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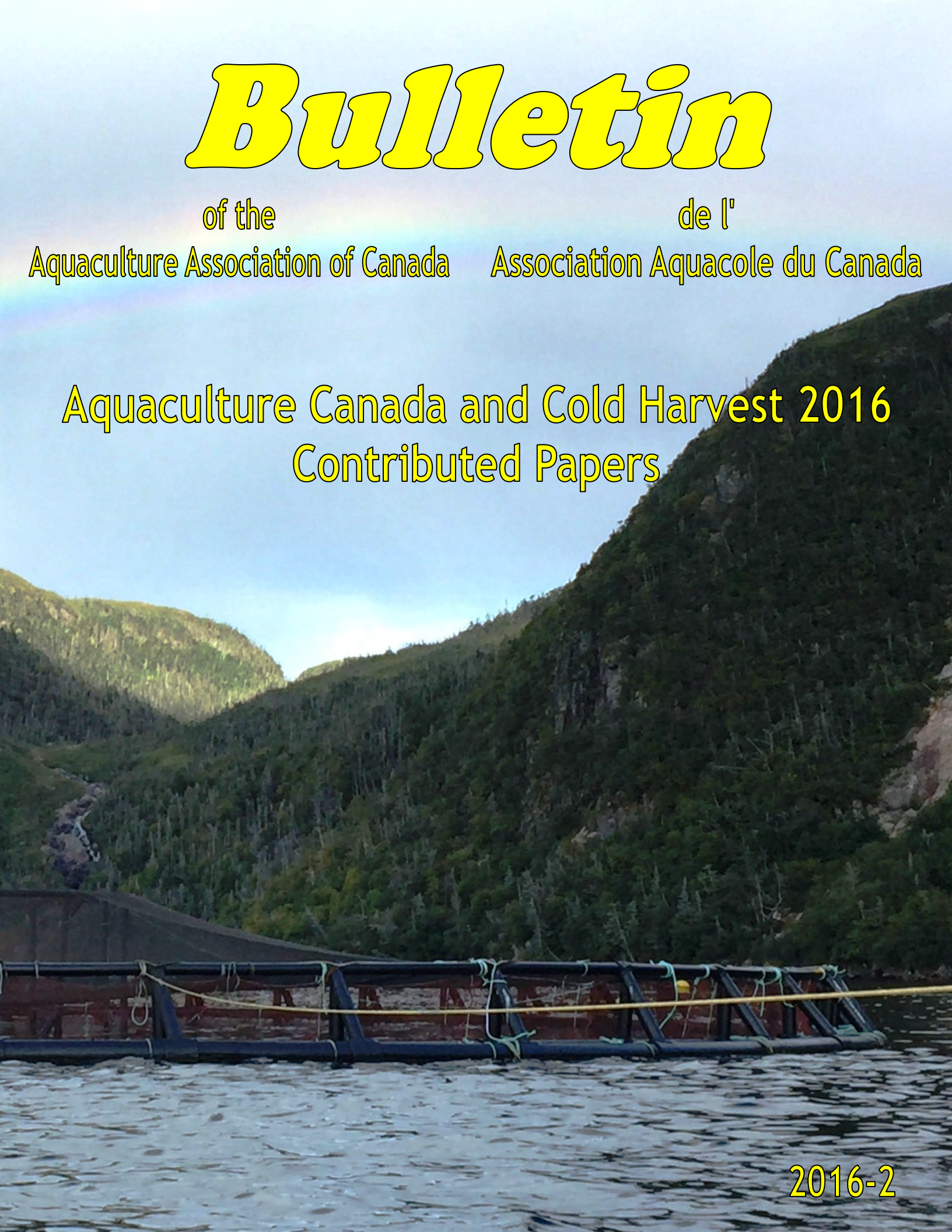

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2016 LIFETIME ACHIEVEMENT AWARD

Rod Carney graduated from the New Brunswick Community College (NBCC) Aquaculture Technician Training Program in 1979. In 1980, he went to work at the International Atlantic Salmon Foundation as a fish culture technician and became assistant manager in 1984. In 1988, Rod became the Research and Production Hatchery Manager for the Salmon Genetics Research Program (SGRP) and was responsible for the production of Atlantic salmon smolts for the burgeoning New Brunswick aquaculture industry. He liaised with and maintained systems and facilities for scientists who through the SGRP were working in the fields of broodstock selection, spawning synchronization, gene transfer, sex reversal, sterilization, light manipulation, and fish identification techniques. In 1997 Rod became an instructor in the Aquaculture Technician Program at NBCC where he taught for 19 years. Rod lives in Saint Andrews with his wife and enjoys soccer, badminton, vegetable farming, and anything that Grand Lake has to offer.

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INTRODUCTION TO THE PROCEEDINGS OF THE ACCH 2016 CONFERENCES

The Aquaculture Association of Canada (AAC) has been hosting the national forum (Aquaculture Canada) on aquaculture science, technology and business of Canadian aquaculture for well over three decades. This year's conference, our 33rd Aquaculture Canada, joined forces with NAIA's Cold Harvest 2016 for the 5th time since 1989. By all indications, interest in aquaculture science and technology has not waned: attendance at the conference and trade show participation were the highest they have been since 2002, suggesting the sector is alive and well in Canada. Over 30 students presented papers at the conference this year, also another record of sorts for the past decade or more. I would like to thank all of our sponsors, contributors, and delegates for making the conference a huge success in bringing academics, government, industry, and suppliers together.

The AAC publishes the proceedings of the conference (voluntary submission of articles by authors). We had quite a few submissions for the proceedings this year, including several graduate student papers. What you see in the next few pages are the outcomes of this effort; I am sure you will be pleased with the breadth and scope of the topics on Canadian aquaculture included therein. As Members of the AAC, you will enjoy receiving these proceedings first, electronically. They will then be made available more broadly on the website at a later date, for widespread dissemination.

Yours in aquaculture,

Cyr Couturier
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Contributed Papers

PREDICTING SEDIMENT ASSIMILATIVE CAPACITY OF ORGANIC WASTES AT MARINE FISH FARMS: IMPLICATIONS FOR ENVIRONMENTAL MANAGEMENT

Francisco Bravo* and Jon Grant

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Abstract

The eutrophication of aquaculture sites due to deposition of particulate organic wastes represents a major factor affecting productive capacity, environmental quality, and social licences of marine fish farm sites. In this study, a mechanistic model was developed to predict sediment assimilative capacity and the transition to hypoxic conditions as a result of organic enrichment. We introduced a new definition of sediment assimilative capacity specific to aquaculture based on the preservation of oxic conditions in sediments. We suggest that the model has extensive application to sustainable environmental management of aquaculture sites, being applicable to all stages of the production cycle (pre-evaluation of new aquaculture sites, operation, fallow, and remediation plans).

Model results were consistent with observations, and highlight the strong dependency of sediment assimilative capacity on organic loading history, hydrodynamics, and benthic biomass, particularly bacteria. No local degradation of organic wastes was predicted in a sedimentary environment exposed to mean bottom currents above 11.3 cm s^{-1}. Below this threshold, sediment assimilative capacity was as high as 10.7 g C_{org} m^{-2} d^{-1}. Any deposition above this value is predicted lead to hypoxic conditions as O_2 demand associated with organic matter degradation and secondary oxidation processes exceed O_2 supply. The combination of diagenetic modelling, optimization analysis, and scenario analysis may contribute significantly to the development of more effective tools for selection and environmental management of marine fish farms.

Introduction

Successful environmental management of marine fish farms implies assurance that production levels are within an area's capacity to handle wastes, despite uncertainties in management systems and/or environmental conditions. Nonetheless, accomplishing these objectives may be rather difficult for farmers when the capacity of the environment to accommodate intensive aquaculture is unknown. This is particularly true regarding the assimilation of excess organic loading coming out from fish cages (faeces and uneaten feed). In this regard, informed science represents a valuable input for strategic development and environmental management of aquaculture sites.

In this study, a mechanistic model of sediment geochemistry at salmon farms was developed with the following objectives: (1) determine maximum organic loads that can be safely assimilated by sediments, (2) predict the transition to hypoxic conditions as a result of organic enrichment, and (3) predict sulfide levels in sediments, a key regulatory variable used in monitoring programs in Canada and the USA (Maine).

Materials and Methods

This tool is based on the combination of a diagenetic modelling, optimization analysis, and scenario analysis (what if?). The numerical model is composed of three modules representing the fish-farm production cycle, water column, and benthos underlying fish cages. Free-stream water current was considered the governing variable due to its role in oxidant availability, sediment type, sediment-water exchange of solutes, and net deposition of particulate material under fish cages. Together these three modules simulate fish farm operations including fish growth, fish culture density, and feeding strategies/methods. The production of organic wastes and their fate once released to the environment are predicted (dispersion in the water column, deposition, degradation and accumulation in sediments) based on local environmental conditions at the fish farm (currents and depth). Using numerical optimization and scenario analysis, the assimilative capacity (AC) and suggested fish densities were estimated for the specific site. Simulations were carried out assuming natural (ambient) deposition, and additional deposition of aquaculture organic wastes (faeces and uneaten food = waste). Ambient organic carbon deposition was incorporated following a simplified sinusoidal cycle that peaks in early March to represent increased deposition associated with spring algal blooms.

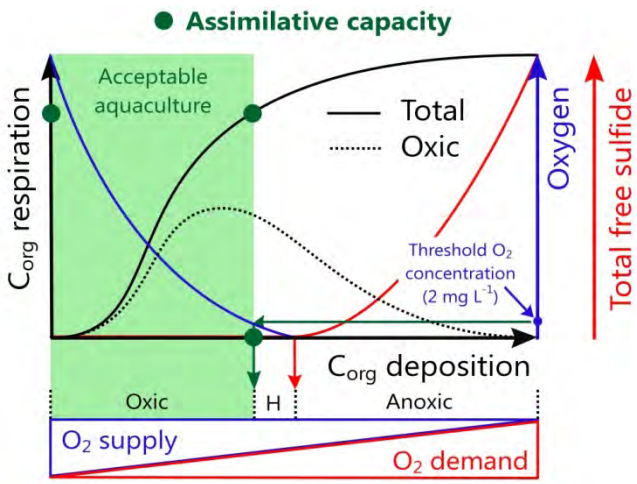

Figure 1
Conceptual representation of assimilative capacity of organic-rich solid wastes in sediments underlying fish farm cages. Total and oxic respiration are represented by black solid and dotted lines, respectively, while O_2 and Total S^{2-} concentration are represented by blue and red lines, respectively. The green dot represents the highest C_{org} loading rate (POC + organic wastes) feasible to be assimilated by benthic communities while avoiding hypoxic conditions (O_2 < 2 mg L^{-1}). Sulfide accumulation in sediments is expected when O_2 demand surpasses O_2 supply and therefore local assimilative capacity.

