ENHANCED HATCHERY PRODUCTION OF STRIPED TRUMPETER, *Latris lineata*, IN TASMANIA THROUGH SYSTEM DESIGN, MICROBIAL CONTROL AND EARLY WEANING

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1 NON TECHNICAL SUMMARY

2004/221	Aquafin CRC - Enhanced hatchery production of Striped Trumpeter, *Latris lineata*, in Tasmania through system design, microbial control and early weaning

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OBJECTIVES:

1. Investigate the control of microbial communities in intensive larval fish culture using ozonation and probiotics.
2. Determine the optimal environmental parameters, and water quality systems and tank design for reducing hatchery mortality and malformations in finfish larvae.
3. To better understand "grey gut syndrome" and the ontogeny of the immune system, including linkages to developments with probionts and immunostimulants.
4. Evaluate formulated diets and their use in early weaning.
5. Evaluate the growth and survival of Striped Trumpeter post-larvae and juveniles reared under semi-commercial conditions.
6. Evaluate the possibility for the culture of Striped Trumpeter using alternative systems and/or sites.

Striped Trumpeter, *Latris lineata*, are one of Australia's finest eating fishes and are native to south-eastern Australia and New Zealand. They have long been considered the best candidate for diversifying sea cage culture in Tasmania. Research into the culture of Striped Trumpeter has been underway since the 1990's and the following report concludes 14 years of continuous research through the Aquaculture CRC (1994 to 2000) and Aquafin CRC (2001 to 2008). The unusually long post-larval or "paperfish" stage lasting up to nine months and requirements for oceanic conditions made them a difficult species to culture. The current CRC project (2005 to 2008) built on the excellent research foundation in three critical areas. First, variability and larval mortality was reduced through improved health and better control of bacterial diseases and parasites. Second, there was further development of live feed, weaning, and grow-out diets, nutritional and other factors, particularly those influencing malformations. Third, the larger-scale assessment of survival and growth of post-larvae and juveniles was undertaken on a semi-commercial scale and culminated in the grow-out of Striped Trumpeter in sea cages.

Improving larval survival was achieved through a better understanding of the microbial flora in culture tanks associated with fresh and frozen algae concentrates. New bacterial identification methods gave superior insights into the complex bacterial communities and how they change during larval rearing. Potential probionts improved survival in live feed cultures of rotifers. Growth and larval survival were better when the probionts were transferred to larvae via rotifers but not when added directly to the culture water. Development of novel filtration and ozonation systems delivered seawater to the marine fish

hatchery which totally removed the need for antibiotics. Ozonation of Striped Trumpeter eggs stopped the vertical transfer of nodavirus from broodstock to the larvae while water treatment prevented myxozoan parasites from becoming established in larval and juvenile fish. Larval feeding rate was optimised by changing the larval visual environment with greenwater, light intensity and tank colour.

Jaw malformations have been a recurrent obstacle in the hatchery production of high quality juvenile Striped Trumpeter. This is a problem shared with many marine finfish species including Yellowtail Kingfish. Nutrition and temperature were examined along with manipulation of the physical culture environment and larval behaviour to reduce jaw malformations. The onset of jaw malformation after metamorphosis in Striped Trumpeter follows changes in larval behaviour from an even distribution throughout the water column to close association with the tank walls, often with vigorous swimming into the walls known as ‘walling’ behaviour. Potential mechanisms for the influence of walling behaviour on jaw malformation are mechanical damage and poor nutrition, via reduced feed intake and increased energy expenditure. The project highlighted the often overlooked importance of hard-surface interactions in the growth and survival of cultured marine fish and demonstrated a cheap and effective technique for assessing tank background colour as a means of reducing malformations in cultured fish. Batches of fish with up to 76% commercially acceptable good jaws were produced.

Weaning of post-larvae from live feeds to formulated diets had been a bottleneck to large-scale production of Striped Trumpeter juveniles. Experimentally, early weaning was achieved from 30 days post-hatch using new improved diets from Europe. The weaning of fish in production trials can now routinely be achieved by 40 days post-hatch. With the excellent improvements in the production of weaned larvae attention was focused on increasing the quality of post-larvae. Prior to the current project almost nothing was known about the optimal rearing conditions for post-larvae. Temperature has a profound effect on fish growth and determining the optimum temperature was important in understanding growth and defining further nutritional characteristics. The optimum temperature for rearing Striped Trumpeter post-larvae changes with age from 16^{o}C to 14^{o}C. The optimum lipid content and diet ration were also examined. Polynomial models predicted a feeding rate of 4 % biomass day^{-1} to be optimal. The increased understanding of post-larval rearing conditions was integrated into a new nursery rearing facility incorporating recirculation systems and temperature control, designed to produce batches of 10,000 post-larvae for future on-growing trials in sea cages.

As the project moved towards commercial-scale grow-out, knowledge of the immune system became more important. Striped Trumpeter were found to produce an antibody molecule similar to that of other teleosts. Initial investigations suggest that they begin to produce antibodies much later than other marine fish. No antibodies have been detected in fish less than six months old. These unusual findings could affect future husbandry and vaccination practices. The holding of fish at higher densities led to the discovery of two new previously undescribed parasitic copepods. The two species were fully described and their life histories examined with the aim of developing effective control and treatment regimes. The first species *Chondracanthus goldsmidi* was described from the gills and opercula and occurred in sufficiently high infestations in tanks to be considered a potential problem. Trials were conducted to establish the efficacy of freshwater, hydrogen peroxide and in-feed medications. The second parasite *Caligus nuenonnae* n. sp. was described from the skin but was not a significant health issue.

Research culminated in sea-cage grow-out trials using hatchery produced Striped Trumpeter. Around 2,500 juvenile fish were stocked in a commercial salmon farm within the Huon River region and on-grown for up to two years. The fish were fed formulated diets in three 20 m sea cages. The trials provided the first hatchery-reared Striped Trumpeter grown to market size in sea cages. Overall survival was good ranging from 66 to 94% and the average weight of harvested fish was 1430±9g. HOG (head-on, gilled, gutted) yield was excellent at 94%. Growth and survival may have been higher at a more marine dominated site. The water salinity was often below full salinity during the winter and sea temperature ranged from 9.4°C to 18.9°C averaging 13.9°C. Over 2 t of fresh farmed fish were sold and market feedback was highly positive. A basic economic analysis of Striped Trumpeter farming suggested it would take 14 years to reach 5,000 t production at which point cumulative profit could eclipse cumulative spend.

The project delivered timely scientific and production outputs. An important component of the project involved post-graduate training. The project supported five post-graduate students. Much of the research has been published, and all of it has been subjected to scrutiny through conferences and workshops. The research results and techniques developed have direct application to other marine species and the Striped Trumpeter team have worked with leading researchers in Australia and overseas to apply the techniques developed on a range of marine species including Yellowtail Kingfish and Southern Blue-fin Tuna.

OUTCOMES ACHIEVED TO DATE

The three main planned outcomes from the project were in large measure achieved:

1. Australian aquaculture has been provided with more systematic ways to control microbial communities, and to evaluate, identify and produce probionts for use in improving hatchery survival rates in finfish. Microbial control has been achieved in culture through the innovative use of ozonated seawater in both live feeds and larval rearing.

2. There will ultimately be a greater choice of new marine fish species available for culture through the efficient technology transfer between research agencies and industry of new products and systems for culturing marine fish larvae.

3. The Atlantic salmon industry has moved significantly closer to diversification into new species, particularly Striped Trumpeter through sea cage trials using cultured fish. In time this should improve their profitability while reducing their risks, hence ensuring their long-term sustainability.

KEYWORDS: Striped Trumpeter, *Latris lineata*, finfish hatchery technology, probiotics, ozone, larval fish malformation, larval weaning, Tasmania

2 ACKNOWLEDGEMENTS

This project formed part of the Research Program of the CRC for Sustainable Aquaculture of Finfish (“Aquafin CRC”), and employed funds invested out of the CRC’s Commonwealth grant and by University of Tasmania and the Tasmanian Government and other Participants of the CRC. We thank the Aquafin CRC CEO Dr Peter Montague and Subprogram Leader Mr Steven Clarke, Tasmanian Aquaculture and Fisheries Institute (TAFI) Director, Professor Colin Buxton and FRDC’s Executive Director, Dr Patrick Hone, and Programs Manager, Crispian Ashby for their strong support of the project. We thank Mr Pheroze Jungalwalla, Executive Officer of the Tasmanian Salmonid Growers Association, for his encouragement and long-term support.

The industry partner Huon Aquaculture Group (HAG) owned by Mr and Mrs Bender provided substantial in-kind support to conduct farm trials. We appreciate the technical support of Dr David Morehead who ran the on-farm trials and never wavered in his optimistic endorsement of Striped Trumpeter as an excellent new species for aquaculture. The feeding, handling and management of fish on the farm was undertaken by Andrew Bourke, Robert Churchill and Colin Johnson.

A Striped Trumpeter Working Group was formed at the start of the project to maximise the chances of success and to provide advice to the Tasmanian Government. It provided advice and support and we thank Prof Colin Buxton (TAFI), Dr Stewart Fielder (NSW Fisheries), Mr Pheroze Jungalwalla (TSGA), Mr Wayne Hutchinson and Mr Steven Clarke (SARDI) for participating.

The project involved a large and talented group of scientists and technicians. At the University of Tasmania we thank Prof Chris Carter, A/Prof Barbara Nowak and A/Prof John Bowman for advice and research on Striped Trumpeter nutrition, health and microbial control. They also provided inspiring student supervision. From the TAFI Fish Health Unit we are grateful to Dr’s Jeremy Carson, Kevin Ellard, Judith Handlinger and Stephen Pyecroft for disease diagnosis and health advice. At TAFI, Marine Research Laboratories we thank Associate Professor Malcolm Haddon for statistical advice. At the CSIRO Marine Research in Hobart we thank Dr Malcolm Brown, Mr Graeme Dunstan and Ms Mina Brock for helping us determine the lipid and vitamin requirements of Striped Trumpeter larvae.

Finally, the project would not have been possible without excellent and dedicated technical support provided at the various institutions. At the TAFI, Marine Research Laboratories in Hobart we thank all staff and students who assisted in the project. In particular, we thank Mr Alan Beech, Dr Matt Bransden, Ms Deborah Gardner, Mr Ross Goldsmid, Ms Anna Overweter, Dr Gavin Shaw, Mr Bill Wilkinson, Mr Karl van Drunen and the weekend staff of the Aquaculture Section. Thanks to Ms Tanaz Jungalwalla and Ms Clare Woolridge for assisting with the production of the Final report. Further detailed acknowledgements can be found at the end of each research chapter.

3 BACKGROUND

A major bottleneck in the development of many new marine fish species for aquaculture in Australia is the production of high quality juveniles (Battaglene and Fielder 1997). According to the Australian Fisheries Statistics in 2008 (ABARE 2009) only three species of fish are cultivated from eggs and reared in sea cages in large numbers. They are, in order of production: Atlantic salmon in Tasmania at 24,248 t; barramundi in Queensland at 2,464 t and yellowtail kingfish in South Australia at 3,801 t. Clearly, the largest production and associated infrastructure is in Tasmania. However, the rapid growth of the Atlantic salmon industry is under threat from increasing costs (particularly food), control and management of disease and predation (especially AGD, Amoebic gill disease, and seals), competition from overseas producers and environmental changes (climatic) (Battaglene and Cobcroft 2003). Experience in other countries indicates the need for the Tasmanian Atlantic salmon industry to support research programs on other commercially valuable finfish species, to provide diversification opportunities and avoid excessive reliance on a single species, Atlantic salmon. A successful industry for Striped Trumpeter would complement the existing industry for Atlantic salmon and provide an alternative should there be a catastrophic disease introduction or major market slump in demand. The Tasmanian Government has long supported the development of an alternative white-fleshed species to augment and diversify the production base. It understands the long-term nature of new species' development and has been highly involved in establishing the salmon industry where it remains a partner in the major hatchery SALTAS (Salmon Enterprises of Tasmania).

