomy analysis of vibriaceae and assessment of pathogenicity to salmonids	\$15,000
2007	ARC	Anthropogenic Influences on the Source, Transformation and Fate of Carbon and Nitrogen in Coastal Waters: A Case Study of the Derwent Estuary	\$875,625
2006	DPIPWE	Marine Links Extension - Aquaculture	\$42,996
2006	Skretting Australia Pty Ltd	The effect of high temperature on apparent digestability of Atlantic Salmon	\$36,864
2006	CSIRO- Commonwealth Scientific & Industrial Research Organisation	Genetic Factors Influencing Resistance to Amoebic Gill Disease (AGD) in Atlantic Salmon	\$39,000
2006	Skretting Australia Pty Ltd	Skretting Australia Salmon Feed Trials	\$35,800
2005	Skretting Pty Ltd	Apparent Digestibility of poultry products to Atlantic salmon – Tasmania	\$14,000
2004	FRDC	Tasmanian Fisheries and Aquaculture Research and Development Plan 2003-2005	\$20,000
2004	University of Tasmania	Can Feeding Entrainment Improve the Level of Food Intake and Growth of Atlantic Salmon Smolt Transferred to Seawater Grow-out?	\$20,000
2004	Skretting Pty Ltd	Nutrient intake and growth performance in Salmon – Project 2	\$28,610
2004	Univeristy of Tasmania	Increased Frequency and Duration of Noctiluca 'Red Tides' in Southern Tasmanian Waters: Possible Range Extension and Impacts on Aquaculture	\$19,300
2004	FRDC	A whole of ecosystem assessment of environmental issues for salmonid aquaculture	\$1,101,828
2003	ARC	Interdisciplinary Network for Aquatic Animal Health	\$10,000
2003	Skretting Pty Ltd	Nutrient intake and growth performance of Atlantic salmon	\$29,924

2003	Department of Education, Science and Training	Management and Control of Amoebic Gill Disease in Atlantic Salmon - International Collaboration	\$36,250
2001	Skretting Pty Ltd	Effect of Fish Oil Replacement on Atlantic Salmon Performance under Tasmanian Summer Conditions	\$35,000
2001	ARC	Why do Fish Die from Gill Diseases?	\$111,000
2001	Aquatas Pty Ltd, Huon Aquaculture, Nortas, Tassal Ltd	Ecology of Moon Jellyfish Aurelia sp. In Southern Tasmania in Relation to Atlantic Salmon Farming	\$180,095
2000	ARC	Why do Atlantic Salmon Die from Amoebic Gill Disease: Cardiac Dysfunction?	\$10,000
2000	ARC	Changes in Sheltered Marine Habitats off Southeastern Tasmania Caused by Human Activity	\$1,360
1999	University of Tasmania	Factors Affecting AGD-related Mortality in Cultured Atlantic Salmon	\$11,830
1999	ARC	Pathophysiology of Amoebic Gill Disease in Atlantic Salmon: a new approach	\$9,000
1998	FRDC	A vision of Tasmania's Aquaculture and Fishing Industry Development - Plans to Achieve it	\$9,445
1996	University of Tasmania	Development and field testing of an algal bloom monitoring buoy for the Australian aquaculture industry	\$6,000
1995	ARC	Effects of environmental factors on the health of Atlantic salmon gills	\$84,565
1994	ARC	An integrated marine aquaculture system using Atlantic salmon and shellfish	\$83,090
1993	FRDC	Optimisation of feed distribution to salmon in sea-cage culture	\$50,319
1992	University of Tasmania	Development of an Algal Bloom Monitoring Programme for the Tasmanian Fish Farming Industry	\$6,000
1992	ARC	Nutritional energetics in Atlantic salmon with special emphasis on protein to energy ratio	\$65,381

## Appendix 3 - IMAS Research Higher Degrees Relevant to Salmon Research

### i) Selection of Relevant Higher Degree Research Projects (Completed)

2019

- Geographic Characterisation and Environmental Detection of Neoparamoeba perurans the Causative Agent of Amoebic Gill Disease
- Causes and Consequences of Hypoxia in Atlantic Salmon Aquaculture PhD

2018

- Assessing the Risks of Marine Debris Ingestion to Procellariiforme Seabirds PhD
- Factors affecting pigmentation quality in Atlantic salmon (*Salmo salar* L.) at elevated temperature PhD
- Evaluating the Extinction Risk of the Endangered Maugean skate (*Zearaja maugeana*), Macquarie Harbour, Tasmania PhD
- Measurement of Productivity in Habitat-forming Seaweeds: The impact of methodology on photosynthetic estimates PhD