A formal definition of assimilative capacity applicable to fish farming is proposed as 'the maximum gross C_{org} deposition rate (g m^{-2} d^{-1}) that can be degraded in a particular sedimentary environment while preventing hypoxia in surface sediments (O_2 < 2 mg L^{-1}). Thus, assimilative capacity is recognized as a finite and time-space dependent variable that varies according to the level of organic enrichment and local environmental conditions. A conceptual representation of assimilative capacity of sediments is provided in Figure 1.

In order to demonstrate model performance, we simulated aquaculture activities and their interactions with the benthos in four different hydrodynamic scenarios, covering poorly to well-flushed environments. These were identified as high and low deposition (H-DEPO and L-DEPO), and low and high dispersion (L-DISP and H-DISP). In order to represent the broad spectrum of husbandry practices at the fish farm, from poor to highly efficient, simulations were run assuming feed loss between zero and 8%. Faeces production was assumed constant throughout the day and dependent on fish defecation (10% of ingested feed).

Results

Our model demonstrates that the balance of fish density, waste production, and hydrodynamics can be used to simulate benthic metabolic response to organic input from fish farming. The model indicates that the capacity of sediment to degrade organic wastes increases considerably in well-flushed environments due to increased renewal of oxidants in bottom waters. In addition, the net deposition of organic wastes decreases with increasing current speed due to dispersion in the water column and secondary resuspension from sediments. No local degradation of organic wastes was predicted in sediments exposed to high tidal currents. Assimilative capacity where deposition occurred was as high as 10.7 g C_{org} m^{-2} d^{-1} (L-DISP). Model predictions of assimilative capacity were consistent with empirical observations by Findlay & Watling (1997), NBDENV (2006), and Abo & Yokoyama (2007) which suggest values up to 11.4 g C_{org} m^{-2}

d^{-1}. Sulfide concentrations at assimilative capacity were always close to ambient, non-impacted levels.

For highly dispersive environments (average current speed 11 cm s^{-1}), assimilative capacity was never exceeded. In less dispersive environments and assuming a feed wastage of 3%, fish may be cultured without hypoxic risk in L-DISP at densities as high as 10.1 kg m^{-3}. As expected in depositional environments, it is not difficult to exceed local AC. The designation of high dispersion is based on predicted resuspension and transport of waste material, and is not a very high current speed for most fish farming sites. We anticipate that many farm areas would be highly dispersive by this criterion, and the relatively low biomass limits suggested herein would not apply.

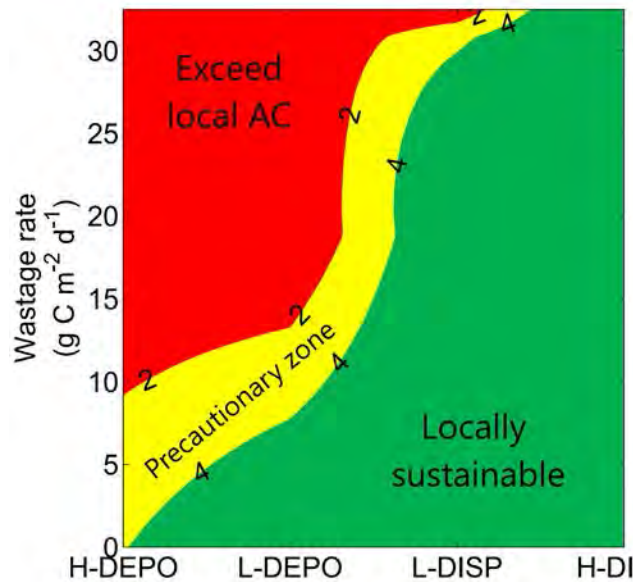

Figure 2
Traffic light model predicting near-field environmental sustainability of aquaculture operations based on hydrodynamic conditions, total organic wastage rate, and benthic response. Environmental performance of aquaculture operations are considered successful if sediment O2 concentration does not decrease below hypoxic levels (O2 < 2 mg L-1) throughout the fish farm cycle.

A traffic light model of near field sustainability of fish farm operations is presented in Figure 2. We test benthic response to wastage rates coming out from fish cages (lost feed plus faeces) as high as 32 g C_{org} m^{-2} d^{-1} (8% feed wastage). A precautionary area (yellow), separating locally sustainable (green) and AC exceeded (red) zones, was defined based on the combination of wastage rate and hydrodynamic conditions leading to O_2 concentration between 2 and 4 mg L^{-1} in the active sediment layer. This figure would need to be re-calibrated to correspond to specific conditions of culture and the environment.

As expected, the area defining sustainable conditions for fish farm operation decreases considerably with increases in wastage rate from the fish cage, as well as toward depositional environments.

Discussion

The model proved useful to (1) estimate the response of the benthic compartment to increased organic waste deposition, (2) determine critical organic loads, and (3) estimate maximum sustainable fish farm densities that can be cultured without affecting local sediment quality. The present model is one-dimensional, but is readily extended to two or three dimensions. We emphasize that assimilative capacity is only applied locally at this stage in keeping with the

regulatory approach, which is farm-based. Exceeding assimilative capacity at the local scale in this study does not have implications for the larger ecosystem.

Applications of assimilative capacity cover the entire fish-farm production cycle, including the *a priori* assessment of new aquaculture sites (e.g., licence or lease applications), throughout operations (e.g., farm management plans), and after ceasing of aquaculture operations (e.g., fallow or site remediation plans). It is noted that other considerations of tidal currents besides waste dispersion must be considered (e.g., flushing of cages to maintain oxygen levels for fish).

We expect that the development of anticipatory management tools, as the one presented here, rather than reactive management tools may contribute significantly to the environmental sustainability of aquaculture operations in Canada and abroad.

Acknowledgements

This study was supported by funds to Jon Grant as the NSERC-Cooke Industrial Research Chair in Sustainable Aquaculture, and to Francisco Bravo by the scholarship program BECASCHILE for doctoral studies. We thank Ramon Filgueira, Bernard Boudreau, Chris Algar, Michelle Simone, Kevin Sorochan, and Martin Bravo for their support and valuable suggestions.

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SPECIES COMPOSITION AND DIVERSITY OF THE GENUS *Saprolegnia* IN FINFISH AQUACULTURE SYSTEMS

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Abstract

The oomycete genus *Saprolegnia* includes a number of animal parasites and opportunistic pathogens, with *S. parasitica* having a significant impact on fresh water fish production. In order to identify factors contributing to disease outbreaks, it is necessary to understand the population structure of this taxa and develop reliable methods for species identification. Species diversity within the genus *Saprolegnia* was evaluated to determine species composition in aquaculture systems. Nucleotide sequence variability in the internal transcribed spacer (ITS)-rDNA region was studied for more than 400 isolates collected in British Columbia. Consistent inter-specific variation supported the designation of species based on ITS sequence. Phylogenetic analyses comparing our results with previous studies support this molecular taxonomic approach to species identification, which does not rely upon transient morphological features. Population genetic diversity within the species *S. parasitica* was evaluated using microsatellite markers. Overall genetic diversity of *S. parasitica* was determined to be low, with evidence for sexual recombination and introduction of new genotypes over time. This study developed molecular diagnostic methods for genus and species identification that will provide useful tools for disease management in aquaculture systems.

Introduction

The genus *Saprolegnia* includes water moulds that belong to the class Oomycota and are placed within the phylum Heterokonta, a taxa that includes macrophytic algae and diatoms. Although members of this genus do not belong with the true fungi, they display fungus-like growth morphology (Robertson et al., 2009).

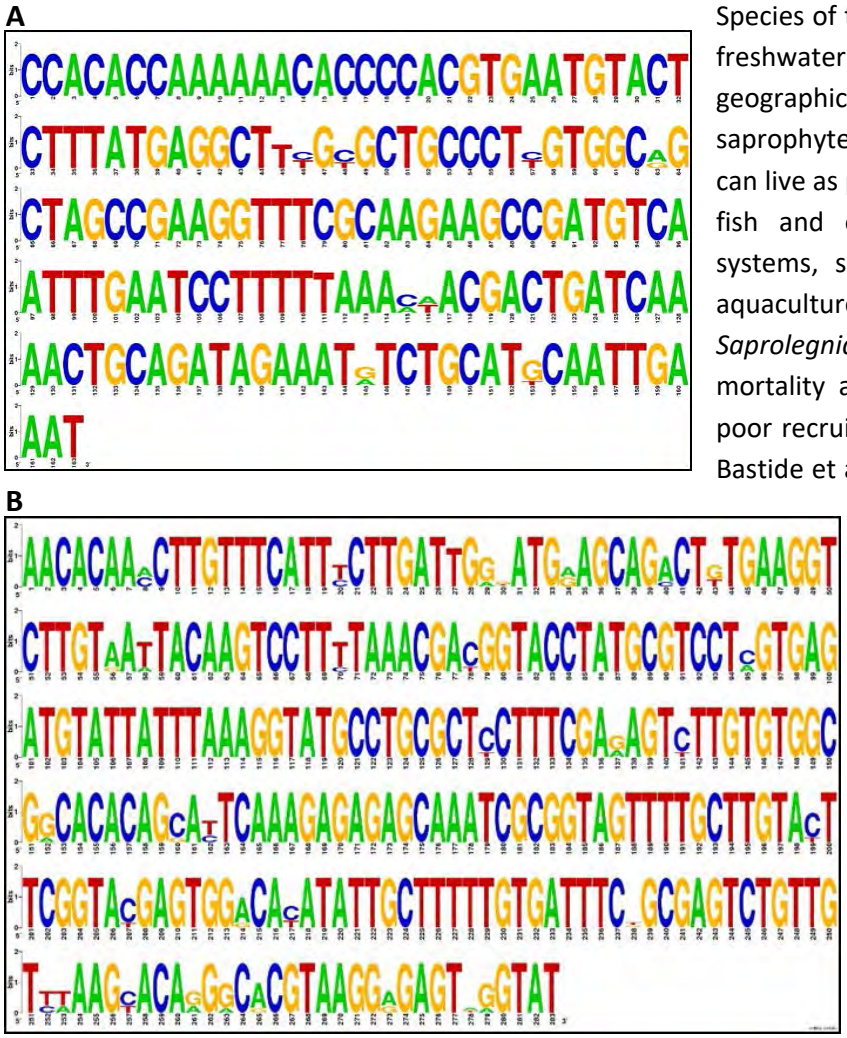

Figure 1
WebLogo representation (Crooks et al., 2004) of a multiple species alignment of nucleotide sequences for the ITS1 (A) and ITS2 (B) rDNA regions. Species aligned include *S. parasitica*, *S. ferax*, *S. diclina*, *S.delica* (I), *S. delica* (II) and *S. asterophora.*

Species of the genus *Saprolegnia* may be found in freshwater systems and damp soils over a wide geographical range. They can exist as saprophytes on various organic substrates, and can live as parasites or opportunistic pathogens of fish and other aquatic species. In artificial systems, such as enhancement hatcheries and aquaculture production systems, infections due to *Saprolegnia* species (Saprolegniosis) can cause mortality at all life stages, thus contributing to poor recruitment and reduced productivity (de la Bastide et al., 2015). Disease outbreaks are often associated with immune stressors, including other infections, routine vaccinations, life-stage transitions, and environmental factors (e.g., temperature extremes).