Striped Trumpeter, *Latris lineata*, were chosen as the best candidate for diversifying sea cage culture in Tasmania in the late 1980's. They are highly prized as one of the best eating fishes in Australia, are also greatly esteemed as sashimi in Japan and have firm white flesh, which is both tasty and fatty (Yearsley 1999). Market research by Tassal Limited into the flesh qualities of "farmed Striped Trumpeter" (wild-caught juveniles reared in captivity to adulthood) has been very encouraging and they rank alongside Australia's best white-fleshed fish in controlled taste tests (Pakes Research 2000). Another potential market advantage is the very high omega-3 polyunsaturated fatty acids (PUFA) concentrations in the flesh of "farmed fish" (Nichols et al. 2001). They also have a limited distribution in southern Australia from Sydney to Kangaroo Island and around Tasmania and New Zealand. Once plentiful in Tasmania the fishery for Striped Trumpeter has now declined from over 100 tonnes to around 50 tonnes per annum. Striped Trumpeter were selected not only for their marketing qualities, but also their docile nature, lack of cannibalism, ability to take formulated feeds and be held in captivity at high densities. However, they have a complex and extended larval phase with a nine month "paperfish" stage and to date they have not proven easy to culture. The knowledge base for Striped Trumpeter is one of the best for a new aquaculture species in Australia. They were one of only two fin-fish species studied for the entire seven years of the Aquaculture CRC (1994 to 2000), following a stringent national screening process. The research providers comprised the Department of Primary Industries and Fisheries, Marine Research Laboratories (MRL), University of Tasmania's School of Aquaculture at Launceston and the CSIRO Marine Research. In 1998 research on Striped Trumpeter was combined under the Tasmanian Aquaculture and Fisheries Institute (TAFI) with continued input from CSIRO. In 2000 the Tasmanian Government, the then largest salmon company Tassal Ltd, and the research providers combined to support the continuation of research through the Aquafin CRC. The research strategy of Aquafin CRC was developed explicitly to deliver the essential technologies needed by the Australian finfish farming industry. The industry partners agreed to invest in a CRC, defined the major goals which they believed a CRC could best achieve, and clearly indicated the weight of effort which should be applied to each of these goals. For five years Tassal Limited was an industry partner with TAFI and CSIRO, providing financial support to both CRC projects. Tassal Limited had a signed MOU (Memorandum of Understanding) with TAFI and CSIRO providing preferential access to methodologies and fish arising from the project. However, in 2003 they withdraw from the project after going into receivership and following major industry restructuring. They have formally relinquished claims to preferential access to methodologies and fish.

The earlier Aquafin CRC project 1B.4 with the support of FRDC (Fisheries Research and Development Corporation) (2001/206) started in 2001 and finished in 2004. It built on the increasingly sophisticated R&D program to develop Striped Trumpeter as an aquaculture species. Excellent progress had been made in understanding and controlling reproduction, arguably the best for any species in Australia. Broodstock collected from the wild are routinely spawned year-round through temperature and photoperiod control (Morehead 1997). Problems with early larval rearing have also been overcome and egg incubation and early larval rearing protocols have been established. The mortality peak associated with first-feeding has been reduced using better live feed production techniques (Brown et al. 1998: Battaglene and Brown 2006) and improving water quality particularly at the air/surface interface (Trotter 2003). Improved swimbladder inflation rates have been achieved using new surface cleaners, optimal temperatures and light conditions (Trotter 2003). Problems with jaw malformations, common to all batches of post-larvae in this and many other species, improved but remain a difficulty (Cobcroft et al. 2001). Some malformations (scoliosis) may be linked to myxozoan, *Kudoa neurophilia*, infections (Grossel et al. 2003). The project met all production targets and over a thousand 100 day-old post-larvae were produced in 2002, 2003 and 2004. Early larval survival has been greatly enhanced with up to 80% survival to Day 20 and 3.5% survival to Day 100. Still the bottleneck to juvenile production remains mortality from flexion to metamorphosis, caused by an apparent primary metabolic disorder with a nutritional and/or health basis. Recent research has focused on investigating the link between deficiencies or imbalances in the three essential PUFAs - docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (AA) - found in live feeds (Sargent et al. 1999). Several commercially available live feed enrichments have been tested on a production scale. Novel experimental emulsions and biochemical approaches have been developed allowing the dietary requirement for DHA to be identified in larvae (Bransden et al. 2005). A significant relationship was found between dietary DHA and larval grey gut syndrome (bacterial gut degeneration), possibly suggesting the importance of DHA as an immunomodulator in early developing Striped Trumpeter larvae. Copepods have been cultured as a supplement to traditional live feeds enriched on novel emulsions and have improved larval rearing success (Morehead et al. 2005). A range of novel diets containing large doses of vitamins C (ascorbic acid) and E (a-tocopherol) have been tested in live feeds and fed to larvae. Increased feed ration has reduced the presence of urinary calculii, increased survival, dry weight and viable larvae. New commercial particulate diets from Europe have shown promise as a weaning diet for post-larvae. Partial weaning may be possible from Day 50 but more research is required to establish the earliest age at which larvae can be weaned. Weaning protocols also need to be devised that maintain adequate water quality. Controlled experiments using antibiotics have demonstrated that high bacterial loads are an important factor in larval mortality. Antibiotics improved survival, increased digestion, and reduced the incidence of grey gut syndrome and the presence of urinary calculii, resulting in more viable larvae. Efforts in 2003 focused on producing fish without antibiotics using a new water filtration and ozonation treatment system. Improved water quality allows greater control of the early bacterial problems, often encountered in Striped Trumpeter culture, and enables better interpretation of experimental results by limiting confounding influences.

The first Aquafin CRC Striped Trumpeter project clearly delivered timely scientific and production outputs. The research results and techniques developed have direct application to other marine species and the Striped Trumpeter team worked with leading researchers in Australia, Scotland, Mexico and Spain to apply the techniques developed on a range of other marine species. The project was reviewed in 2003 by an independent international panel (Anon 2003), and as part of the second year Aquafin Review. Full review reports are available from the Aquafin CRC, but the following extract summarises the highly favourable findings: "The review team was impressed by the high standards of the research and the methodological approach to the Striped Trumpeter project. The research team operates from a strong scientific base and exhibits a high level of dedication to the challenging research. The research team in the opinion of the external reviewers has covered all bases and has encompassed relevant larval rearing and production information from around the world." Both reviews made a series of recommendations for future research that have been incorporated into the current project. In a shared view of the future it was agreed that research was required in three key areas to take full advantage of the momentum and resources established. First, the health of post-larvae needed to be improved through better control of bacterial diseases and parasites. Research in this area is by no means unique to Striped Trumpeter and a general lack of understanding of factors influencing larval survival and growth combined with poor seawater supplies means that many Australian hatcheries and research facilities struggle to reliably supply seedstock. Indeed, many commercial hatcheries routinely used large quantities of prophylatic antibiotics. Bacterial load reduction using antibiotics is not acceptable in terms of fish, environmental or human health and the catastrophic effects of indiscriminate use of antibiotics in aquaculture are well known (Weston 1996). In the past 10 years there has been growing interest in the use of probiotics to improve the health of aquatic animals. Use of probiotics shows increasing promise as a strategy that will reduce hatchery mortality and has been used successfully in crustacean hatcheries (Gomez-Gil et al. 2000). However, the diversity of species raised in hatcheries and the specifics of the bacterial interactions means that there is no universal solution or magic bullet. The development of probiotics needs to go hand in hand with better system design and water quality management, including ozonation, to achieve microbial control. The second key area is the further development of live feed, weaning, and grow-out diets, leading to a better understanding of nutritional and other factors, particularly those influencing malformations. The third, is the larger-scale assessment of survival and growth of post-larvae and juveniles on a semi-commercial scale, leading ultimately to the grow-out of Striped Trumpeter in sea cages.

This report is presented in two books. The first as a series of scientific abstracts framed by introductory information about the project and a summary of the research conducted. The full research papers are provided in Book 2 and are either published or intended for publication. The full paper citation (or stage in publication) and authorship details are provided. Important information relating to the project is also included in Appendices 1 to 3. The first research Chapter 7 provides a detailed review of research into the culture of Striped Trumpeter at the start of the project, the next four Chapters 8-11 relate to the selection of potential probionts and microbial differences in green and clearwater culture. Chapters 12 and 13 examine the effects of turbulence, algal-induced turbidity and tank colour on larval growth, survival and behaviour. Chapter 14 reports on early larval weaning and the use of new micro diets. Chapters 15 and 16 investigate optimal temperatures, lipid levels and rations for culture of post-larvae. Jaw malformations and the important role of larval behaviour in relation to walling are experimentally examined in detail in Chapters 17 and 18. The characterisation of immunoglobulin in Striped Trumpeter is provided in Chapter 19. The identification and control of parasites affecting Striped Trumpeter post-larvae and juveniles is examined in Chapters 20 to 23. The final research Chapter 24 provides a summary of the farm trials giving details of the growth and survival of juveniles stocked into sea cages. The Intellectual Property from the current project 2004-221, 1B.4(b) and the earlier project 2001-206, 1B.4(a) has been assigned to UTAS taking effect from 1st July 2008 to allow development of new projects (Appendix 1 and 2). Ethical approval to conduct the research was provided by the University if Tasmania Animal Ethics Committee under permits A8191, A8705, A8719, A8975.

4 NEED

The project was essential for four main reasons. First, experience in other countries, now rapidly developing new species like Atlantic cod, turbot, haddock and halibut, indicates the need for a new coldwater species to complement the existing salmon industry in Australia, which is facing significant challenges. Major stakeholders, the Tasmanian Government and Tasmanian Salmonid Growers Association fully supported the development of Striped Trumpeter as an alternative species, recognising that it was a long-term investment. The Tasmanian Aquaculture Research Advisory Group and TasFRAB (Tasmanian Fisheries Research Advisory Board) sanctioned the project as a top priority. Overcoming the Striped Trumpeter larval mortality bottleneck was identified in the Tasmanian Fisheries and Aquaculture Strategic Research Plan 2005-2009 as a high priority. Second, the project addressed the two objectives of the Aquafin CRC, Production Subprogram (Subprogram Hatchery Technology) by increasing the availability of species suitable for aquaculture and improving the quality of fingerlings for farm stocking. It also filled an expertise gap for the CRC following the Tuna Propagation Program demise by further developing a team capable of tackling difficult to rear marine fish and tapping into research institution in-kind contributions. The Aquafin CRC Board and JMAC (Joint Management Advisory Committee) requested the submission of the proposal. Third, there was an identified need for the research at a generic level where there was a necessity for both, a more systematic way to match the nutritional profile of live feeds with the requirements of new species of marine larvae (FRDC Hatchery Feeds R&D Plan high priority), and the development of system design and probiotics for the control of disease or improved health of hatchery and farmed aquatic animals (key area for research (7.2.2) in the Aquatic Animal Health Subprogram Strategic R&D Plan 2002-2007). Fourth, it facilitated capacity building and collaboration among the key institutions developing new marine species and an excellent training opportunity for post-graduate students, hatchery technicians and scientists.

5 OBJECTIVES

1. Investigate the control of microbial communities in intensive larval fish culture using ozonation and probiotics.
2. Determine the optimal environmental parameters, and water quality systems and tank design for reducing hatchery mortality and malformations in finfish larvae.
3. To better understand "grey gut syndrome" and the ontogeny of the immune system, including linkages to developments with probionts and immunostimulants.
4. Evaluate formulated diets and their use in early weaning.
5. Evaluate the growth and survival of Striped Trumpeter post-larvae and juveniles reared under semi-commercial conditions.
6. Evaluate the possibility for the culture of Striped Trumpeter using alternative systems and/or sites.