2017

- The Effect of L-tryptophan on Aggressive Interactions in Barramundi (*Lates calcarifer*), and Food Intake of Atlantic Salmon (*Salmo salar*) during Seawater Transfer PhD
- Using Macrophytes to Improve the Understanding and Management of Metal Contaminated Estuaries PhD
- Assessing Anthropogenic Impacts on Reef Communities: Patterns, indicators and processes PhD
- Comparative Histopathology of Mucosa Associated Lymphoid Tissue in the Gills of Atlantic Salmon *Salmo salar* and Southern Bluefin Tuna *Thunnus maccoyii* PhD

2016

- Investigations of Skeletal Anomalies in Triploid Atlantic Salmon (*Salmo salar* L. 1758) in Freshwater with Particular Focus on Lower Jaw Deformity (LJD) PhD
- Host Response to Amoebic Gill Disease in Atlantic Salmon and Blood Fluke Infection in Pacific Bluefin Tuna PhD
- Development and Validation of Biomarkers in a Finfish Species from Southern Australian Contaminated Waters PhD
- The Application of Benthic Imagery From an Autonomous Underwater Vehicle to Broad-Scale Ecological Monitoring PhD
- Determining the Effects of Nutrient Enrichment on Macroalgae-Dominated Reefs (Observational, Experimental and Predictive Capabilities) PhD
- Unravelling the Importance of Benthic Mineralisation and Nutrient Cycling in Macquarie Harbour, Tasmania Masters
- Factors Influencing Fish Diets in Reef Food Webs PhD
- Staying Ahead of the Game: A framework for effective aquaculture decision-making PhD

2015

- Population Connectivity in Marine Macroalgae PhD
- Evaluation of Immersion Immunisation of Atlantic Salmon (*Salmo Salar*) against Yersiniosis PhD

- Farming Macroalgae to Mitigate Coastal Nitrification from Finfish Aquaculture: a modelling study PhD
- Oral Immunoprophylaxis using Microencapsulated Antigens as a Disease-management Strategy in Farmed Finfish Populations PhD
- Ecophysiology of Habitat-Forming Seaweeds in a Changing Environment PhD
- Antimicrobial peptides: Immunomodulatory and therapeutic potential for use in Atlantic salmon (*Salmo salar*) Masters
- Extoparasites and Associated Pathogens Affecting Farmed Salmon During Marine Grow Out PhD

#### 2014

- Using the Mucosal Response to Recombinant *Neoparamoeba perurans* Attachment Proteins to Design an Experimental Vaccine against Amoebic Gill Disease (AGD) PhD [Completed 2014]
- Novel Chlamydia-like Agents of Epitheliocystis in Wild and Cultured Australian Finfish PhD [Completed 2014]

#### 2013

- Relationships and Interactions between Temperate Reef Fish Communities, Physical Habitat Structure and Marine Protection PhD [Completed 2013]
- Quantifying and Predicting Benthic Enrichment: Lessons Learnt from Southern Temperate Aquaculture Systems PhD [Completed 2013]
- Natural and Anthropogenic Ecosystem Regime Variance Within a Tide-dominated Estuary: A Late Anthropocene Paleo-reconstruction PhD [Completed 2013]
- Novel Methods for Quantifying Movement Behaviour of Free-ranging Fish from Telemetry Data PhD [Completed 2013]
- Testing and Evaluating Non-Extractive Sampling Platforms to Assess Deep-water Rocky Reef Ecosystems on the Continental Shelf PhD [Completed 2013]

#### 2012

- Rare Marine Macroalgae of Southern Australia PhD [Completed 2012]
- Trade-offs Between Biodiversity Conservation and Maintaining Fisheries Yield from Australian Marine Environments; Approaches Using the Atlantis Ecosystem Modelling Framework PhD [Completed 2012]
- Detection of Aquareovirus in Farmed Tasmanian Atlantic Salmon (*Salmo Salar*) PhD [Completed 2012]
- Computational Techniques for Automated Tracking and Analysis of Fish Movement in Controlled Aquatic Environments PhD [Completed 2012]
- The Effects of Catchment Landuse on Estuaries: Using Macroinvertebrates as Bio-indicators Masters [Completed 2012]
- Biosecurity Under Uncertainty: The Influence of Information Availability and Quality on Expert Decision-making for Risk Outcomes PhD [Completed 2012]