Studies of Saprolegniosis are hampered by the difficulty in identifying the causal agent; the morphological taxonomy of this genus is defined primarily by the sexual structures, which are rarely observed with vegetative growth during infection. Early studies have been plagued by frequent misidentification of species and a confused taxonomy (Sandoval-Sierra et al., 2014; Diéguez-Uribeondo et al., 2007). Our approach was to apply molecular tools for reliable species identification using nucleotide sequence information. These tools will allow us to answer important questions regarding species composition and assist disease management efforts in artificial systems. Our priority areas of research include; (1) developing molecular methods for species identification; (2) determining species composition in aquaculture systems; (3) describing the genetic variability of the primary pathogen species; (4) developing diagnostic methods for species detection and quantification; and (5) evaluating host – pathogen interactions in a model system.

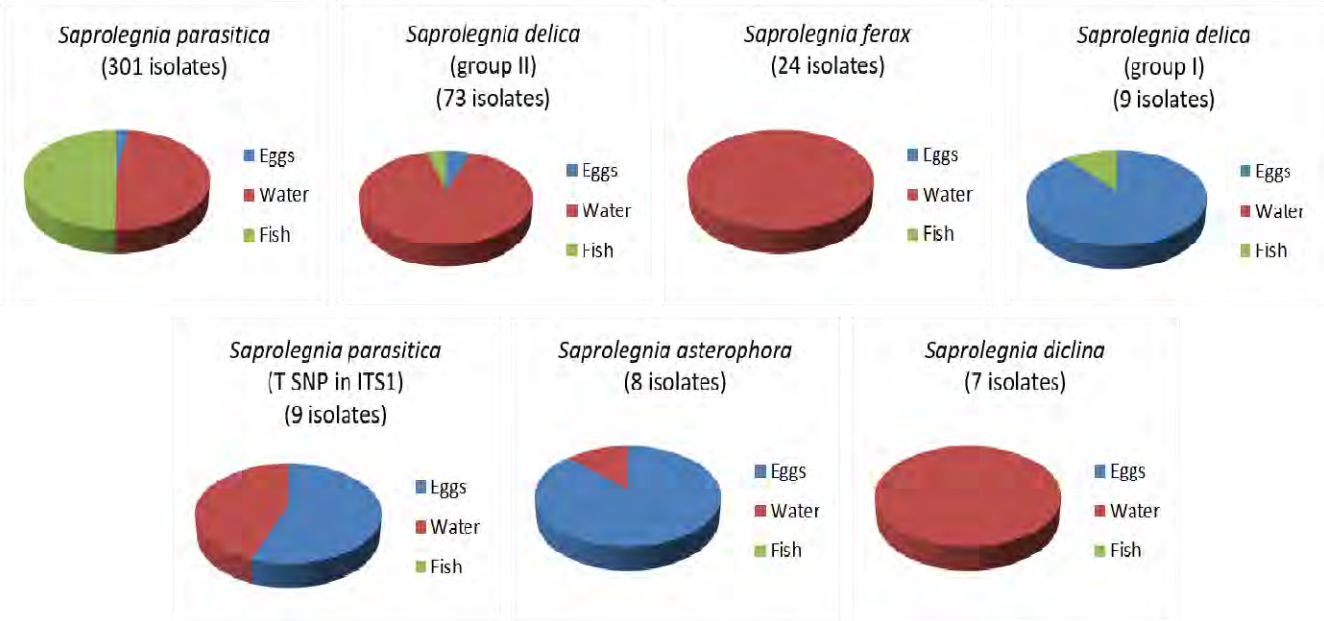

Figure 2
Summary of species identified, their relative abundance, and substrate origin for confirmed isolates of the genus *Saprolegnia* identified in a 15-month study of aquaculture facilities.

Materials and Methods

Most of the protocols for field and laboratory work have been described previously in de la Bastide et al. (2015) and Naumann et al. (In prep.). The details of some protocols are proprietary and have consequently not been provided in this publication.

Results and Discussion

Molecular methods for species identification: The identification of species of the genus *Saprolegnia* has always been challenging, due to a paucity of distinguishing characters. Our strategy was to clarify species identification by characterizing nucleotide sequence variability among species for the ITS-rDNA region. By use of confirmed voucher sequences (Diéguez–Uribeondo et al., 2007) and our sequence data collection, we determined that ITS nucleotide sequence comparisons are a reliable method for distinguishing *Saprolegnia* spp. Sequence polymorphisms included single nucleotide polymorphisms and short deletions (Fig. 1). We established a reference library of sequence data that facilitated our study of species diversity in freshwater aquaculture facilities.

Species composition in aquaculture systems: A survey of BC aquaculture facilities was conducted to describe the species diversity and composition in systems supporting salmon species. Water, tissue and egg samples were collected over a 15-month period from eight sites to isolate pure cultures from these sources and confirm their species identity. Pure cultures were propagated to obtain mycelium for DNA extractions and subsequent PCR amplifications of the ITS-rDNA region for sequence analysis and confirmation of species identity. This study identified 431 *Saprolegnia* spp. isolates and compared the relative abundance of each species from these sources (Fig. 2).

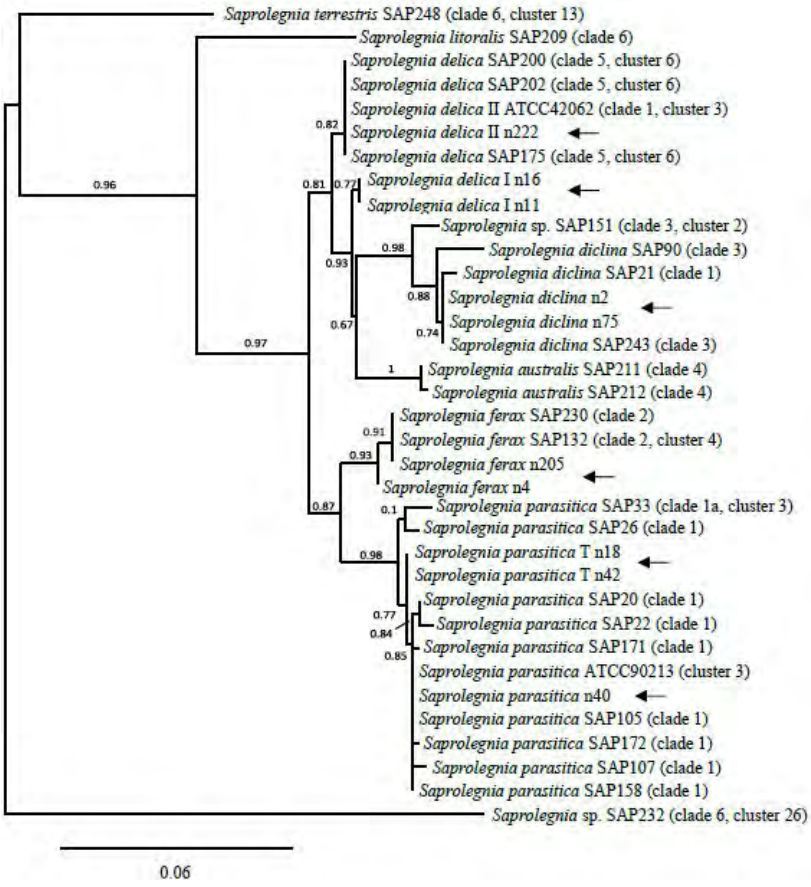

Figure 3
Phylogenetic analysis of confirmed isolates from our aquaculture system study (arrows) and their relatedness to voucher sequences from the study of Sandoval-Sierra *et al.* (2014). Confirmed isolates from the current study include *S. parasitica* (n18, n40, n42), *S. ferax* (n4, n205), *S. diclina* (n2, n75), *S. delica* I (n11, n16) and *S. delica* II (n222).

A total of 6 distinct species/taxa were identified in this collection, with the most abundant being *Saprolegnia parasitica*, comprising 310 isolates from all sources examined; about half of these isolates were obtained from infected fish or eggs, while the remainder were isolated from water. In contrast, the other species detected were much less abundant and were isolated primarily from water, the exception being *Saprolegnia asterophora* and *Saprolegnia delica* (group II) isolates, which appeared to favour eggs as a substrate. In order to confirm the species identity of these Canadian isolates, reference sequences from our collection were compared to voucher ITS sequence data in a large-scale phylogenetic analysis (Fig. 3). This analysis confirmed that our species designations were in agreement with the accepted clade system of Sandoval-Sierra et al. (2014).

Genetic variability of the primary pathogen species: Our analysis to date has supported the importance of *S. parasitica* as the primary pathogen of this genus affecting fish in aquaculture systems. Consequently, our focus has been centred on improving our understanding of this species. This has included a separate study of intraspecific variability to assess the population structure of

this species in aquaculture systems, as well as the development and refinement of diagnostic protocols for the detection of *S. parasitica* from different sources.