6 REVIEW OF STRIPED TRUMPETER CULTURE

Publication Title: Advances in the culture of Striped Trumpeter larvae: a review

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Publication status: Aquaculture (2007) 268:195-208

6.1 Abstract

Striped Trumpeter, *Latris lineata*, was chosen as the best new candidate for sea cage culture in Tasmania in the late 1980s. It has a complex and extended post-larval or 'paperfish' stage lasting up to 9 months and has historically proven difficult to culture. Excellent progress has been made in understanding and controlling reproduction and broodstock are spawned year-round through photothermal control. Problems with early larval rearing have been overcome and egg incubation and early larval rearing protocols have been established. A mortality peak associated with first-feeding has been reduced using better live feed production techniques and improved water quality. Using antibiotics showed that high bacterial loads were an important factor in larval mortality. A new water filtration and ozonation system has removed the need for antibiotics. Larval nutrition research focused on the link between potential deficiencies or imbalances in the three essential PUFA in live feeds: docosahexaenoic acid, eicosapentaenoic acid and arachidonic acid. Novel experimental emulsions were applied with dose response experimental designs to identify the dietary requirement for selected PUFA and vitamins. Despite the advances in live feed enrichments, the live feeds, particularly *Artemia*, were found to have sub-optimal lipid profiles. Copepods were cultured, as a supplement to traditional live feeds, and improved larval rearing success. Costs to scale up production and to control extensive cultures presently restrict the usefulness of copepods. Important breakthroughs have occurred in health with the detection and control of nodavirus, myxozoan and bacterial disease. Ozone disinfection of eggs and sterilisation of hatchery seawater have been important control measures. Another bottleneck to production has been mortality of larvae from notochord flexion to metamorphosis. System changes to reduce nocturnal movements and a better understanding of optimal live feed densities, and weaning onto formulated diets, have improved survival and growth. High rates of jaw malformation remain a challenge and no definitive cause has been established. Reduced rates of malformations have been associated with one or a combination of high feed rates, lower larval densities and temperatures, and reductions in 'walling' behaviour. Future research is aimed at finding ways to reduce malformations, develop probiotics and early weaning strategies, control parasites and scale-up production to assess performance of juveniles in sea cages.

See **Book 2 - Chapter 7** for full study details.

7 BACTERIAL FLORA IN LIVE OR CONCENTRATED ALGAE AND CLEARWATER

Publication title: Bacterial flora and larval performance in the culture of Striped Trumpeter *(Latris lineata)* larvae using live or concentrated algae or clearwater

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Publication status: unpublished

7.1 Abstract

Marine fish larvae are susceptible to mortality caused by bacterial infection during larval rearing. This study investigated performance and cultivable bacteria of Striped Trumpeter larvae reared to 15 days post-hatch (dph) in clearwater or greenwater based on live microalgae or a concentrated algal paste (instant). BTB-Teepol (BTBT) agar had better recovery of environmental Vibrios than TCBS and was used as the presumptive *Vibrio* selective media. Larval growth was higher in live algae (7.1 ± 0.3 mm standard length, SL, 330 ± 28 µg larva^{-1} dry weight, DW) than in clearwater (6.6 ± 0.3 mm SL, 268 ± 22 µg larva^{-1} DW) or instant algae (6.6 ± 0.3 mm SL and 232 ± 30 µg larva^{-1} DW). However, survival was higher in both live algae and clearwater (35 ± 9 and 38 ± 5%, respectively) than in instant algae (7 ± 4%). Mortality of larvae reared in instant algae was consistently high from 12 dph and coincided with significantly higher cultivable bacteria in the larvae (presumptive *Vibrio* 0.2 colony forming units [cfu] µg^{-1} larva DW at 3 dph to 650 cfu µg^{-1} larva DW at 15 dph) and in larval culture tanks (presumptive *Vibrio* peak at 8 x 10^{5} cfu ml^{-1} at 7 dph and decline to 2 x 10^{3} cfu ml^{-1} at 15 dph). Bacterial isolates (*n* = 515) were classified as presumptive Vibrios (39%), with eight well-defined species and eight unspeciated types with distinct phenotypes (75% similarity level), and glucose non-fermenters (61%), with 19 unspeciated types with discrete phenotypes (82% similarity level). Potential pathogenic isolates included *Vibrio anguillarum* and *V. ichthyoenteri*, and a strain with probiotic properties, *V. alginolyticus*, was identified. There was a correlation between bacterial flora of the larvae and the culture environment, demonstrated by a more complex flora associated with larvae held in live algae compared to instant algae or clearwater. Live microalgae is recommended for greenwater culture of Striped Trumpeter to provide for good growth and survival. Concentrated algal paste products should be used with caution as greenwater for marine fish larval rearing as they may lead to potentially harmful bacterial proliferation and larval mortality.

See **Book 2 - Chapter 8** for full study details.

8 MICROBIAL COMMUNITIES CULTIVATION-INDEPENDENT APPROACHES

Publication Title: Microbial communities of post hatch Striped Trumpeter (*Latris lineata*) larvae, held under different rearing conditions, determined using cultivation-independent approaches

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Publication status: unpublished thesis

8.1 Abstract

A knowledge of the microbial bacterial community of the larvae, seawater and live feeds was believed to be necessary in order to develop an understanding of what represents a healthy microbial ecology relevant for larval fish rearing success. 16S rRNA bacterial clone library and terminal restriction fragment length polymorphism (TRFLP) analysis was utilised to examine the microbial community associated with Striped Trumpeter (*Latris lineata*) larvae cultured under different "green water" conditions. It was discovered that the larvae-associated microbial diversity was restricted but varied considerably between culture conditions. Most bacteria detected belonged to class *Alphaproteobacteria* (predominantly the *Roseobacter* clade), *Gammaproteobacteria* (genus *Psychrobacter* and *Pseudoalteromonas*) and *Actinobacteria* (primarily genus *Microbacterium*). No association was found between larval survival and microbial community structure. Similar results were obtained using TRFLP analysis, though it was found that the larval microbial community was distinct from the bacterial community present in the surrounding water.

See **Book 2 - Chapter 9** for full study details.

9 ASSESSMENT AND TRACKING OF BACTERIAL PROBIONTS

Publication Title: Assessment and tracking of bacterial probionts within a Striped Trumpeter (*Latris lineata*) larvae rearing system

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Publication status: unpublished thesis

9.1 Abstract

Potential probiotic candidates were identified by using antimicrobial *in vitro* plate testing against known pathogenic *Vibrio* species, with six out of 25 isolates tested selected for further testing. In *Artemia* challenge trials, it was determined that *Pseudoalteromonas agarivorans* ST18 and *Aliivibrio fischeri* ST7 had the least effect on *Artemia* survival. To further assess the probiotic capability of strains ST18 and ST7, rotifer and *Artemia* cultures were challenged with pathogenic strain *V. proteolyticus* V760 mixed with strains ST18 or ST7. Strain ST18 was found to have a probiotic effect in that cultures containing both V760 and ST18 were not significantly different from the control system but produced significantly better survival compared to the pathogen-only treatments. To further investigate ST18 and ST7 in a mixed cultured system terminal restriction fragment length polymorphism (TRFLP) analysis was applied to monitor the change in bacterial community. Through tracking probiont strain specific terminal restriction fragments (TRF) the probionts could be distinguished within the microbial community associated with rotifers and appeared to be readily taken up by rotifers. However, in *Artemia* experiments, uptake of the probionts appeared to be less successful.

See **Book 2 - Chapter 10** for full study details.

10 ADDITION AND TRACKING OF PROBIONTS

Publication Title: Addition and tracking of probionts to yolk-sac and first-feeding Striped Trumpeter (*Latris lineata*) larvae

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Publication status: unpublished thesis

10.1 Abstract

The protective capacity and most effective delivery mode of putative probionts *Pseudoalteromonas agarivorans* ST18 and *Aliivibrio fischeri* ST7 was investigated in the rearing of yolk sac and first-feeding Striped Trumpeter (*Latris lineata*) larvae. In these experiments 4500 larvae were randomly stocked into 24, 300 l black hemispherical fiberglass tanks at 1 dph and held under static conditions for 5 days after which 300% daily water changes was applied. Terminal restriction fragment length polymorphism (TRFLP) was used to monitor the changes in bacterial community. The addition of strain ST18 to yolk sac larvae showed no significant reduction in survival (70 ± 6%) versus a control group (83 ± 5%) reared without potential probionts being added. The addition of strain ST7 with and without strain ST18 was found to be more disadvantageous (58 ± 7% and 55 ± 8% survival respectively). By tracking distinct 16S rRNA-derived TRFs, strain ST18 was specifically detected in treatments where it was added by both bioencapsulation and by direct addition. When strain ST18 was added directly to the water it resulted in decreased survival, due to the high bacterial load and possibly potential oxygen demand. The introduction of ST18 to the larvae bioencapsulated in rotifers resulted in the introduction of comparatively smaller numbers of bacteria that did not compromise the growth of the developing larvae.

See **Book 2 - Chapter 11** for full study details.

11 EFFECT OF ALGAL-INDUCED TURBIDITY AND TANK COLOUR

Publication Title: Feeding by Striped Trumpeter, *Latris lineata*, larvae, in response to changes in prey and larval density, algal-induced turbidity and tank colour

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Publication status: Aquaculture Research (in review)

11.1 Abstract

Maximising the contrast between potential prey items and the background against which they are viewed is particularly important for larvae that are reliant upon vision for feeding. This study investigated the effect of background environment on prey intake by Striped Trumpeter larvae that had either experience, or no experience, of feeding in a specific visual environment. Initial experiments investigated the effect of prey and larval density on rotifer prey intake and feeding rates over time. Rotifer intake increased linearly during the first 1.5 h post-feeding when fed at a rotifer density of 10 ml^{-1}. All subsequent experiments were therefore completed using prey densities ≤ 5 ml^{-1} and feeding periods of not greater than 2 h in order to ensure that prey consumption was not constrained by digestive tract capacity. Background colour significantly influenced prey intake with larvae consuming 5 times more rotifers in blue compared with black aquaria. However, larvae used in this experiment came from a blue culture tank and therefore had experience of feeding in a blue tank. A second experiment investigated the hypothesis that the change in tank colour resulted in the poor feeding in black tanks. Results showed that larvae fed best in the visual environment of which they had prior experience, and poorly in a new unfamiliar environment. The influence of changing visual environment was tested further using larvae with and without experience of feeding in an algal-induced green water environment. Larvae with experience of feeding in green water consumed significantly more rotifers in green water than larvae from a clear water environment. Larvae from a clear water environment fed equally well when shifted to either a clear or green water environment. However, larvae from a green water environment had significantly reduced feeding rates once shifted to a clear water environment. Striped Trumpeter larvae appear to develop a “search image” which is highly specific to the environment in which they are feeding. Quickly changing the contrast between prey and background, and the subsequent visual image available to the larva, resulted in reduced prey intake. The ability to strongly influence feeding rates via changes to the visual environment of larvae in culture highlights the importance of optimising factors that contribute to visual discrimination of potential prey items. In particular, larval rearing systems should be designed such that transfer of larvae between systems minimises any change to the visual environment.

See **Book 2 - Chapter 12** for full study details.

12 EFFECTS OF TURBULENCE AND TURBIDITY

Publication Title: Effects of turbulence and turbidity on growth, survival and rotifer intake of Striped Trumpeter, *Latris lineata*, larvae

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Publication status: Aquaculture (in review)

12.1 Abstract

Both turbulence and turbidity can influence predator-prey interactions and therefore have longer-term effects on growth and survival of larvae. Much research has concentrated on the effects of turbulence and turbidity on short-term processes such as foraging behaviour, or long-term effects on growth and survival. Few studies have investigated simultaneously short and long-term effects or the interaction of turbulence and turbidity on larval performance in culture. Thus, the effects of turbulence induced by aeration and turbidity induced by algal cell addition, on growth, survival and rotifer intake of Striped Trumpeter, *Latris lineata*, larvae were investigated. Experiments were conducted in replicate 300 l black hemispherical tanks with turbulence created via aeration from the centre bottom of each tank. Turbulence levels higher than 200 ml min^{-1} aeration resulted in significantly reduced growth, survival and rotifer intake of larvae from first feeding to 14 days-post hatching (dph), and no larvae survived under static conditions after 12 days. Prey intake increased in larvae 10 - 14 dph in comparison with larvae 6 and 8 dph and prey intake in older larvae 14 dph increased at the higher turbulence level of 800 ml min^{-1} in comparison to younger larvae. Furthermore, larvae 10 dph were more capable of coping with an increased aeration level of 400 ml min^{-1} than larvae receiving aeration at 400 ml min^{-1} from 6 dph. There was no interaction between turbulence and turbidity on growth, survival or prey intake. Larvae reared in turbid (green water) out performed those in clear water. Improvement in growth, survival and feed intake was evident for larvae reared in green water provided by either live algal cells, or concentrated algal paste of *Nannochloropsis oculata*. Green water reared larvae, despite being of the same size 10 dph as clear water reared larvae, were better equipped to cope with an increase in aeration from 200 to 400 ml min^{-1} than larvae reared in clear water. Combining the best level of aeration, 200 ml min^{-1}, with green water, resulted in the highest growth and survival of Striped Trumpeter achieved to date. It remains to be tested whether a turbulence level below 200 ml min^{-1} would provide for further improvement to growth and survival. Further investigation into increasing turbulence with age to gain maximal growth rates requires more attention primarily in regard to timing and strength of turbulent increases.