#### 2011

- Select High Value Australian Finfish: Residues and Contaminants of Importance to Public Health and Market Access PhD [Completed 2011]
- Alternatives to Fish Oil Substitution - An Assessment of Strategies for Sustaining n-3 Long Chain Polyunsaturated Fatty Acids (n-3 LC-PUFA) Levels in Salmonids PhD [Completed 2011]
- The Tag Location Problem PhD [Completed 2011]
- Responses of Temperate Mobile Macroinvertebrates to Reef Habitat Structure and Protection from Fishing PhD [Completed 2011]

2010

- The Identification and Development of Probiotics for Use in Marine Fish Hatcheries Masters [Completed 2010]
- Nitrogen Uptake by Phytoplankton in the Huon Estuary: With Special Reference to the Physiology of the Toxic Dinoflagellate *Gymnodinium Catenatum* Masters [Completed 2010]
- Assessment of Resistance to Amoebic Gill Disease in the Tasmanian Atlantic Salmon Selective Breeding Population PhD [Completed 2010]

2009

- Analysis of Marine Animal Behaviour from Electronic Tagging and Telemetry Data using State-space Models PhD [Completed 2009]
- Qualitative Modelling to Aid Ecosystem Analyses for Fisheries Management in a Data-Limited Situation PhD [Completed 2009]
- Phosphorous Requirement of Atlantic Salmon (*Salmo Salar* L.) at Elevated Temperature PhD [Completed 2009]
- The Aetiology of Amoebic Gill Disease (AGD) and Aspects of the Host Immune Response to Infection PhD [Completed 2009]

2008

- Benthic Respiration and Nutrient Cycling in the Huon Estuary (Southern Tasmania) PhD [Completed 2008]
- Oral Treatments for Amoebic Gill Disease (AGD) in Atlantic Salmon, *Salmo salar* PhD [Completed 2008]
- Amoebic Gill Disease of Atlantic Salmon: Resistance, Serum Antibody Response and Factors that May Influence Disease Severity PhD [Completed 2008]
- Molecular Assessment of Resistance to Amoebic Gill Disease PhD [Completed 2008]

2007

- The Assessment of Omega 3 Oil Sources for Use in Aquaculture - Alternatives to the Unsustainable Harvest of Wild Fish Stocks PhD [Completed 2007]
- Factors Affecting Feed Intake, Aggression, Growth and Condition around Transfer to Sea in Atlantic Salmon (*Salmo salar*) PhD [Completed 2007]
- Vaccination of Atlantic Salmon (*Salmo salar*) against Marine Flexibacteriosis PhD [Completed 2007]
- Interactions between *Neoparamoeba* spp. and Atlantic Salmon (*Salmo salar* L.) Immune System Components PhD [Completed 2007]

2006

- Role of Bacteria in Amoebic Gill Disease PhD [Completed 2006]
- The Recovery of Benthic Communities Following Organic Enrichment: Examples from Caged Finfish Aquaculture sediment recovery from Atlantic Salmon (*Salmo salar* L.) marine aquaculture operations PhD [Completed 2006]
- Ecology of Moon Jellyfish *Aurelia* Sp, in Southern Tasmania in Relation to Atlantic Salmon Farming PhD [Completed 2006]
- Potential Risk Factors of Amoebic Gill Disease in Tasmanian Atlantic Salmon PhD [Completed 2006]
- The Pathophysiology of Amoebic Gill Disease (AGD) in Atlantic Salmon (*Salmo salar* L.) PhD [Completed 2006]
- Potential Biological Control Agents for the European Green Crab, *Carcinus maenas*, in Australian Waters PhD [Completed 2006]

2005

- Immune-regulatory Genes in Amoebic Gill Disease: Potential for Immunomodulation PhD [Completed 2005]

2004

- Improving the treatment of amoebic gill disease in salmonids with soft freshwater and the mucolytic drug L-cysteine ethyl ester PhD [Completed 2004]
- Pathology of amoebic gill disease in Atlantic Salmon (*Salmo salar* L.) PhD [Completed 2004]

2002

- Epidemiology of amoebic gill disease PhD [Completed 2002]
- The culture of copepods as live food for marine fish larvae PhD [Completed 2002]
- Spatial utilization by herbivores in Tasmania PhD [Completed 2002]

2001

- Ecological risk assessment PhD [Completed 2001]
- Marine product replacement in Atlantic salmon feeds PhD [Completed 2001]
- Integrated open-water mussel and salmon culture PhD [Completed 2001]