The assessment of population structure examined a subset of our *S. parasitica* collection (87 isolates) sampled over a 15-month period and evaluated the genetic variability of this population by using microsatellite markers. This study detected a low level of genetic variability that did not vary over time and was likely the product of infrequent sexual events, and/or the periodic introduction of new *S. parasitica* genotypes on colonized eggs and fish (Naumann et al., in prep.).

Diagnostic methods for species detection and quantification: As our work with this species proceeded, we quickly recognized the need for a rapid and reliable diagnostic to detect *S. parasitica* in various sample types, including water, tissue, and eggs. Our current protocols are PCR-based and do not require the isolation of a pure culture to obtain a DNA template for amplification. By use of species-specific primers that target a unique locus, we are able to confirm species identity from tissue or egg samples, and quantify species inoculum load in water samples collected from an aquaculture system. The individual species of *S. parasitica*, *S. ferax*, and *S. diclina* may be detected and quantified by a quantitative PCR (qPCR) approach, by use of a standard curve based on zoospore concentration in water (Fig. 4).

These diagnostic methods are useful for a number of reasons. They will allow the early detection of *S. parasitica* for timely decisions regarding prevention

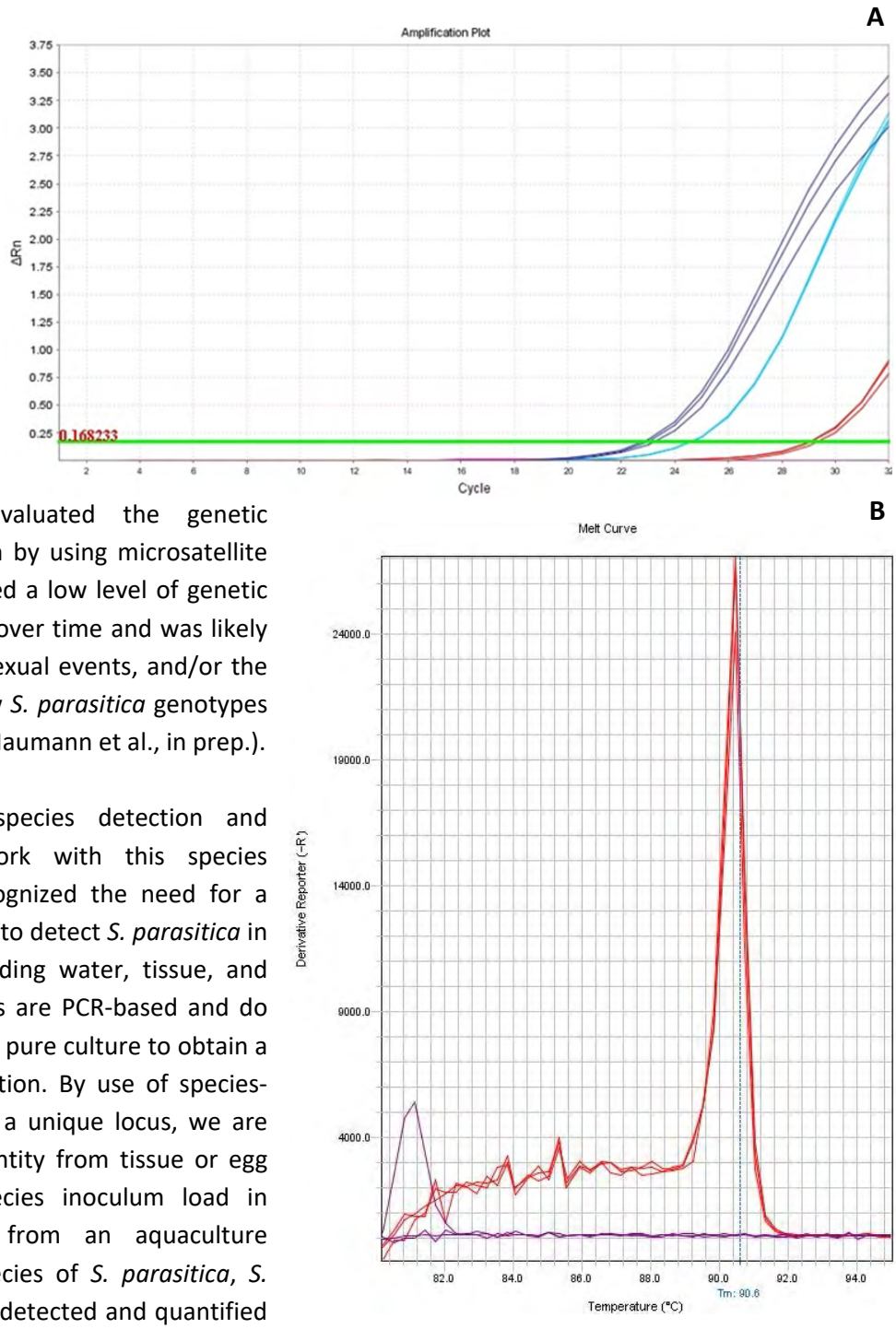

Figure 4
Quantitative PCR (qPCR) analysis using species-specific primers to detect and quantify species of the genus *Saprolegnia*. This example demonstrates the detection *S. parasitica* and *S. ferax*, with the amplification plot (A) allowing quantification of inoculum load, and the melt curve analysis (B) confirming species identity.

and treatment. The diagnostics will identify sources of the inoculum (water supply, egg, and fish stocks) and confirm the efficacy of any treatment measures. They may be used to monitor the effectiveness of existing water-treatment systems (e.g., UV, ozone, biofilters), especially in recirculating aquaculture systems. Diagnostics will also be useful in monitoring *S. parasitica* populations over the course of fish production cycles to determine the potential risk of outbreaks at different life-stages.

Host-pathogen interactions between Atlantic salmon and *Saprolegnia parasitica*: Our current research is examining host-pathogen interactions in order to develop new approaches to disease management. Our major project objectives include the development of a reliable challenge system in which to evaluate host-pathogen interactions, the mapping of biomarkers that describe the host response at different phases of infection, and the identification of unique genes and/or pathways in *S. parasitica* that are up-regulated in the presence of the host at both the pre- and post-infection phases.

Through gene expression analysis of both the host and the pathogen under controlled conditions, we hope to obtain a better understand of the infection process for *S. parasitica*, as well as identify unique gene targets for the future development of control measures. At the same time, this study will improve our understanding of host response to *S. parasitica* under different conditions; these will include both optimal, as well as stressful living conditions that are often experienced by fish in aquaculture systems. By understanding these interactions, we hope to address this persistent disease problem that degrades fish health and reduces the productivity of finfish aquaculture systems.

Acknowledgements

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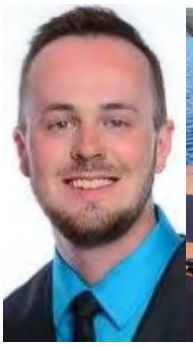


THE USE OF GIS TOOLS TO AID IN THE UNDERSTANDING OF THE MARINE ENVIRONMENT: EXAMPLES FROM THE NEWFOUNDLAND FISHERIES AND OCEANS AQUACULTURE SECTION

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Abstract

Geographic Information Systems (GIS) are computer applications that are used to analyze and graphically present multiple forms of spatially and temporally varying data. GIS programs such as Ocean Data View (ODV), ArcGIS, and QGIS provide sophisticated tools to examine the marine environment. The use of such tools is, therefore, particularly suited to the study of the marine environment, which is a place very much variable in both space and time. The aquaculture section of the Fisheries and Oceans Northwest Atlantic Fisheries Centre (DFO-NAFC) have, over the years, made extensive use of those tools and developed methods to analyse geographical data of Newfoundland's (NL) marine

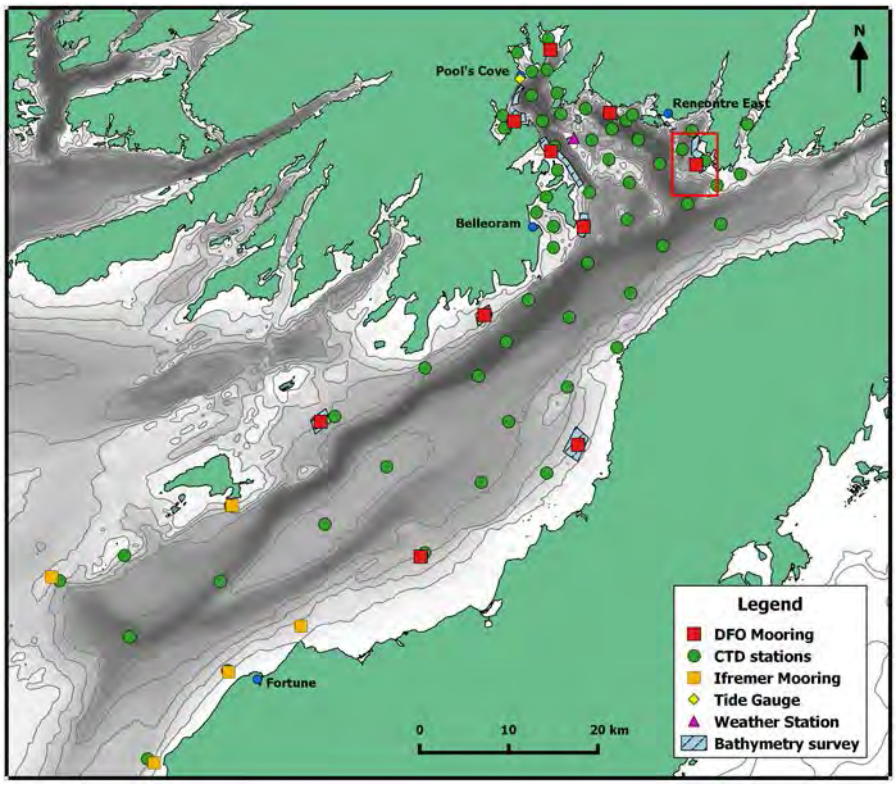


Figure 1
Summary of the field work completed on the south coast of Newfoundland (Fortune Bay) in May 2016 and created using QGIS. Red square represents area defined in Figure 3.

environment. In this article, examples of such analyses and graphical products are presented in relation to the physical oceanography and aquaculture activities of the Coast of Bays, an area of the south coast of Newfoundland.