See **Book 2 - Chapter 13** for full study details.

13 WEANING STRATEGIES FOR POST-LARVAE

Publication Title: Weaning strategies for Striped Trumpeter (*Latris lineata*) post-larvae culture

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Publication status: Journal of the World Aquaculture Society (2010 in press)

13.1 Abstract

The Striped Trumpeter (*Latris lineata*) is a native fish being developed for aquaculture in Tasmania, Australia. Striped Trumpeter have a long post-larval stage and rearing has been reliant on the long-term use of *Artemia*. Two experiments were conducted to investigate weaning strategies. The first experiment used 52 days post hatch (dph) post-larvae previously reared on *Artemia* from 16 to 52 dph enriched with either low or high ascorbic acid. The treatments were *Artemia*, a microdiet or co-feeding. The post-larvae were reared until 67 dph. The *Artemia* treatment yielded significantly higher mean survival, followed by co-feeding and the microdiet. Feeding *Artemia* yielded significantly heavier post-larvae and the microdiet produced significantly smaller post-larvae. A second experiment used 41 dph post-larvae and investigated the effect of co-feeding duration prior to feeding with *Artemia* and microdiet as controls. Co-feeding periods of 5, 10 and 15 days were tested. Co-feeding for 5 days and microdiet feeding yielded significantly poorer survival compared to *Artemia*. *Artemia* feeding yielded significantly heavier post-larvae. In both experiments, the diets did not have a significant effect on jaw morphology. These experiments are the first to examine weaning strategies for Striped Trumpeter post-larvae and suggest co-feeding post-larvae from 40 dph and feeding microdiet exclusively at 50 dph.

See **Book 2 - Chapter 14** for full study details.

14 EFFECT OF TEMPERATURE ON POST-LARVAE

Publication Title: Effects of temperature regime on growth and development of post-larval Striped Trumpeter (*Latris lineata*)

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Publication status: Aquaculture (2010) 305:95-101

14.1 Abstract

The Striped Trumpeter (*Latris lineata*) is a promising new candidate for diversification of aquaculture in temperate regions of Australasia. Striped Trumpeter is also of scientific interest due to an unusually prolonged post-larval phase. The research was aimed at identifying the optimal temperature for rearing post-larval Striped Trumpeter approaching metamorphosis. Three-hundred-day-old post hatch post-larvae (12.1 ± 0.2 g, 114.0 ± 0.5 mm, mean ± SE) were reared at 12, 14, 16 and 18 ^{o}C, over 84 d. Survival, growth and metamorphosis into juveniles were recorded every 21 d. Fish were fed to apparent satiation and reared in oxygen saturated water (95.9 ± 2.6 %). At 14 ^{o}C, fish exhibited the best growth, had significantly higher lipid content and the majority (> 90 %) of the population metamorphosed into juveniles. The performance of fish reared at 16 ^{o}C was similar to those at 14 ^{o}C but the carcass had a significantly higher protein content and a significantly smaller proportion of the population (66.2 ± 3.0 %) metamorphosed into juveniles. Growth rate models predicted that growth was maximised between 12.9 ^{o}C (thermal growth coefficient) and 14.4 ^{o}C (specific growth rate). At 12 ^{o}C, fish showed the highest food conversion efficiency and all of the fish metamorphosed into juveniles. At 18 ^{o}C, fish showed the poorest growth, metamorphosis, and protein and energy retention. This is the first study on the effects of temperature on growth and development of Striped Trumpeter post-larvae. The results have important implications for aquaculture and fisheries management of Striped Trumpeter, in particular the rearing of post-larvae in hatcheries, timing of stocking into sea cages especially prior to metamorphosis, and for wild stock recruitment models.

See **Book 2 - Chapter 15** for full study details.

15 FEED RATION AND POST-LARVAE

Publication Title: The effects of ration and dietary lipid on growth of post-larval Striped Trumpeter (*Latris lineata*)

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Publication status: unpublished

15.1 Abstract

Striped Trumpeter (*Latris lineata*) is a new candidate species for adoption by the Tasmanian aquaculture industry. In preparation for future trials in sea cages, an experiment was conducted to investigate feeding strategies and the effects of dietary lipid inclusion level. Post-larval Striped Trumpeter (8.1 ± 0.1 g fish^{-1}) were reared using a combination of 33 %, 67 % or 100 % satiation rations and low (18 %) or high lipid (24 %) diets at a constant temperature of 15 ^{oC}. The diets were formulated to only vary nutritionally in the fish oil component. The 33 % and 67 % rations were determined by adjusting proportionally to the feed intake of fish fed to satiation (100 % ration) at the start of each week. Fish were reared for 63 d and at the end of the experiment three fish representing post-larvae (incomplete metamorphosis) and three fish representing juveniles (complete metamorphosis) were taken from each replicate tank (n = 4) and measured for whole body chemical composition. The 100 % (22.7 ± 1.0 g) and 67 % ration (21.1 ± 1.1 g) produced similar weight gain; the 33 % ration (15.4 ± 0.7 g) produced significantly smaller fish at the end of the experiment. Food conversion efficiency was highest at the 33 % ration (40.9 ± 3.2 %) compared to the 67 % (37.6 ± 3.0 %) and 100 % (30.1 ± 2.7 %) rations, which were similar. The 67 % (22.6 ± 0.0) and 100 % (22.7 ± 0.0) ration showed significantly higher proportions of fully metamorphosed individuals compared to the 33 % ration (8.6 ± 0.0). Dietary lipid did not have a significant effect on growth nor on food conversion efficiency. Data from starvation trials on similar sized fish reared at similar temperatures were incorporated to estimate optimum rations. The optimum ration for late stage post-larvae was found to be 4 % biomass d^{-1}. A dietary lipid content of 24 % of dry matter produced post-larvae with significantly higher carcass lipid content (5.8 ± 0.3 % of wet weight). Post-larvae were shown to have significantly higher carcass ash content and significantly lower carcass lipid content compared to juveniles. Metamorphosis into juveniles required fish to reach a minimum weight of 23 g and a carcass lipid content of at least 4 %. Metamorphosis of the majority of post-larvae (> 50 %) was predicted to occur at weights above 20 g and carcass lipid content of 7 %. All post-larvae were predicted to have metamorphosed by 40 g. This is the first experiment to investigate the effects of ration and different dietary lipid levels on post-larval Striped Trumpeter metamorphosis and growth. The results emphasise the need for proper feed management to increase growth and feed efficiencies to shorten the post-larval rearing period.

See **Book 2 - Chapter 16** for full study details.

16 JAW MALFORMATION LINKED TO WALLING

Publication Title: Jaw malformation in Striped Trumpeter *Latris lineata* larvae linked to walling behaviour and tank colour

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Publication status: Aquaculture (2009) 289:274-282

16.1 Abstract

Jaw malformations are a recurrent obstacle in the hatchery production of high quality juveniles of many marine finfish species. Whilst nutrition and temperature are often cited as the most likely causes, this study investigated manipulation of the physical culture environment and larval behaviour to reduce jaw malformations. The onset of jaw malformation after metamorphosis in Striped Trumpeter *Latris lineata* follows changes in larval behaviour from an even distribution throughout the water column to close association with the tank walls, often with vigorous swimming into the walls known as ‘walling’ behaviour. Larvae were reared through metamorphosis, 16 to 44 days post-hatching (dph), in twenty four 300 L hemispherical tanks with six different wall colours, black, blue, green, marble (a black, grey and white mottled pattern), red and white. Walling behaviour, jaw malformation and swim bladder hyperinflation were assessed. The highest proportion of severely malformed jaws at 44 dph occurred in red tanks, followed by green, white, blue, black and marble. More fish walled in coloured tanks (25-44%) than in black and marble tanks (9.6 and 3.4%, respectively). The proportion of fish with jaw malformations at 44 dph was positively correlated with fish walling behaviour. Both black and marble tanks had more than 50% of fish with normal jaws at 44 dph, and close to 80% with no or very minor malformations. Growth and survival to 44 dph were highest in the black (15.7 ± 1.3 mm fork length, 7.9 ± 0.9 mg dry weight, 71 ± 6%) and marble (15.6 ± 1.2 mm, 7.6 ± 0.5 mg, 58 ± 17%) tanks, compared with the lowest values in red tanks (14.2 ± 1.1 mm, 6.4 ± 0.4 mg, 11 ± 6%). Swim bladder hyperinflation, an apparent stress response, was greatest in red tanks and surface mortality was positively correlated with the proportion of fish with hyperinflated swim bladders. Potential mechanisms for the influence of walling behaviour on jaw malformation are mechanical damage and poor nutrition, via reduced feed intake and increased energy expenditure. The study highlights the often overlooked importance of hard-surface interactions in the growth and survival of cultured marine fish and demonstrates a cheap and effective technique for assessing tank background colour as a means of reducing malformations in cultured fish.

See **Book 2 - Chapter 17** for full study details.

17 JAW MALFORMATION INDUCED BY WHITE TANKS

Publication Title: Jaw malformation in Striped Trumpeter larvae is correlated with culture conditions, white tanks and clearwater, walling behaviour and live feed enrichment

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Publication status: unpublished.

17.1 Abstract

A high incidence of jaw malformation has hindered the production of quality Striped Trumpeter *Latris lineata* juveniles and has been correlated with walling behaviour in *Artemia*-fed larvae. In this study, Striped Trumpeter were reared from first-feeding in different coloured tanks (black or white), culture conditions (clear or greenwater) and fed different diets (enriched or non-enriched rotifers and *Artemia*), to examine the effects on behaviour and jaw malformation to 29 days post-hatching (dph). The highest incidence and severity of jaw malformations occurred in clearwater and enriched diet treatments, being significantly more common in white (70 ± 15%) than in black tanks (26 ± 15%). In black tanks, jaw malformation was significantly more common in larvae fed enriched diets (18 ± 14%) than in those fed non-enriched diets (8 ± 8%) and in clearwater (19 ± 14%) versus greenwater (7 ± 6%). At the end of the rotifer feeding phase, larvae were significantly larger in black (8.44 ± 0.42 mm, 0.79 ± 0.09 mg) than white tanks (7.51 ± 0.52 mm, 0.55 ± 0.06 mg), and longer in greenwater (8.74 ± 0.23 mm) than in clearwater (8.44 ± 0.42 mm). In the *Artemia* feeding phase, larval growth was slower and mortality was higher in non-enriched treatments compared with those fed enriched diet. Larval length and survival were higher in black (9.4 ± 0.9 mm, 26 ± 13%) than in white tanks (8.0 ± 1.0 mm, 10 ± 10%) and higher in enriched (9.3 ± 1.0 mm, 24 ± 16%) than in non-enriched (8.1 ± 1.0 mm, 12 ± 10%) diet treatments, whilst survival was higher in greenwater (46 ± 18%) versus clearwater (26 ± 13%). Swimbladder inflation was significantly higher in fish reared in black (50 ± 20%) than in white (25 ± 11%) tanks and in greenwater (95 ± 5%) versus clearwater (25 ± 11%). The average proportion of larvae walling over the duration of the experiment was higher in white than black tanks, and higher in enriched than non-enriched treatments, with lowest walling in greenwater. There was no consistent relationship between feed intake and jaw malformation. However, there was a significant positive correlation between walling and the incidence of jaw malformation at 29 dph in larvae fed enriched diets, but not in larvae fed non-enriched diets. The use of greenwater, black tanks and enriched live feeds are required for good growth, development and survival of Striped Trumpeter. The study emphasises the importance of reducing walling in the culture of oceanic larvae and may have direct application in the rearing of other marine fish with similar malformations such as yellowtail kingfish.