2000

- Optimisation of Feed Distribution to Sea Caged Fish with an Emphasis on Atlantic Salmon (*Salmo salar* L.) Masters [Completed 2000]
- Analysis and toxicity of free copper in seawater PhD [Completed 2000]
- Ecotoxicology of Contaminated Marine Sediments PhD [Completed 2000]

## ii) **Selection of Relevant Higher Degree Research Projects (Current)**

PhD Students:

- Clarifying and Optimising the Environmental Benefits of IMTA [Commenced 2019]
- Are We Getting Through? Evaluating the Success and Impact of Science Communication and Engagement Activities [Commenced 2019]
- Ecology and Conservation Biology of Red Handfish [Commenced 2019]
- Tracing the Fate of Fish farm Derived Nutrients in the Broader Marine Environment [Commenced 2019]
- Developing a Process Model for Recirculating Aquaculture System (RAS) Technology Incorporating a Fugacity Model to Track Waste Signal [Commenced 2018]
- The Effect of Aquaculture on Marine Microbial Communities [Commenced 2018]
- Skin Health in Chinook Salmon Farmed in New Zealand [Commenced 2018]
- Identification of a Feed Intake Marker in Atlantic and Chinook Salmon using Proteomics and Transcriptomics [Commenced 2018]
- Identification of Candidate Vaccine Antigens to Protect Atlantic Salmon from Amoebic Gill Disease [Commenced 2018]
- Impact of nutritional and environmental factors on the proteome and gene expression in Atlantic salmon [Commenced 2018]
- Relationship between Gut Microbiome and Health in Chinook Salmon [Commenced 2018]
- Gill Necrosis in Farmed Atlantic Salmon [Commenced 2018]
- Examining the Physiological Tolerance of the Prey of the Maugean Skate in Macquarie Harbour to Low Dissolved Oxygen [Commenced 2018]
- Understanding How the Microbiome of Host and Pathogen Affects AGD Pathogenesis [Commenced 2018]

- The Ecological Significance and Habitat Restoration Effectiveness of Taut Ecomoooring: Assessing the recovery and temporal changes of epibenthic community and threatened species distribution [Commenced 2018]
- Top Predators and Feeding People from the Ocean: Natural Behaviour, Habituation, and the Foraging Ecology of Fur Seals in Tasmania [Commenced 2017]
- Development of a New Size - Structured Ecological Model for Assessing Human Impacts on Coastal Food Webs [Commenced 2017]
- Relative Values of the Coastal and Marine Environment: Ecosystem service valuation in multi-use governance contexts [Commenced 2017]
- Developing Remote Operated Vehicles (ROVs) as a Platform for Robust Video-Based Surveys of Biodiversity in Deep-Water Habitats across the Tasmanian Continental Shelf [Commenced 2017]
- Enhancing Decision Making in a Multi-Use Management Environment by Capitalising on Available Information: Linking data sources through GIS to provide meaningful decision support outputs [Commenced 2017]
- Broad-Scale Assessment of the Role of Macrofauna in Reef Food Webs [Commenced 2016]
- The Nature, Dynamics and Complexity of Environmental Conflict in Governance and Corporate Structures; How can positive change be effectively communicated? [Commenced 2016]
- Participation and Political Representation: A critique of 'participation' in marine governance [Commenced 2016]
- Mapping Marine Ecosystem Services to the Total Economic Value Framework [Commenced 2016]
- Considerations for Monitoring River Catchments in relation to Potential Environmental Impacts from Intensive Aquaculture Systems [Commenced 2016]
- An Integrated Study of a Rapidly Changing Continental Shelf Ecosystem: Linking physical conditions, prey field dynamics and top predator behaviour [Commenced 2015]
- Influence of *Ecklonia radiata* patch characteristics on associated communities [Commenced 2015]
- Prediction, Characterisation, and Detection of Regime Shifts in Ecological Systems [Commenced 2014]
- Treatment of Amoebic Gill Disease (AGD) Caused by *Neoparamoeba Perurans* in *Salmo Salar* (Atlantic Salmon) [Commenced 2013]
- Tasmanian Coastal Waste and Marine Debris: An investigation into causal factors and educational strategies to address coastal waste and marine debris in Tasmania [Commenced 2013]
- Examining the Effect of Fear, Risk Effects and Secondary Effects of Predation on Social Behaviour in Fish in the Context of Aquaculture and Conservation [Commenced 2013]
- Analytical Tools for Estimating Predator Diets in Marine Ecosystems [Commenced 2008]

Masters Students:

- The Under-Utilisation of Australia's Living Marine Resources [Commenced 2019]