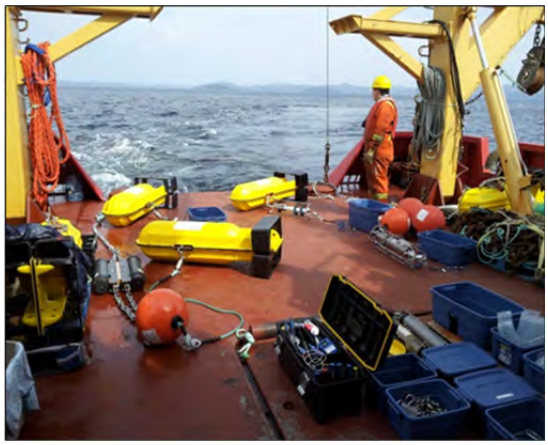


Figure 2
Mooring deployed on board CGGS Vladykov (Fortune Bay).

Introduction

Geographic Information Systems (GIS), such as Ocean Data View (ODV, Schlitzer, 2016) and Quantum GIS (QGIS, 2016), are powerful and freely available computer applications used to analyze and graphically present multiple forms of spatially and temporally varying data.

ODV utilizes three forms of interpolation gridding: Quick, Weighted-Average (WA) and Data-Interpolating Variational Analysis (DIVA, Troupin et al., 2012). Unlike the other forms of gridding available with ODV, DIVA can take the natural physical barriers into account such as coastlines and sub-marine sills which are critical parameters for realistic representations of water masses.

QGIS has multiple forms of interpolation gridding, with Inverse Distance Weighting (IDW) and Triangulated Irregular Network (TIN) being some of the most widely used methods (Lloyd, 2010). IDW employs the sampled points (e.g., observations) to produce a grid whereby the influence of a point declines as distances increase from centre. TIN, on the other hand, utilizes neighbouring points to make up a mesh of irregular triangles minimizing small angles (i.e., Delauney's Triangulation).

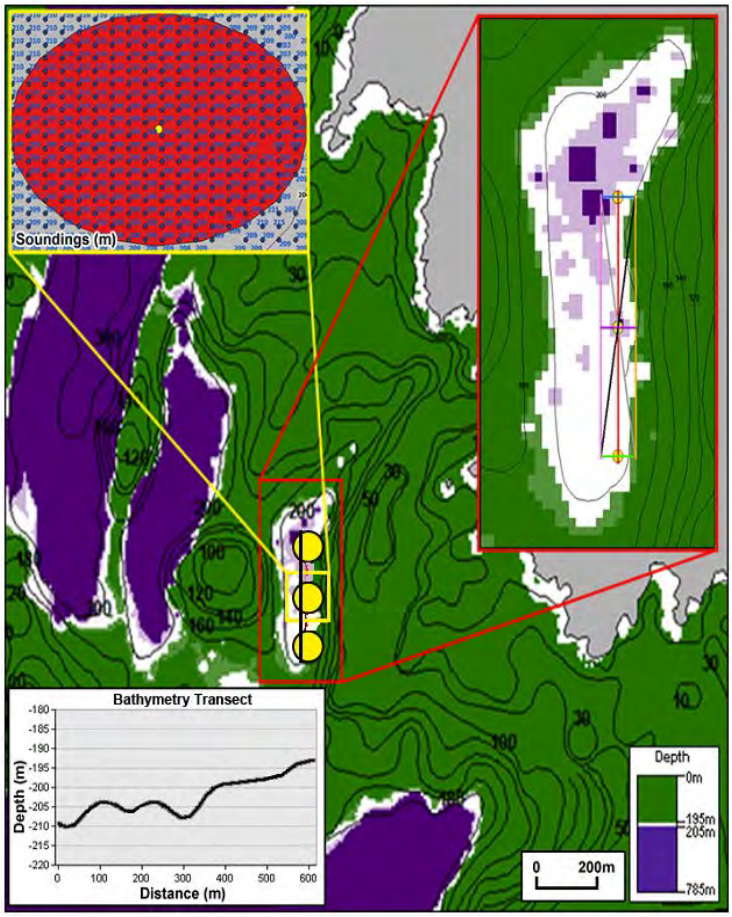


Figure 3
Determining a mooring deployment site to plan ship route, optimum bathymetry slope, and depth. Yellow dots represent target sites and the red circle is deployment uncertainty (50-m radius).

The NAFC Aquaculture section is currently undertaking a number of studies related to the growth of the aquaculture industry on the south coast of Newfoundland (Fig. 1). For most

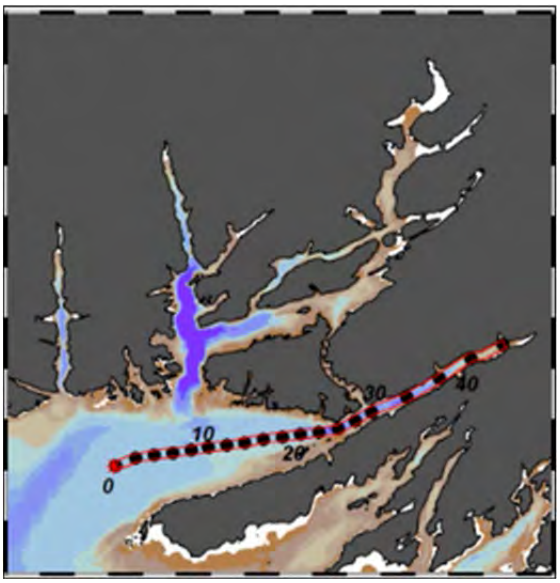


Figure 4
Hermitage Bay map showing the transect used in DIVA interpolation (Fig. 4), spatial & temporal comparison.

(if not all) of these studies, a sound understanding of the physical environment (e.g., ocean circulation and variation of the water column stratification) is critical and requires the use of GIS tools. This article presents examples of this use, in particular with respect to field activities and spatial interpolations.

Ultimately, the data collected is being analysed and used to produce input data fields (forcing and initial conditions) to run a hydrodynamic, numerical model, which will provide with the necessary spatial resolution to better understand potential water linkage between aquaculture sites as well as to provide key baseline data for aquaculture management on aspects such as, but not limited to, site selections and carrying capacity as well as diseases, parasites, and waste dispersion.

Field Work Examples

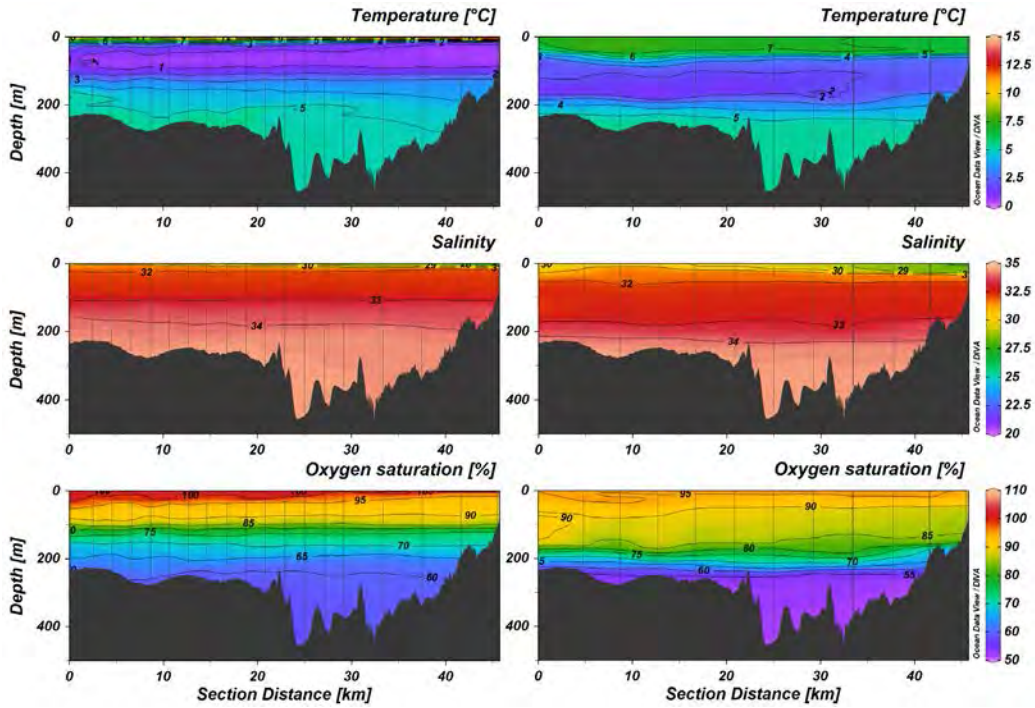
In the context of field work, GIS tools are used by the section for program planning, execution, and reporting. GIS tasks include the creation of transects providing depth profiles (thus, slopes) to plan boat trajectory during mooring deployments (Fig. 2) and the creation of detailed zooms of the depth soundings to ensure appropriate site depth (Fig. 3).

When deploying moorings, significant efforts go into the choice and determination of the targeted site. This is of particular importance to ensure the safety of mooring lines and expensive instrumentation (e.g., enough clearance for shipping activities) and to ensure the collection of meaningful observations (i.e., suitability or representativeness of the site for the parameters being measured).

Spatial Interpolation Examples

The DIVA interpolation, unlike other methods available from ODV, can take natural physical barriers such as coastlines and bathymetry into account to provide a more accurate representation of the water masses spatial distribution (Figs. 4 & 5).

Figure 5
DIVA interpolation of the Hermitage Bay transect between June (A) and November (B) using ODV.