See **Book 2 - Chapter 18** for full study details.

18 CHARACTERISATION OF IMMUNOGLOBULIN

Publication Title: Purification and partial characterisation of Striped Trumpeter (*Latris lineata*) systemic immunoglobulin for the purpose of polyclonal anti-serum production

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Publication status: Aquaculture (2009) 287:11-17

18.1 Abstract

The Striped Trumpeter (*Latris lineata*) has been identified as a new species for diversification of the Tasmanian finfish culture industry. It is a deep water species with an unusually long, oceanic post-larval developmental stage and therefore, has not been easy to culture. Recent break throughs in the hatchery phase of culture have enabled the first sea cage grow-out trials. As the culture of Striped Trumpeter moves towards commercial-scale grow-out, knowledge of the immune system as it relates to disease resistance and vaccination is becoming more important. This study began at the basic level of immunoglobulin (Ig) characterisation and then moved onto the creation of anti-serum, which was used as an immunological tool to investigate the onset of the antibody response in the Striped Trumpeter. Similar to many other teleost species, Striped Trumpeter Ig is composed of a light chain of *Mr* 28 ± 3 kDa and a dominant heavy chain of *Mr* 86 ± 7 kDa. As seen in many other species of teleosts, these heavy and light chains form a tetrameric molecule weighing approximately 926 kDa. Purified Striped Trumpeter Ig was used to create polyclonal anti-serum directed against the light chain. The anti-serum was then used to investigate the ontogeny of the antibody response. Using Western blot analysis, Ig could not be detected until larvae were 225 days post-hatch (dph). This is later in terms of days post-hatch than other fish examined and could affect future husbandry and vaccination practices for this species.

See **Book 2 - Chapter 19** for full study details.

19 CLONING AND EXPRESSION ANALYSIS OF PRO-INFLAMMATORY CYTOKINES

Publication Title: Cloning and expression analysis of three Striped Trumpeter (*Latris lineata*) pro-inflammatory cytokines, TNF-α, IL-1β and IL-8 in response to infection by the ectoparasitic, *Chondracanthus goldsmidi*

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Publication status: Fish and Shellfish Immunology (2009) 26:773-786

19.1 Abstract

This study reports the cloning and sequencing of three Striped Trumpeter (*Latris lineata* Forster) pro-inflammatory cytokines, TNF-α, IL-1β and IL-8, as well as their differential expression in response to an infection by the ectoparasite *Chondracanthus goldsmidi*. The Striped Trumpeter TNF-α transcript consisted of 1093 bp, including a 759 bp ORF which translated into a 253 aa transmembrane peptide. The sequence contained a TACE cut site, that would produce a 167 aa soluble peptide containing the TNF ligand family signature. The IL-1β sequence consisted of 963 bp, including a 774 bp ORF which translated into a 258 aa protein. The protein lacked both a signal peptide and an ICE cleavage site, but did contain the IL-1 family signature. The sequence for the chemokine IL-8 contained 906 bp, with an ORF of 297 bp, which translated into a 99 aa protein. The protein lacked an ELR motif as is common with many teleost IL-8 sequences. The differential expression of the three cytokine genes in parasitised fish was investigated via quantitative real-time PCR. A significant up-regulation of all three pro-inflammatory cytokines was found in the gills, which were the site of parasite attachment. Examination of head kidney cells revealed a significant up-regulation of TNF-α, but not IL-1β or IL-8. Conversely, the spleen cells showed significant up-regulation of both IL-1β and IL-8, but not TNF-α. These findings allow for more detailed investigations of the Striped Trumpeter immune response.

See **Book 2 - Chapter 20** for full study details.

20 IDENTIFICATION AND CONTROL OF PARASITES

Publication Title: Identification and control of parasites in a new species for aquaculture: A case study with Striped Trumpeter.

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Publication Status: World Aquaculture (2009) 40(1):30-32

20.1 Abstract

This story takes place in Tasmania, but where exactly is Tasmania? It is Australia's southern most island state situated directly on the 'roaring forties', which has a very changeable weather pattern throughout the year with snowfalls not uncommon in summer. This cool to mild climate is ideal for temperate agriculture (stone- and deciduous fruit) and aquaculture. The Tasmanian aquaculture industry began in the mid 1940's with the cultivation of Pacific oysters. It has since expanded to include other shellfish, such as abalone, scallops and mussels. Today the largest Tasmanian aquaculture industry involves the culture of over 18,000 t of Atlantic salmon per year, which are farmed in sea cages. Since the beginning, the salmon industry has experienced various health challenges, including amoebic gill disease (AGD), which is caused by amoebic protozoans. Treatment of this disease using freshwater is relatively simple but expensive and progress is being made in the development of a vaccine. Atlantic salmon are also reared in sea temperatures close to their thermal maxima in late summer and it makes sense for the industry to diversify into other marine species. The industry is particularly interested in developing an alternative native white-fleshed fish species. Striped Trumpeter, *Latris lineata,* was identified in the 1980's as a possible aquaculture species because of its tolerance to handling, high stocking density as well as the superior flesh quality, which is high in omega fatty acids and highly regarded as sashimi.

See **Book 2 - Chapter 21** for full study details.

21 A NEW SPECIES OF COPEPOD *CALIGUS NUENONNAE*

Publication Title: A new species of copepod (Siphonostomatoida: Caligidae) parasitic on the Striped Trumpeter, *Latris lineata* (Forster), from Tasmania

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Publication status: Zootaxa (2009) 1971:59-68

21.1 Abstract

A new species of caligid copepod (Siphonostomatoida), *Caligus nuenonnae* n. sp., is described based on material collected from the body surface of Striped Trumpeter [*Latris lineata* (Forster)] reared at the Tasmanian Aquaculture and Fisheries Institute, Marine Research Laboratories, Australia. *Caligus. nuenonnae* n. sp**.** is characterised by the following combination of features: 1) female genital complex with a mid-lateral indentation and highly concave posterior margin; 2) 1-segmented abdomen in the female that is about one-third the length of the genital complex; 3) distinctly broader first abdominal somite relative to the second abdominal somite in the male; 4) antenna with a spatulate process on the proximal segment; 5) recurved postantennal process without a basal accessory process; 6) female maxilliped with a proximal ridged protrusion on the corpus; 7) sternal furca with widely separated, apically truncate tines; 8) distal exopodal segment of leg 1 with a lateral flange on each apical spine and an accessory process on apical spines two and three; 9) leg 3 protopod with two adhesion pads on the dorsolateral surface; 10) leg 4 exopod 2-segmented, with I-0; I, III armature; 11) terminal exopodal segment of leg 4 with the outer apical spine being slightly shorter than the middle apical spine; 12) male maxillulary dentiform process with numerous small points embossed on the surface; and 13) male legs 5 and 6 represented by distinct lobate projections.

See **Book 2 - Chapter 22** for full study details.

22 DESCRIPTION OF *CHONDRACANTHUS GOLDSMIDI* N. SP

Publication Title: The first chondracanthid (Copepoda: cyclopoida) reported from cultured finfish, with a revised key to the species of *Chondracanthus*

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Publication status: Journal of Parasitology (2007) 93(4):788-795

22.1 Abstract

A new species of the Chondracanthidae (Copepoda: Cyclopoida), *Chondracanthus goldsmidi*, is described based on material collected from the naso-branchial region of Striped Trumpeter (*Latris lineata* [Forster]) cultured at the Tasmanian Aquaculture and Fisheries Institute, Marine Research Laboratories, Australia. This represents the first report of a chondracanthid copepod infecting cultured finfish and the first metazoan parasite from cultured Striped Trumpeter. *Chondracanthus goldsmidi* n. sp. can be distinguished from its female congeners by the absence of lateral processes on the head and presence of 3 pairs of lateral trunk outgrowths, 3 mid-dorsal body outgrowths (of which the first 2 are rounded), a small and subcylindrical antennule, and unornamented legs 1 and 2. A revised key to the 39 valid species of *Chondracanthus* is provided.

See **Book 2 - Chapter 23** for full study details.

23 HOST RESPONSE TO GILL PARASITE

Publication Title: Host response to the chondracanthid copepod *Chondracanthus goldsmidi*, a gill parasite of the Striped Trumpeter, *Latris lineata* (Forster), in Tasmania

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Publication status: Journal of Fish Diseases (2010) 33(3):211-220

23.1 Abstract

The chondracanthid copepod, *Chondracanthus goldsmidi* is an ectoparasite of gills, inner opercula and nasal cavities of cultured Striped Trumpeter, *Latris lineata* (Forster). Whilst often present in high numbers (up to 60 parasites per host), little is known about its effect on Striped Trumpeter. In this study *C. goldsmidi* was associated with extensive epithelial hyperplasia and necrosis. Pathological changes were most pronounced near the parasite's attachment site, with papilloma-like growths surrounding the entire parasite resulting in deformation of the filament. The number of mucous cells increased near the parasite attachment sites on both the opercula and gills. Mast cells were absent in healthy gills; in contrast numerous mast cells were identified in the papilloma-like growths. Immunostaining identified piscidin-positive mast cells in the papilloma-like growths, presenting the first evidence of piscidin in the family Latridae.

See **Book 2 - Chapter 24** for full study details.

24 FARM TRIALS

Publication Title: Performance of hatchery reared Striped Trumpeter (*Latris lineata*) on-grown in sea cages

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Publication status: unpublished

24.1 Abstract

Four groups of high quality hatchery produced Striped Trumpeter juveniles were successfully transported and stocked into sea cages in the Huon River region between October 2006 and October 2007. A total of 2,483 fish were stocked from three hatchery cohorts ranging in mean size from 64 to 193 g. The fish were successfully on-grown for 427 to 793 days. The fish were fed a selection of formulated diets through automated Aquasmart feeders in three 20 m sea cages within separate predator exclusion nets. The trials provided the first hatchery reared Striped Trumpeter grown to market size in sea cages. Overall survival was 66, 93 and 94% for the three cohorts. Males from all three cohorts were precociously spermiating during spring 2008. Females remained sexually immature. The water salinity was often below full salinity during the winter. Sea temperature at the cage sites throughout the period at sea ranged from 9.4°C to 18.9°C and averaged 13.9°C. Three copepod parasites *Chondracanthus goldsmidi, Caligus nuenonnae* and *Ceratothoa imbricata* were detected on farmed fish but none proved problematic and fish were not treated. The data collected were used for growth model predictions. The results suggest that southern Tasmanian conditions especially cage culture in a site dominated by freshwater inflow are not optimal for growth. The model predicted that Striped Trumpeter stocked at 100 g would attain a weight of 1.6 kg within three years at sea. The model suggests that the variation of growth with temperature was not significant. Future trials would be best carried out at higher temperatures in a marine dominated site. The average weight of harvested fish was 1430 ± 9g. HOG (head-on, gilled, gutted) yield was excellent at 94%, while fillets were 61% total recovery for a basic trim and 55% for a full trim. The product can be hot and cold-smoked, with hot-smoked product testing most favourably. Over 2 t of fresh farmed fish were sold and market feedback was highly positive. A basic economic analysis of Striped Trumpeter farming suggested it would take 14 years to reach 5000 t production at which point cumulative profit could eclipse cumulative spend.

See **Book 2 - Chapter 25** for full study details.

25 BENEFITS AND ADOPTION

25.1 Industry benefits

The benefits and beneficiaries of the research closely match those identified in the original application. The industry to benefit most from the planned outcomes is the Atlantic Salmon aquaculture industry in Tasmania because it is now closer to having a viable alternative cage culture species endemic to Tasmania. This is a very important Australian seafood industry estimated to produce 26,000 tonnes of Atlantic Salmon in 2007/08 at an estimated farm gate value of $270 million. Fish are farmed towards the upper thermal limit and growth rates are extremely fast, with production taking around 30 months (Battaglene et al. 2008). The importance of the salmonid industry diversifying into alternative species is further highlighted by concerns over climate change and one of the possible mitigation strategies will be to move into alternative species tolerant of higher water temperatures (Battaglene et al. 2008). Further impetus is given to finding a suitable alternate fish to farm by the need for the Tasmanian salmonid industry to move further offshore. This move is driven by most inshore and sheltered sites being close to their maximum carrying capacity. Striped Trumpeter are ideally suited to more oceanic conditions and do not require freshwater bathing for the control of Amoebic Gill Disease (AGD), the major health challenge for the salmon industry. However, a number of new challenges have been identified with Striped Trumpeter culture including the lack of a commercial marine fish hatchery, development of new markets, control of new diseases and possible integration problems with the culture of salmonids (Battaglene et al. 2008).