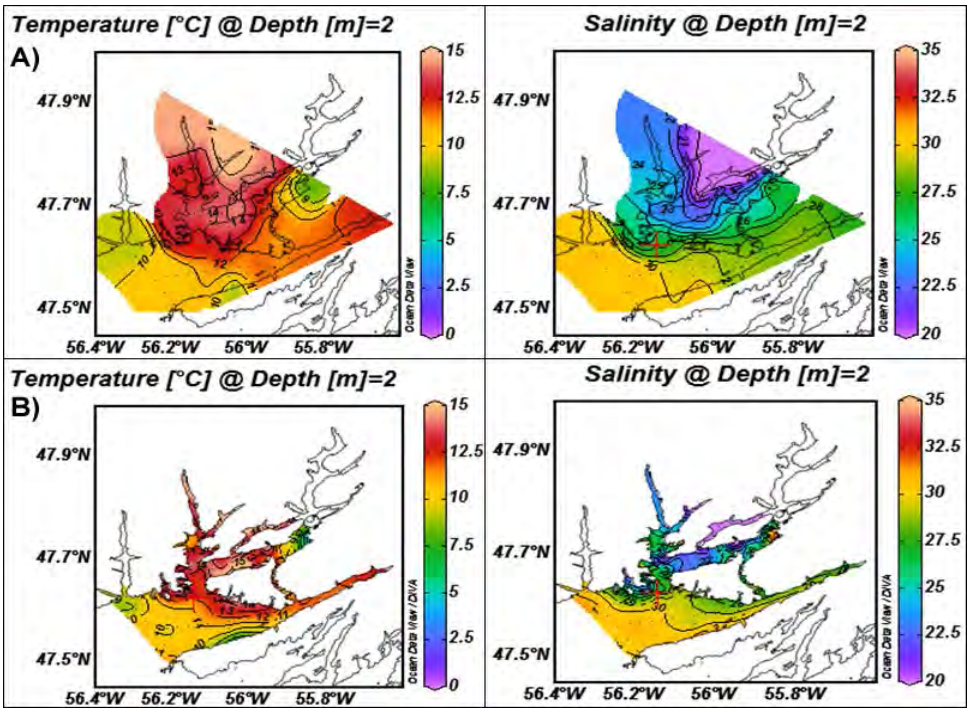


Figure 6
WA (A) vs. DIVA (B) interpolation produced using ODV.

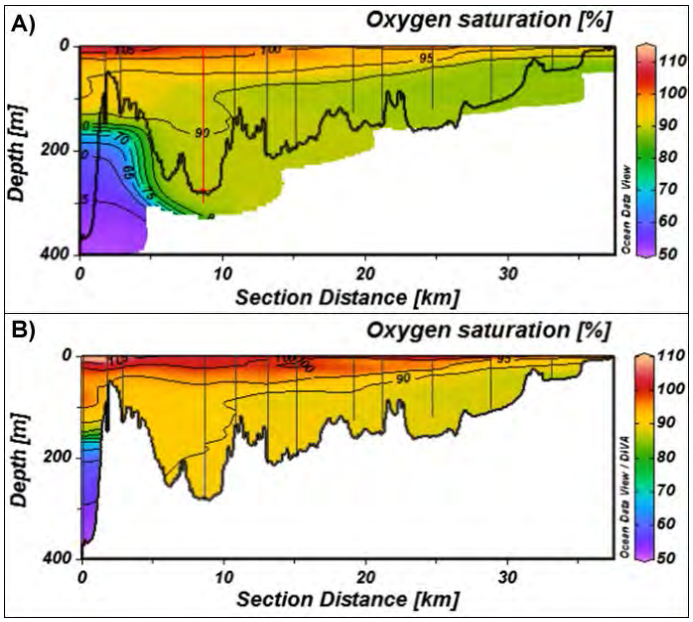


Figure 7
WA (A) vs. DIVA (B) interpolation produced using ODV.

Figures 6 and 7 show examples of WA and DIVA interpolation produced using ODV and how using the coastlines (Fig. 6) and bathymetry (Fig. 7) as physical barriers can provide a more accurate representation of water masses and avoid unrealistic 'slippage of water' from one area to another. For instance, note the changes in oxygen saturation (Fig. 7) from the right (due to excessive horizontal average from the WA technique) and the 'artificial' transfer of deep water, with low oxygen saturation, from the left-hand side of the sill to the right (i.e., 'crossing' the sill).

An another example of a common interpolation challenge is presented in Figure 8, which illustrates how the variability and/or scarcity of bathymetric observations can lead to interpolation issues:

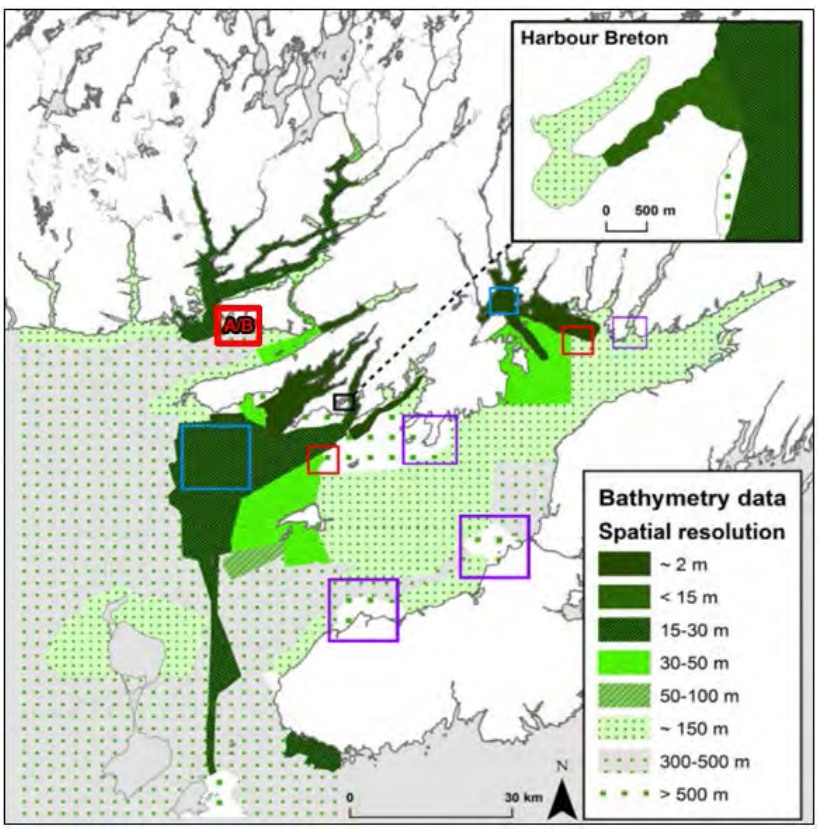


Figure 8
Spatial resolution of bathymetry data for the Coast of Bays region.

- THE GOOD: Blue boxes represent regions with similar and high data resolutions, leading to a good interpolation (Fig. 8).

- THE BAD: Red boxes (Fig. 8) show regions with data of varying resolutions which can cause unrealistic artifacts (e.g., spotting and overestimation in areas with depth < 50 m; Fig. 9).

- THE UGLY: Purple boxes display regions with data of very low resolution which results in very significant errors (Fig. 8).

Acknowledgements

The authors would like to thank the researchers and technical staff from the aquaculture and biotechnology sections at the Northwest Atlantic Fisheries Centre (NAFC) of Fisheries and Oceans Canada for their help and effort in the collecting of data.

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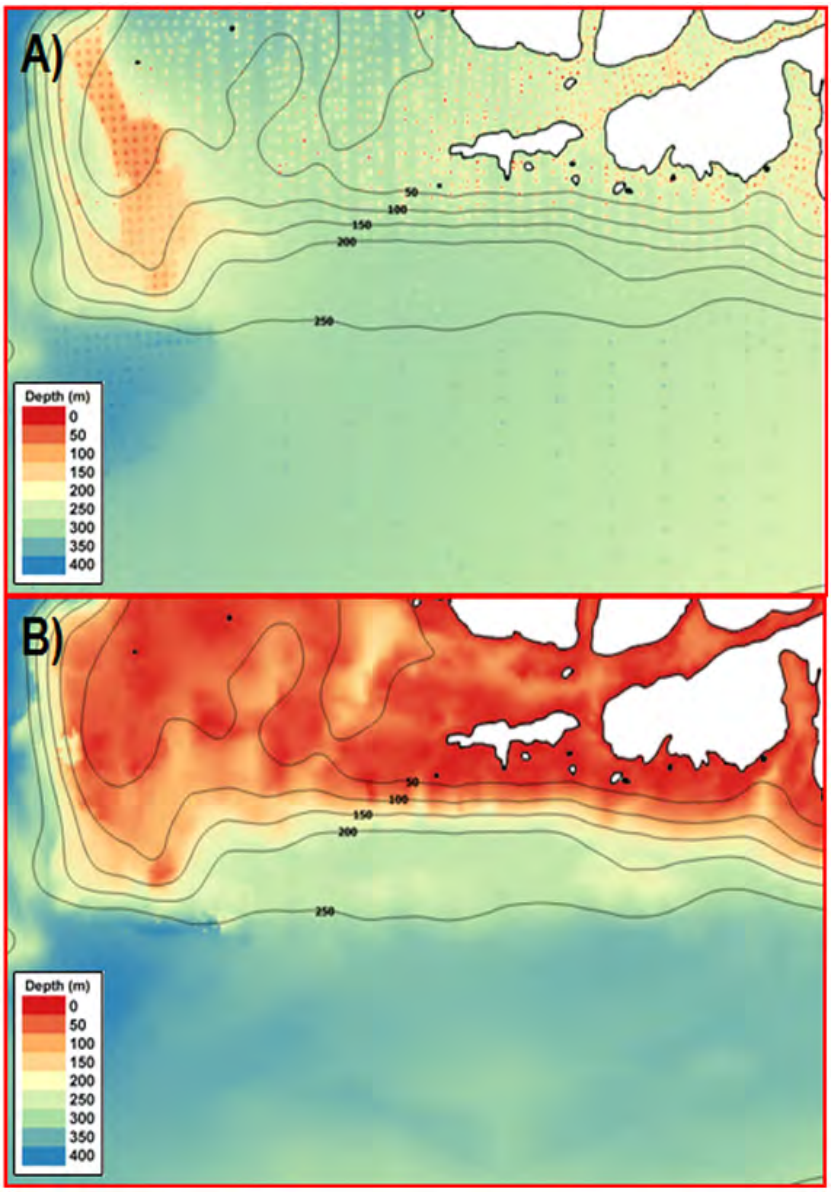

Figure 9
Varying degrees of resolution can cause problems with interpolation (red box): IDW (A) and TIN (B).

THE EFFECT OF BUTYRIC ACID IN MICROPARTICULATE DIETS ON THE SOMATIC GROWTH PERFORMANCE AND SURVIVAL OF EARLY JUVENILE STRIPED BASS (*Morone saxatilis*)

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Abstract

Butyric acid (BA) is a respiratory fuel produced by bacterial fermentation in the digestive tract of all vertebrates that can improve digestion, gut health, and somatic growth. To test the hypothesis that BA can improve the growth performance of striped bass, early juveniles (39 days post hatch) were fed a diet containing BA at 0.0, 0.5, or 1.0% for ten days. Mean body weight (85 mg initial) among bass fed either 0.5 or 1.0% BA increased three-fold by day ten (247 mg final), and was significantly greater than the control (176 mg final) (p = 0.016). The striped bass consuming either 0.5 or 1.0% BA doubled their body length (20.7 mm initial) after ten days, which was significantly greater than the control (30.1 mm final) (p = 0.022). For both length and weight, there was no significant difference between the 0.5 and 1.0% treatments, only between BA and no BA. Moreover, there was a significant difference in the specific growth rate for 0.5 and 1.0% treatments compared to the control (p = 0.017) and the survival of bass consuming either treatment diet was significantly greater than the control (83 vs. 77%; p = 0.032).

Introduction

The short-chain fatty acid, butyric acid, has been recognized as a potential dietary additive to remedy the growth issues that hinder the rearing of marine fish. Butyric acid has been linked to the upregulation of a di- and tripeptide

transporter in the digestive tract of all vertebrates (Smith et al., 1998). There was a significant increase in the transcription level of the intestinal Peptide Transporter 1 (Pept1) of European sea bass (*Dicentrarchus labrax*) when fed a diet with 2.0% butyric acid (Rimoldi et al., 2013). Sodium butyrate in the diet of triploid red crucian carp (*Carassius carassius*) improved growth and protein absorption, which was linked to increased levels of Pept1 (Liu et al., 2014). In sea bream (*Sparus aurata*), butyric acid in the diet resulted in an improvement in growth performance (Bendito-Palos et al., 2014), Butyric acid is novel in aquaculture; however, its effects on growth and intestinal health have been reported in a variety of vertebrates, including rats (*Rattus rattus*) and pigs (MacIntyre et al., 1993; Manzanilla et al., 2006).

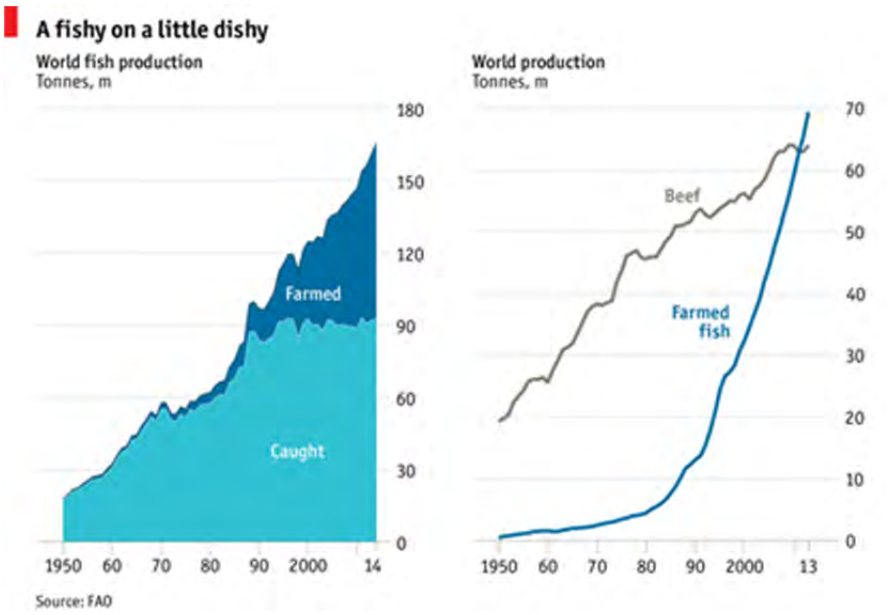

Figure 1
World production of caught and farmed fish (tonnes) over the last 65 years, including a comparison to beef production (FAO).

Total global aquaculture production reached over 60 million t in 2012, and continues to increase by 1 million t per year (Fig. 1; FAO, 2014). One major concern is the current production bottleneck that plagues the expansion of marine finfish aquaculture whereby less than 10% of the eggs hatched may survive to market-sized adulthood (Fernández et al., 2008). Striped bass were used as a model species of marine fish to investigate the underlying mechanisms that underpin growth, survival, and nutritional mechanisms of fish growth within the aquaculture industry (Duarte et al., 2009).

The critical point in the production cycle of an aquaculture species is the first exogenous feeding stage, which occurs for striped bass at approximately day 4 post hatch. They consume live zooplankton that are nutrient deficient, until they can be weaned onto a microparticulate diet (Terova et al., 2013). Intervening at this critical point is essential; determining the age at which marine fish can be weaned onto a formulated microdiet that can satisfy larval nutrient demands may promote growth, potentially resulting in improved growth and survival of early juveniles.

Current studies focus on butyric acid and adult fish, therefore, including butyric acid at specific percentages for juvenile striped bass weaning may significantly improve the growth and survival of striped bass, which may have positive implications for many other species of marine finfish within the aquaculture industry.

Materials and Methods

The trial ran for ten days, with striped bass that were 39 days post hatch (dph). There were a total of 9 tanks; 3 replicate tanks for each of the 3 diets (0.0% BA, 0.5% BA, and 1.0% BA). The tanks were cleaned and siphoned twice per day, during which time the number of mortalities were counted and recorded while being removed from the tanks.

The volume of the black tanks was 155 L, and they were 27 cm in diameter. Each tank was stocked with 200 early juveniles. The tanks were on a flow through system and the temperature and salinity were monitored twice per day and were held constant at 20°C and 22 parts per thousand (ppt), respectively. All tanks received 24-hour light at a constant intensity of 20 lux. Vibratory feeders were programmed to dispense meals every two hours for 24 hours a day, alternating with hand feeding took place every 2 hours between 6 am and 8 pm for the entire trial.

In order to determine the specific growth rate, the following equation was used: (lnWf-lnWi x 100)/ t, where lnWf was the natural logarithm of the final weight, lnWi was the natural logarithm of the initial weight, and t was the time (in days) between lnWf and lnWi. The gains in length and weight were calculated by taking an average of 30 individuals from each treatment before and after the trial. The percent survival was determined by counting mortalities each day of the trial, as well as counting the survivors at the end of the trial and determining the difference between the initial stocking number of 200 juveniles and what remained at the very end. The following calculation was used: S/I x 100, where S was the number of survivors and I was the initial number.

A one-way analysis of variance was used to determine any statistical significance between the variation of length gain, weight gain, specific growth rate, and survival. A Tukey post hoc test was then conducted to analyze the significance between the treatments to determine if 0.5% was significantly different from 1.0%.

Results

After ten days, the 39 dph striped bass fed either of the treatment diets experienced a three-fold increase in weight (Fig. 2), and the length nearly doubled (Fig. 3). A one-way analysis of variance showed that the mean weight gain and length gain was significantly higher among the 0.5% BA and 1.0% BA treatments compared to the control with 0.0% BA (p = 0.016 and p = 0.022). Similarly, the specific growth rate of the striped bass after ten days was significantly greater among the treatment groups compared to the control (p = 0.017). In order to determine if the treatments were significantly different from each other, a Tukey multiple means comparison test was performed, and revealed that 0.5% BA and 1.0% BA were not significantly different from each other for length, weight gain, or specific growth rate.

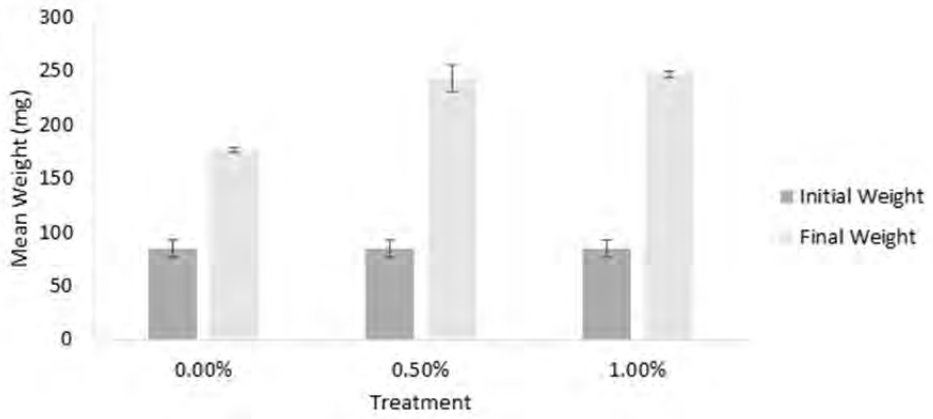

Figure 2
Mean initial weight (mg) with standard error of 39 day old striped bass early juveniles, and mean final weight (mg) with standard error after ten days (49 day old) of receiving 0.0% BA, 0.5% BA, or 1.0% BA.

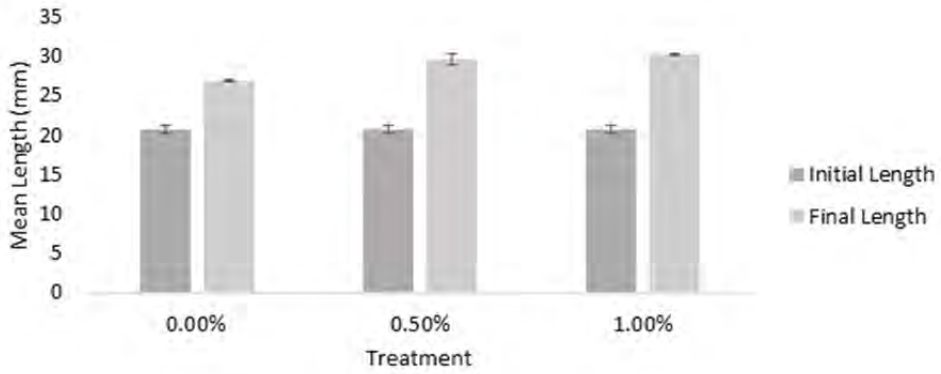

Figure 3
Mean initial length (mm) with standard error of 39 day old striped bass early juveniles, and mean final length (mm) with standard error after ten days (49 day old) of receiving 0.0% BA, 0.5% BA, or 1.0% BA.