The emerging marine finfish and rock lobster culture industries throughout Australia also benefit from the research and technological advances. While most of the benefits have been derived by Tasmania, they have also flowed to New South Wales, Queensland, South Australia, Northern Territory and Western Australia through the transfer of technology and knowledge to the new marine fish species being developed in each state. In particular, the development of novel techniques for water treatment, enrichment of live feeds, ozone disinfection of eggs, and the use of coloured and marbled wall patterns has been of direct relevance to Barramundi, Mulloway, Kingfish, Snapper, Whiting and Grouper culture. The research on ozone disinfection has been extended to the Yellowtail Kingfish and prawn industries. The identification of malformations, their onset and solutions to reduce them have also been directly extended to the yellowtail kingfish industry.

Collaborative projects with international institutions have extended the benefits and adoption to New Zealand through joint studies of malformations of cultured Kingfish, and to Scotland (Atlantic Cod) and Spain (Senegal Sole) through joint research on lipid requirements.

The specific benefits of the project are as follows:

1. Provision of good quality hatchery produced Striped Trumpeter juveniles for assessment of performance in semi-commercial cage conditions.
2. A better understanding of the bacterial flora of larval culture tanks and methods to manage bacterial communities through ozonation and probionts.
3. Improved culture environment (turbidity, turbulence and tank colour) for the production of high quality Striped Trumpeter larvae.

4. A better understanding of weaning and the lipid, vitamin and ration requirements of Striped Trumpeter post-larvae.
5. Characterisation of parasites of Striped Trumpeter with potential to impact sea cage culture.
6. Description of the development of the immune system with a view to vaccine development.
7. Transfer of Striped Trumpeter hatchery technology to other finfish hatcheries in Australia, particularly the use of ozone and identification and reductions in jaw malformations.
8. Increased training of skilled hatchery technicians and graduate and post-graduate students.

One area where research was less successful than planned was in the identification and use of probionts, partially due to the late start of the PhD candidate and the refocussing of research efforts in other areas as per the working group recommendations prior to the commencement of the project.

25.2 Adoption and communication

The information from the project has been diligently and extensively communicated as outlined in the communication plan. In brief, the communication has been as follows:

1. Detailed and timely research progress reports as defined in contracts with AQUAFIN.
2. The production of a hatchery manual where the hatchery protocol information has been captured but not widely distributed due to Intellectual Property (IP) caveats.
3. Attendance at some 22 conference and workshops with over 28 presentations to industry and research groups (see Table 25.1).
4. Popular press releases, including TV, radio and print media and grey literature articles in Aquasplash and other trade journals (see Table 25.1 and references in Appendix 3).
5. Some 16 refereed scientific articles have been published in international peer reviewed journals (See Appendix 3).

Table 25.1

Date	Where	Participants	Affiliation	Outcome	Output
A. Conferences and workshops					
Jul 04	Launceston	Bransden Shaw	TAFI	Attendance at the IP and Commercialisation Workshop sponsored by the Aquafin CRC	IP register updated
Oct-04	Sydney	Battaglene Bransden Cobcroft Shaw	TAFI,	Attendance at the Australasian Aquaculture Conference, Sydney, 26-29 th September 2004	Co-chair session, 8 abstracts
Oct-04	Sydney	Battaglene Bransden Cobcroft Shaw	TAFI, +	Attendance Second Hatchery Feeds and Technology Workshop run by Dr Kolkovski (WA Fisheries) and Battaglene (TAFI), Rimmer (QDPI), comparison of methods	Proceedings, paper and Strategic Plan

May-05	Bali, Indonesia	Battaglene	TAFI	World Aquaculture Society Conference international collaboration	Chair session 1 abstract
Jul 05	Hobart	Battaglene Cobcroft Overweter Shaw	TAFI	Attendance Aquafin CRC Conference, national collaboration	Chair session 2 abstracts and presentations
Oct-05	Ghent Belgium	Battaglene Bransden Cobcroft Shaw	TAFI ARC	Attendance LARVI 05 conference International collaboration	Invited presentations, 4 abstracts, 2 posters, 3 papers
Oct-05	Ghent Belgium	Battaglene Cobcroft Shaw	TAFI ARC	Attendance Rotogen (Rotifer) workshop	Invited, type id for rotifers at TAFI
Feb-06	MRL Hobart	Battaglene Cobcroft Andrews Nowak Heyward Aiken	TAFI, CSIRO TASSAL	Attendance Fish Parasitology Workshop, greater knowledge of parasites	Collection and id of parasites
Aug-06	Adelaide	Battaglene Cobcroft Andrews	TAFI	Attendance at the Australasian Aquaculture Conference, Adelaide, 27-31 st August 2006	3 abstracts, 3 presentations, 2 posters
Aug-06	Adelaide	Cobcroft Battaglene	TAFI	Attendance at the Skretting Spectrum Marine Fish Hatchery Session, Adelaide, 30 th August 2006	1 abstract, 1 presentation
Nov-06	Canberra	Cobcroft	TAFI	Attendance FEAST forum, French contacts established	Aquasplash article
Feb-07	MRL Hobart	Battaglene Cobcroft Andrews Nowak Dykova Gardner Covello	TAFI, CSIRO	Attendance Fish Protozoan Parasitology Workshop, greater knowledge of parasites	Collection and id of parasites
Feb-07	MRL Hobart	Battaglene Andrews Covello Cameron	TAFI,	Attendance Epidemiology Workshop	Epidemiology literature
Apr-07	Wageningen Netherlands	Covello	TAFI	Attendance Fish immunology and vaccine workshop	Aquasplash article?
May-07	Barossa Valley, South Australia,	Andrews Choa De'Pannone	TAFI	4th Aquafin CRC conference, 15th to 17th May.	3 abstracts
Sep-07	Melbourne	Battaglene Nowak	TAFI +	Attendance at the FRDC Nodavirus Workshop	1 abstract, strategic plan
Sep-07	Grado Italy	Andrews Nowak	TAFI	13th EAFP International Conference on Diseases of Finfish and Shellfish	1 abstract
Sep 07	Viterbo Italy	Andrews Nowak	TAFI	7th International Symposium on Fish Parasites, 24th to 28th September.	1 abstract

Jun-07	Stirling Scotland	Covello	TAFI	7th International Symposium on Fish Immunology	1 abstract
Jul-2008	Adelaide	Andrews	TAFI	ASP & ARC/NHMRC Research Network for Parasitology Annual Conference	1 abstract
Sep-2008	Reykjavik, Iceland	Covello	TAFI	International Conference on Fish Diseases and Fish Immunology,	1 abstract
Aug-2008	Brisbane	Battaglene Cobcroft	TAFI	Attendance & session chairs at the Skretting Australasian Aquaculture Conference, 3-6 August.	Session chairs 1 abstract
Aug-2008	Brisbane	Battaglene Cobcroft	TAFI	Advances in marine fish hatchery technology in Australia	Workshop facilitators, 2 abstracts
Aug-2008	Cairns QLD	De'Pannone John Bowman	TAFI TIAR	12th International Symposium on Microbial Ecology – ISME- Microbial Diversity – Sustaining the Blue Planet	1 abstract
Jun-2009	Prague Czech Republic	Covello	TAFI	11th Congress of the International Society of Developmental and Comparative Immunology	1 abstract
Sep-2009	Prague Czech Republic	Covello	TAFI	Diseases of Fish and Shellfish, 14th European Association of Fish Pathologists Conference, 14-19, Prague, (F3).	1 abstract

B. Promotion

May 05	Hobart	Battaglene Bransden	TAFI	Presentation to stake holders at Aquaculture Review and RAG	Promotion
May 06	Hobart	Battaglene	TAFI	Presentation to stake holders at Aquaculture Review and RAG	Promotion
Aug-06	Hobart	Battaglene	TAFI	ABC interview for science week and to promote Striped Trumpeter project	Promotion
May 07	Hobart	Battaglene	TAFI	Presentation to stake holders at Aquaculture Review and RAG	Promotion
Apr 08	Hobart	Cobcroft	TAFI	Presentation on hatchery malformation research to stake holders at Aquaculture Review	Promotion
Apr 08	Hobart	Battaglene Buxton Montague	TAFI Aquafin CRC	Media promotion of project by Hon Minister Llewellyn, include TV and newspaper articles	Promotion
Jun 08	Hobart	Cobcroft	TAFI	Media promotion of project through Hook Line and Sinker TV program	Promotion

C. Visits and training

May 05	Launceston, TAS	Battaglene Cobcroft	TAFI, UTAS	Lectures on swim bladder and eye development in fish	Student recruitment
Nov 05	Cairns, QLD	Goldsmid	TAFI, QDPI	Collaborative talks and visits to institutions and aquaculture facilities holding broodstock	Trip report
Oct-05	Trondhiem, Norway	Battaglene Cobcroft Shaw	TAFI UST SINTEF	Understanding of Norwegian research and industry development	Farm visits, Presentations, research facility tours, Aquasplash article
Jun 06	Port Lincoln, South Aust	Andrews	TAFI SARDI	Field trip to identify parasites in SBT	Exchange of information
Feb-06	Hobart	Battaglene Shaw Overweter	TAFI, NDA	Ability to manipulate and build Databases	Internal report
May 06	Launceston, TAS	Cobcroft Shaw	TAFI, UTAS	Lectures on swim bladder and eye development in fish	Student recruitment
Aug-06	MRL,Hobart	Midtlyng	VESO Norway	Better understanding of Norwegian health research especially vaccine development	Presentation
Feb 07	MRL, Hobart	Buxton Battaglene Bender Morehead Evans Ford	TAFI, HAG, DPIW	Collaborative research project proposal plan	Draft project proposal
Jun 07	Port Lincoln Arno Bay	Battaglene Cobcroft	Clean Seas TAFI	Exchange of information on malformations in larval fish.	Draft SfCRC project proposal
Jun-07	Port Lincoln	Andrews	TBOA	Parasites collected off SBT	Training for student
May 07	Launceston, TAS	Battaglene Cobcroft	TAFI, UTAS	Lectures on swim bladder and eye development in fish	Student recruitment
Aug-Oct 2007	Norway Italy	Andrews	TAFI	Six week study tour, experimental design of parasite trials learnt, attendance at two conferences (see above)	Training for student
Apr-Jun 2007	Aberdeen Scotland	Covello	TAFI SFIRC	Ten week study visit at the Scottish Fish Immunology Research Centre	Training for student
Sep-Nov 2007	Port Lincoln Port Augusta South Australia	Battaglene Cobcroft Overweter Goldsmid	TAFI Clean Seas	Three trips of two staff each trip for one week to train Clean Seas staff in ozonation and identification of malformations in kingfish	See SfCRC project for outputs

D. Reviews and working groups

Jul-04	Hobart	Battaglene, Buxton, Hutchinson, Fielder, Jungalwalla	TAFI, SARDI, NSW Fisheries, TSGA	Working group discussions and planning for Striped Trumpeter trials in other institutions and for proposal review	New project endorsed

E. Major Collaborations

2005	Hobart	Bartels	Abtec	Grow-out of Striped Trumpeter in recirculation systems	Grow-out for 2 years
2005	Hobart	Bartels	Abtec	Collaboration on breeding of banded morwong	Larval rearing of banded morwong to metamorphosis
2006	Sydney		Ecotox	Supplied eggs and rotifers to undertake toxicity tests for Gunns Pulp Mill proposal	Demonstrated transport of eggs and larvae, external funds received
Jul-2006	MRL, Hobart	Chong	Universiti Sains Malaysia	International collaboration on gene expression through AMI Australia Malaysia Fellowship	Paper
Feb-Mar 2006	France	Cobcroft	TAFI, IFREMER	Isolated Striped Trumpeter genes and learnt new molecular techniques	Paper Trip report, Aquasplash article, ARC Linkage proposal
2007	Sydney		Ecotox	Supplied eggs and rotifers to undertake toxicity tests for water purification project	Demonstrated transport of eggs and larvae, external funds received
Mar-2007	Malaysia	Cobcroft	Universiti Sains Malaysia TAFI	International collaboration on gene expression	Paper
Sep- Nov 2007	Arno Bay Port Augusta	Battaglene Cobcroft	Clean Seas TAFI	Malformation study funded through the Seafood CRC	Reported through Seafood CRC

25.3 Educational benefits

An important component of a successful CRC project involves post-graduate training. The Striped Trumpeter project has been particularly successful in this area having supported five PhD students. A brief summary of the students, their project title and progress to date follows.

Dr Gavin Shaw became a formally adopted PhD candidate within the Aquafin CRC in 2002 and was supervised by Dr Tish Pankhurst and A/Prof Stephen Battaglene. His research thesis was entitled “Feeding behaviour of Greenback Flounder and Striped Trumpeter larvae in greenwater culture”. The early component of his research was conducted at the School of Aquaculture in Launceston on Greenback Flounder. He started research at MRL on Striped Trumpeter in March 2003. He completed his studies in March 2006 and has presented results at the Aquafin CRC Conference and 2nd Year CRC Review in 2003, at the Australasian Aquaculture Conference in 2004, and at Larvi 05 in Belgium in 2005 and has published one paper with another three submitted for publication. His research has provided the project with a better understanding of feeding behaviour in relation to prey density and highlighted the benefits of greenwater culture. He is currently employed by Skretting Australia Pty Ltd as a Technical Services Manager.

Mr Mark De' Pannone an Australian PhD student on an Aquafin CRC scholarship started in August 2005 and was supervised by A/Prof John Bowman and A/Prof Stephen Battaglene. His research thesis was entitled "The identification and development of probionts for use in marine fish hatcheries" is currently being evaluated. He has presented his results at the 4th Aquafin CRC conference, in May 2007 in the Barossa Valley, South Australia and the 12th International Symposium on Microbial Ecology in Cairns, Australia, in August 2008. His thesis is currently being reviewed.

Dr Bryan Choa an international PhD student from the Philippines was formally adopted into the Aquafin CRC in October 2005 and was supervised by Prof Chris Carter and A/Prof Stephen Battaglene. His research thesis was entitled "Culture of Striped Trumpeter (*Latris lineata*) post-larvae". He started research at MRL in April 2005. He has presented results at two Aquafin CRC conferences and had presentations accepted for the 13th International Symposium on Fish Nutrition and Feeding in Brazil in 2008. His research has provided the first weaning and feeding experiments with post-larvae and critical information on optimal temperatures, body composition and feeding ration. He completed his degree in May 2010.

Dr Melanie Andrews an international PhD student from South Africa was adopted into the Aquafin CRC in March 2006 and was supervised by A/Prof Barbara Nowak, A/Prof Stephen Battaglene and Dr Jennifer Cobcroft. Her research thesis was entitled "Arthropod parasites of cultured Striped Trumpeter *Latris lineata* (Forster 1801), and potential treatments". She has presented her results at two Aquafin CRC conferences, the Australasian Aquaculture Conference in 2006 and two international conferences in Italy in 2007 and published four scientific papers. Her research has described and identified two new copepod parasites and examined control and treatment methods. She completed her degree in January 2010.

Dr Jennifer Covello an international PhD student from Canada was adopted into the Aquafin CRC in February 2006 and was supervised by A/Prof Barbara Nowak, Dr Richard Morrison and A/Prof Stephen Battaglene. Her research thesis was entitled "Aspects of the Striped Trumpeter (*Latris lineata*) Immune System". She presented her results at three international conferences and spent ten weeks on an overseas study tour at the Scottish Fish Immunology Research Centre learning new techniques. She has published two scientific publications describing the development of the immune system in Striped Trumpeter. She completed her degree in March 2010.

Several students spent time with the Striped Trumpeter research team on short-term (2-3 weeks) work experience placements including: two from Friends (July 2006), one from Rosny College (Oct 2007), two from Hobart College, one from UTas Zoology Honours, 2 from TAFE (Tomoko Chida and Nan Kang, Nov 2004), Mark Jensen, Kwan Tzu N in Sept 2006 from UTas School of Aquaculture. The students provided general assistance in many areas of our work, including handling broodstock, larval rearing, bacteriology, larval sampling, histology to assess grey gut syndrome, larval quality tests, and water quality monitoring. They in return received valuable work place experience and training.

26 FURTHER DEVELOPMENT

The research undertaken through the Aquafin CRC was further developed in three projects.

1. A four year project developed with FRDC to conduct larger sea cage trials using cultured Striped Trumpeter entitled "Optimising size of fish and time of release of cultured Striped Trumpeter (*Latris lineata*) into sea cages in Tasmania" (FRDC:

2008-201 total cash funding of $1.674 million, $837,470 from FRDC and $560,00 from Huon Aquaculture Company (HAC) and $277,470 from Tasmanian DPIW).

2. A three year approved Australian Research Council (ARC) Linkage grant entitled "Reducing skeletal malformations in cultured marine fish using gene expression, improved nutrition and advanced system operation" to extend the expertise on larval rearing to other marine fish hatcheries and to reduce malformations in cultured juveniles. (ARC: LP0882042 total cash funding of $619,307, $450,000 from ARC and $169,307 from Tasmanian DPIW, HAC, Clean Seas, Skretting Australia and Nutrakol).
3. A one year scoping study funded by the Seafood CRC entitled "Yellowtail Kingfish juvenile quality: Identify timing and nature of jaw deformities in Yellowtail Kingfish and scope the likely causes of this condition". (Seafood CRC 2007-718 total cash funding of $97,157 with support from Clean Seas).

The successful development of hatchery protocols to produce healthy juvenile Striped Trumpeter and the results of preliminary sea cage trials at HAC generated considerable interest in Striped Trumpeter culture. A proposal for a new project was developed with the full support of the Aquafin CRC, TSGA (Tasmanian Salmon Growers Association) and Tasmanian Government. A Technology Transfer Business Plan for Striped Trumpeter commercialisation was developed and contained a background intellectual property register, IP management provisions to license the Centre IP to the University of Tasmania for the remainder of the Aquafin CRC Grant Period, with the assignment of IP to UTAS taking effect from 1st July 2008. These arrangements for transfer of rights to UTAS following the windup of the CRC in June 2008 provided the security necessary for industry to participate in a new project. The Huon Aquaculture Company (HAC) had an MOU with TAFI and collaborated on cage trials since December 2006 (see Chapter 25). HAC was endorsed by the CRC and TSGA as the best placed and most committed company to progress Striped Trumpeter aquaculture following a formal call for expressions of interest. The new FRDC project proposal was ranked a high priority by the Tasmanian FRAB and Tasmanian Aquaculture and Fisheries RAG (Research Advisory Group). The objectives of the new research project were to:

1. To establish if Striped Trumpeter post-larvae can be grown faster in tanks or sea cages.
2. To determine the optimal size and condition of Striped Trumpeter for stocking in commercial sea cages.
3. To evaluate the best season to stock Striped Trumpeter into sea cages to obtain maximum growth and survival.
4. To examine health risks and treatment options for parasitic, viral and bacterial diseases encountered during farming trials.

The project proposal to continue research on Striped Trumpeter was submitted to FRDC on the 1st November 2007. A positive response from the FRDC was provided in March 2008. It built on 20 years of research through the FRDC and two consecutive CRC's which has enabled excellent control of reproduction and the pilot-scale production of high quality Striped Trumpeter juveniles. This was a project that had been well backed with both research and industry resources and had been extraordinarily productive and innovative in outputs. If successful it would have allowed more efficient production of juveniles and established cost-effective methods for transfer to sea cages, facilitating capacity building and collaboration among the key institutions developing new marine species and provide an excellent training opportunity for hatchery technicians and farmers. The cost of producing Striped Trumpeter juveniles is potentially higher than that of other farmed marine fish because they do not metamorphose till 50 g in weight. If a way could be found to stock post-larvae of 5 g into sea cages or to rear them more cost-effectively in land based systems it will greatly improve the economic potential of large-scale culture. The new project aimed to identify any major impediments to commercial farming and allow a fuller economic assessment of the potential farming benefits allowing informed decisions to be made on large-scale capital investments in marine fish hatcheries and grow-out facilities. The projected value to Tasmania of a potential Striped Trumpeter industry was estimated at $40 million per annum in 1994 (Searle and Zacharin 1994). A revised economic assessment was to have been undertaken which would have taken into account higher fish consumption, hatchery production and grow-out information. Unfortunately, after gaining FRDC approval the industry partner withdrew from the project during the world economic difficulties in February 2009. The Striped Trumpeter project is currently under review by the Tasmanian Government and likely to be scaled back or terminated by the end of 2010.

The ARC and Seafood CRC projects have supported the transformation of existing marine fish aquaculture industries, promoted the sustainable use of Australian species in aquaculture, and responded to climate change by enabling the salmon industry that is challenged by global warming to have the ability to diversify to alternative temperate species. Despite the growing understanding of factors that contribute to malformation, they remain a chronic issue for the international finfish hatchery industry. Without the production of healthy seedstock, the development of Australian sea cage culture, intensive shore-based recirculation grow-out systems and expanding saline water culture are threatened. The costs of hatchery production are proportionally very high in fish farming and currently limiting expansion. Malformations add greatly to the cost of farming by increasing grow-out time, reducing survival, adding infrastructure and labour costs, reducing marketability and fish quality. Variability among batches of fish is common, requiring complete culling or expensive grading. Accurate estimates are difficult to obtain but >40% malformed fish have been suggested for some Australian hatcheries and even in the more established European industry ~17% of all juveniles are malformed (Sweetman 2004). Improved hatchery production will increase the capacity of the industry to work on more difficult, but potentially economically rewarding tropical, deep sea and oceanic species. The projects' aim to break down the barriers between hatcheries while preserving confidentiality, and document the occurrence and describe skeletal malformations in larvae reared in hatcheries using different water sources, rearing protocols and personnel. Importantly, the research had agreed industry commitment, which allowed the information accessed to underpin the research program. Protection of IP will be carefully managed through patents on products/systems and release of information only with the full consent of all parties. Community perceptions of the aquaculture industry are adversely affected by the production and sale of malformed fish. The information from the projects will be a positive marketing tool for those industries where malformations are reduced, and a starting point for improvement of larval quality and monitoring for others. The studies will allow the Australian industry to move closer to the standardised assessment of larval quality, as a routine element of quality control, as is currently practised by the Mediterranean aquaculture industry (Sweetman 2004).

27 PLANNED OUTCOMES

The three main planned outcomes from the project were in large measure achieved:

1. Australian aquaculture has been provided with more systematic ways to control microbial communities, and to evaluate, identify and produce probionts for use in improving hatchery survival rates in finfish. Microbial control has been achieved in culture through the innovative use of ozonated seawater in both live feeds and larval rearing.

2. There will ultimately be a greater choice of new marine fish species available for culture through the efficient technology transfer between research agencies and industry of new products and systems for culturing marine fish larvae. Research and/or production hatcheries in SA, WA and NZ have requested information and advice from the Striped Trumpeter project team. Greater communication among hatcheries has been achieved through development of a successful ARC Linkage project, research with yellowtail kingfish through the Seafood CRC, and a key role in the planning and running of the third marine fish hatchery workshop held in Brisbane in August 2008.

3. The Atlantic salmon industry has moved significantly closer to diversification into new species, particularly Striped Trumpeter through the first sea cage trials using cultured fish. In time this should improve their profitability while reducing their risks, hence ensuring their long-term sustainability.

28 CONCLUSION

The project was reviewed by a Striped Trumpeter Working Group in 2004 and modified to reflect a more holistic approach with less emphasis on probiotics and more on the use of ozonation, disease control and improved weaning. A Charter for the project and all the necessary approvals were obtained on the 9th of October 2004.

1. *Investigate the control of microbial communities in intensive larval fish culture using ozonation and probiotics.*

A large-scale replicated experiment investigated the effects of two types of greenwater (fresh or instant algae), as well as clearwater on growth, survival and bacterial proliferation during rotifer feeding. Fresh algae produced the largest larvae and survival was not significantly different to clearwater. Instant algae produced fewer fish and without ozonation is not a good algae substitute for fresh algae in greenwater rearing. Bacterial loads were highest in instant algae. Libraries of bacteria were developed and assessed for potential probionts along with a number of other candidates from the Fish Health Unit collection. Research was refocused into better understanding the microbial dynamics of culture environments and the development of probiotics progressed through the development of clone libraries for bacterial 16S genes. A new method that allows greater data mining of bacterial community fingerprint patterns called automated ribosomal intergenic spacer analysis (ARISA) was adopted. Another technique Terminal Restriction Fragment-length Polymorphism (T-RFLP), similar to ARISA was also used to distinguish closely related bacterial species. Clone libraries and T-RFLP of the six key water treatments were successfully completed and 16s similarity trees compiled. Two isolates ST18 and ST7 inhibited *Vibrio* sp in plate tests and were further evaluated for probiotic potential in live feeds (rotifers and *Artemia*). Pilot testing of ST18 and ST7 in isolation and combination showed no detrimental effects on live feeds. T-RFLP was used to trace the probionts in live feeds and RT-PCR was used to quantify the probiont populations that the rotifers and *Artemia* had taken up.

2. *Determine the optimal environmental parameters, and water quality systems and tank design for reducing hatchery mortality and malformations in finfish larvae.*

The proportion of Striped Trumpeter post-larvae with jaw malformations improved with consecutive cohorts of fish produced in 2005. In 2006 we achieved the highest level of normal and minor malformations to Day 100 (76%) of any production batch to date, with consistently high levels at Day 200. This was repeated with another batch in 2007 that had a similar result to Day 100 (75%). These results are promising, particularly in light of an overall increased survival, such that many more normally formed fish were produced. Three large-scale replicated experiments were conducted to improve survival and reduce malformations. They also provided large numbers of fish samples for jaw structure assessment. The first experiment completed in 2005 investigated the effect of vitamin C addition and *Artemia* density on larval morphology and jaw development during metamorphosis. The addition of Vitamin C to the *Artemia* enrichment diet reduced the severity and incidence of malformation in older post-larvae after weaning. Tissue concentrations of Vitamin C in larvae were achieved up to 1600 μg AsA g^{-1} DW. Optimal feeding rates during the *Artemia* phase were determined and this information has reduced the cost and labour associated with production trials. The second experiment completed in June 2006 tested the effects of background tank colour on Striped Trumpeter larvae and post-larvae during the *Artemia* feeding period. The experiment demonstrated that tank wall colour had a significant effect on growth, survival, distribution and walling behaviour. Fish reared in black and marble tanks exhibited a lower proportion of walling behaviour, they grew longer, had higher survival and had a lower incidence of malformations than fish in other tank colours. The incidence and severity of malformation remained relatively constant from Day 30 in the black and marble tanks with ~80% of fish with no or minor malformations (scores 0 + 0.5), but became progressively worse in tanks with high levels of walling behaviour, especially red, green and white tanks. The absence of other significant skeletal malformations, besides that of the jaw elements, is strong evidence that physical contact with the walls is the primary cause of malformations in Striped Trumpeter, although compromised nutritional status and infections appear to exacerbate the problem. The third experiment examined the effect of culture conditions (tank wall colour and greenwater) and live feed enrichment on growth, survival, behaviour, and jaw malformation of Striped Trumpeter larvae.

3. *To better understand "grey gut syndrome" and the ontogeny of the immune system, including linkages to developments with probionts and immunostimulants.*

This objective relates to the health of larvae and post-larvae. No experiments were conducted specifically on “grey gut syndrome”. The syndrome is associated with bacterial infection or accumulated lipid in enterocysts. It was recorded for all larvae samples taken during large-scale experiments and production trials and commonly occurred in fish with high intake of enriched live feeds, typical of the lipid accumulation type and not directly linked to mortality events. Health research investigated the metazoan parasites of Striped Trumpeter post-larvae and juveniles. A range of previously undescribed parasites are known to cause problems in rearing juvenile Striped Trumpeter and will need to be controlled during farming operations. The research first described and identified the main parasites and then investigated control and treatment methods. Two copepod parasite species have been found commonly on cultured Striped Trumpeter (*Chondracanthus goldsmidi* n. sp. and *Caligus nuenonnae* n.

sp.). Both species have been carefully described and their taxonomy established with the assistance of recognised experts in the field. Bathing treatments for controlling *C. goldsmidi* have been investigated. The use of freshwater and hydrogen peroxide bathing is effective at reducing but not eliminating *C. goldsmidi*. The tolerance of Striped Trumpeter to freshwater is short at around 30 minutes. In-feed treatments with emamectin benzoate (25 to 100 mg kg^{-1}) are currently being trialled in infected juvenile fish.

The specific immune response of the Striped Trumpeter was investigated with particular focus on the development of the antibody response and vaccination against bacterial disease. The research was divided into three main areas: characterization of the antibody molecule (Ig), ontogeny and vaccination. Similar to many other teleost species, Striped Trumpeter Ig is composed of a light chain and a dominant heavy chain. The heavy and light chains form a tetrameric molecule weighing approximately 926 kDa. Purified Striped Trumpeter Ig was used to create polyclonal anti-serum directed against the light chain. The anti-serum was then used to investigate the ontogeny of the antibody response. Using Western blot analysis, Ig could not be detected until larvae were 225 days post-hatch (dph). This preliminary investigation has shown that the ontogeny of the antibody response in larval Striped Trumpeter may occur later than in other marine teleosts. It is hypothesised that the unusually long larval stage of this species may play a part in the delayed onset, as the production of antibodies may be linked to developmental changes as post-larvae become striped juveniles. Further work developed sensitive RT-PCR primers and *in situ* hybridisation probes targeted to recombination activating gene (RAG) and Ig molecules. These studies further examined the timing of a functional antibody response in the Striped Trumpeter.

4. Evaluate formulated diets and their use in early weaning

Two experiments in 2005 demonstrated that post-larvae can be weaned around Day 40. Experiments in 2006 demonstrated that weaning can be achieved as early as Day 30 using new improved diets from Europe. The weaning of post-larvae in production trials is now routinely done at 40 days of age. However, the research into larger post-larvae was constrained by the design of the larval rearing tanks in the fish hatchery. A new experimental room was designed and established specifically to conduct research on post-larvae, complete with a 32 tank replicated system with temperature control and computer operated automated feeders and lights. During 2006 a growth experiment was conducted on post-larvae using a range of temperatures (12 to 18°C). The optimal temperature for culture was found to be from 14 to 16°C. Once fish metamorphosed into juveniles temperature effects on growth rates were less apparent. This suggests that to achieve optimal growth, juveniles should be stocked in sea cages after they have undergone final metamorphosis. Research on feed ration found no significant difference between a 67% and 100% satiation ration on growth performance of post larvae, indicating that the optimum feeding regime is to slightly underfeed. Lipid content (9 and 14% fish oil) had no effect on growth and analysis of flesh quality is ongoing. Experimental research results have been incorporated into production trials and fish are fed Gemma Micro from Day 30 to Day 50 and weaned off *Artemia* by Day 50.

5. Evaluate the growth and survival of Striped Trumpeter post-larvae and juveniles reared under semi-commercial conditions

Production trials were run concurrently with large-scale experiments. In 2004 a production run produced 3,400 fish to Day 75 a survival rate of 11% with fish fully weaned off live feeds by Day 50. All of the advances made in 2005 culminated in the most successful production run ever, resulting in 23.4% survival to Day 100 well above the Day 50 target of >20%. Total production of Day 100 post-larvae in 2005 was 5,415, up from the 2,157 produced in 2004. In 2006 we produced 8,149 post-larvae to Day 100 in two trials. A trial in a 25,000 L tank started in 2006 produced ~3,000 fish to Day 275 in June 2007. In 2007, one production trial produced > 5,000 fish to Day 50 and ~12,000 post-larvae were produced to Day 100. Growth of post-larvae was monitored in all trials and the benchmark for Day 80 post-larvae is 30 mm FL and at Day 100 it is 43 mm TL. The production of high quality juveniles generated increased interest from industry and the first transfer of a trial shipment of hatchery-produced post-larvae was undertaken in December 2006, followed by another three shipments in 2007. Growth and survival of fish in sea cages were good. Fish were harvested after two years and over 2 t of fresh product sold with good consumer feedback.

6. *Evaluate the possibility for the culture of Striped Trumpeter using alternative systems and/or sites*

Considerable progress was made in obtaining the necessary approvals to translocate eggs to NSW DPI and SARDI. Appropriate risk assessments have been undertaken. The working group recommended that this work be continued to the point where eggs were transferred to determine whether they survive transport and hatch. Several shipments of eggs and larvae have successfully taken place to Ecotox Pty Ltd in Sydney in 2006 and 2007. However, the working group reached the conclusion that any attempt to culture Striped Trumpeter at alternative hatchery sites, particularly on the mainland, had a high risk of failure and was not pursued further.

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APPENDIX 1: INTELLECTUAL PROPERTY

The Intellectual Property from the project 2004-221, 1B.4(b) is documented in the AQUAFIN CRC Project Intellectual Property Register. It contains sections on Centre, Background and Third party IP, confidentiality agreements with consultants, staff and students. An agreement for the transfer of all intellectual property and data from the project 2004-221, 1B.4(b) was made by all AQUAFIN CRC participants to the University of Tasmania taking effect from 1st July 2008 to allow development of new projects.

APPENDIX 2: STAFF LIST

Staff engaged in the project were:

Name	Position	Qualifications	Project funded/ In-kind	Time
Dr Stephen Battaglene (TAFI)	Associate Professor, Aquaculture Section Leader, Principal Investigator	PhD, 20 years experience rearing marine fish larvae	In-kind	50
Mr Alan Beech (TAFI)	Senior Technical Officer (Aquaculture facilities)	Diploma of Aquaculture, over 13 years aquaculture research	In-kind	50
Dr John Bowman (AgSc)	Associate Professor Agricultural Sciences	PhD, 15 years bacterial research	Project	5
Dr Matthew Bransden (TAFI) Till Aug 05	Postdoctoral Scientist	PhD, 10 years aquaculture research, salmonid & larval fish nutrition	Project	100
Dr Jeremy Carson (TAFI)	Senior Scientist	PhD, > 20 years microbiology experience	Project In-kind	12.5 12.5
Dr Chris Carter (TAFI)	Professor, Head of Aquaculture School	PhD, fish nutritionist	In-kind	5
Dr Jennifer Cobcroft (TAFI)	Hatchery Manager / Postdoctoral Scientist	PhD, 12 years aquaculture research, broodstock and larval rearing	Project	100
Ms Debrah Gardner (TAFI) 2007 only	Hatchery Manager	B.Sc., M.Sc., 13 years aquaculture experience	Project	100
Mr Ross Goldsmid (TAFI)	Technical Officer (Broodstock)	Aquaculture Diploma, 12 years maintaining broodstock and larval rearing	Project In-kind	50 50

Ms Anna Overweter (TAFI)	Technical Officer (Live feeds and hatchery)	B.Sc.App.Sci. (Hons) 7 years aquaculture experience	Project In-kind	50 50
Dr Gavin Shaw (TAFI) 2006 only	Hatchery Manager	PhD, 5 years aquaculture experience	Project	100
Technical Officers (TAFI)	Technical Officers, Weekend staff	Associate Diploma or greater	Project	20
Ms Young (FHU)	Technical Officer	B.Sc. Degree	Project	50
Mr Bill Wilkinson (TAFI)	Senior Technical Officer (Algae)	M.Sc. Aquaculture, over 13 years culturing algae	In-kind	50

APPENDIX 3: PROJECT PUBLICATIONS

Publications from research started during the previous Aquaculture CRC and completed during the Aquafin CRC are marked by **
Student publications not directly funded by the CRC are marked by ***

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