There was a significant increase in the survival of the 39 dph striped bass after ten days, as it nearly doubled in the treatments (Fig. 5). A one-way analysis of variance indicates that the survival was significantly higher among the 0.5% BA and 1.0% BA treatments compared to the control with 0.0% BA (p = 0.032). To determine if the treatments are significantly different from each other, a Tukey's

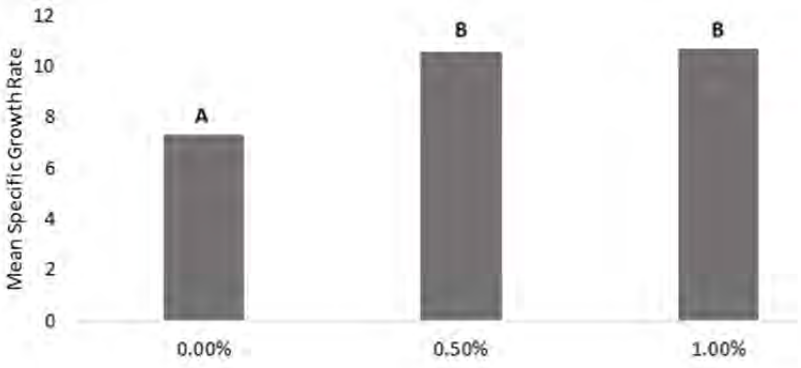

Figure 4
The mean specific growth rate for each treatment (0.0%, 0.5%, and 1.0% BA) of early juvenile striped bass (39 dph) over a ten-day trial. Different letter groupings indicate a significant difference between treatments.

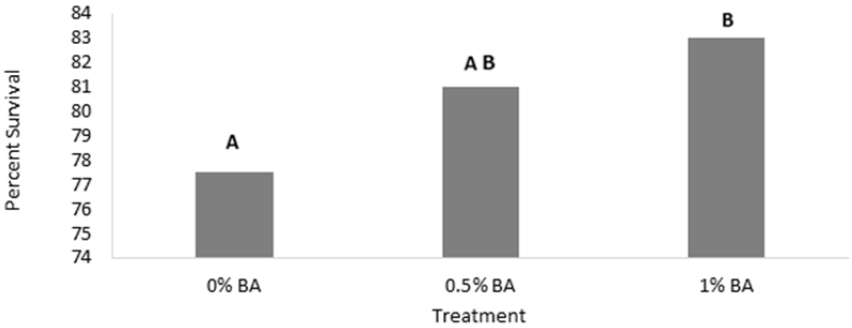

Figure 5
Percentage survival of striped bass early juveniles after the ten-day experimental trial. Different letter groupings indicate significant differences among treatments, while similar letter groupings indicate that there are no significant differences among treatments.

test was performed, and indicated that 0.5% BA and 1.0% BA are not significantly different from each other.

Discussion

Successful changes in growth were observed in this trial, which was conducted on striped bass at 39 dph. At this stage, the striped bass are no longer considered larvae but rather early juveniles. Although the initial goal of the experiment was to intervene with a butyrate microparticulate diet at an earlier life stage, the results are still indicative of positive growth, which can also be seen across a wide variety of different species of teleost fish.

In Nile tilapia (*Oreochromis niloticus*), fingerlings fed sodium butyrate experienced a significant increase in specific growth rate, final weight, and total weight gain (Ahmed and Sadek, 2014). A second treatment of sodium butyrate including probiotics commonly used in aquaculture called Protexin, also resulted in an improved growth performance (Ahmed and Sadek, 2014). Similarly, the early juvenile striped bass also experienced a significantly improved growth performance when butyric acid was included in the diet compared to the control diet (Ahmed and Sadek, 2014). In terms of specific growth rate, total weight gain, and total length gain, striped bass grew significantly better while consuming butyric acid in a microparticle than when there was no dietary butyric acid (Figs. 1 & 2). In the Nile tilapia, the promotion in growth performance was due to an increase in blood glucose levels and intestinal glucose absorption, facilitating nutrient uptake in the fingerlings caused by probiotics and butyrate (Ahmed and Sadek, 2014). These results suggested that not only does butyrate have a positive impact on growth performance but that it

may also have positive health benefits for the fish, especially when coupled with probiotics.

Dietary butyric acid can be introduced into fish feed in a few ways: partially protected, free, or microencapsulated. In the case of this experiment, the butyric acid was partially protected and included in a microparticle (Robles et al., 2013). Microencapsulation and partial protection are designed to create a gradual release down the digestive tract, whereas free butyric acid may only become available in the anterior portion of the digestive tract (Robles et al., 2013). Studies have been conducted on juvenile gilthead sea bream (*Sparus aurata*) whose treatment diet consisted of 3.5 mm pellets containing 21% partially protected butyric acid. The results of this study revealed a significant increase in weight in the treatment groups compared to the control (Robles et al., 2013).

Common carp juveniles (*Cyprinus caprio*) were used in an experiment involving a microencapsulated sodium butyrate (MSB) diet in which there were three treatments: a control with no supplementation, a 1.5-hour release and a 3.0-hour release treatment (Liu et al., 2014). Both of the treatments with the MSB supplement experienced significant changes in growth, intestinal indices, and feed conversion rates; however, there was only a significant difference between the two release times (MSB1.5 and MSB3.0) with respect to weight gain throughout the 10-week trial (Liu et al., 2014). Although not microencapsulated, the butyric acid treatments in our striped bass experiment differed by the percentage of butyrate inclusion (0.0, 0.5, or 1.0%). These results correspond to the results from the juvenile common carp, as the growth performance of the striped bass was significantly improved among the treatment groups than the control. Similarly, there were no significant differences between the two treatments for growth.

There are studies that have not found any positive correlation between these growth factors and butyric acid, particularly when fed with fishmeal or soybean meal to rainbow trout (*Oncorhynchus mykiss*). In this case, digestibility and the feed conversion ratio were negatively affected (Gao et al., 2011). This is an area of research that should continue to grow and examine these indices at varying ages of fish, as well as across many different species. Understanding ratios, supplements, and age of administration would help to fill the knowledge gap that exists in fish nutritional requirements.

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LUMPFISH AS CLEANER FISH: GAPS IN KNOWLEDGE AND RESEARCH NEEDS

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Abstract

The use of lumpfish (*Cyclopterus lumpus*) as cleaner fish offers an attractive alternative to the use of medicines to treat sea lice infecting farmed salmon, but the species is new to aquaculture and little is known about factors limiting production. Research on lumpfish at CSAR (Swansea University) started in 2014 when 5,000 juveniles were reared for the incipient lumpfish aquaculture industry. Since then, CSAR has produced over 3 million eggs, and > 2 million juveniles, making it one of the largest producers of lumpfish in the UK. The lumpfish facility consists of a 60-m³ recirculating aquaculture system, plus a quarantine room for the reception of broodstock. The system operates with 15 or 30 upwelling incubators, eight 1,500-L tanks, and six 4,000-L tanks with a nominal capacity of 3.6 million weaned larvae. Lumpfish research at CSAR is focussing on genetics, larval weaning, health and welfare, some results of which are presented here. Our gap analysis indicates that the development of a selection programme for improved disease resistance, all year production, and better delousing efficiency represent the next steps for the rapidly expanding lumpfish industry.

Figure 1
CSAR's dedicated, 750-m² building housing two large recirculation facilities and laboratories.

Introduction

The Centre for Sustainable Aquatic Research (CSAR) is a 750-m² recirculation aquaculture system for education, training, and research based at Swansea University's College of Science (Fig. 1).

CSAR covers both pure and applied research themes, ranging from theoretical and modelling work to experimental and field trials with

Figure 2
CSAR's algal photo bioreactors and microalgae research facilities (UK's largest University algal research facility).

organisms including bacteria, microalgae, shellfish, finfish, and aquaculture engineering. The Centre has a range of dedicated laboratories, including two algal photo bioreactors (PBRs) (Fig. 2), a tropical recirculating facility, a killifish aquaria laboratory, a salmonid recirculating facility, and a zebrafish unit (Fig. 3).

Figure 3
Left: one of the two main recirculating aquaculture systems. Right: tropical fish laboratory.

In 2014, Swansea University and Marine Harvest Scotland began a 5-year R & D partnership to develop the commercial production of lumpfish for use as cleaner fish by the salmon farming industry. This included two jointly funded scholarships on sustainable aquaculture (a MRes and a PhD). Production in 2014 was modest at 5000 juveniles, but it rapidly expanded in 2015 and 2016. As with many other facilities working with this novel species, many initial hurdles had to be overcome, but lessons were learned and survival increased dramatically. Standard operating procedures were developed to achieve full traceability, improve hatching rates and increase alevin survival (Fig. 4).

Figure 4
Left: broodstock being PIT tagged to ensure family identification and full traceability.
Middle: vertical egg incubators developed at CSAR. Right: Newly hatched larvae ready for first feeding.
Bottom: tropical fish laboratory.

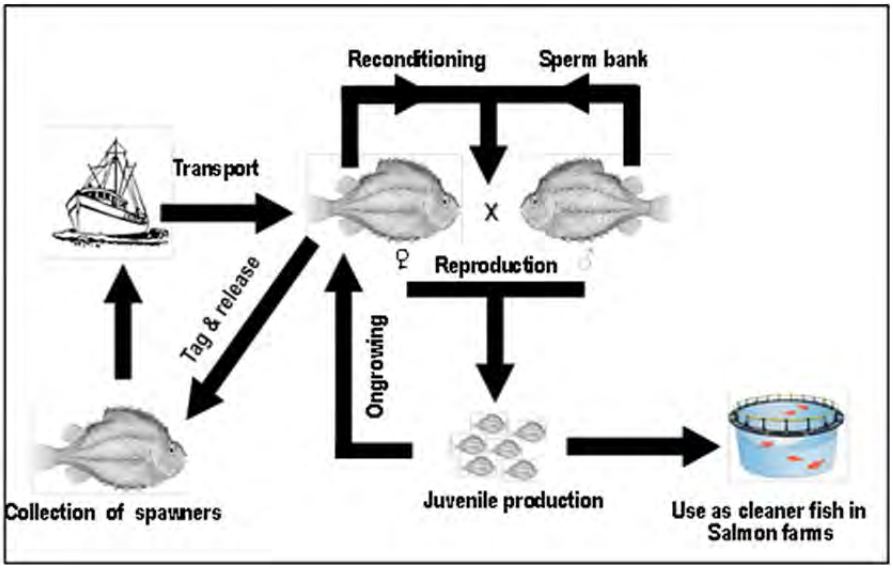

Figure 5
Main areas of lumpfish research being developed at CSAR. Each stage shows areas where targeted research can be used to increase survival and improve sustainability (adapted from Powell et al. 2017).

To date, CSAR has produced over 3 million eggs and 2 million weaned juveniles, up to 10 g in mass, which have been sent to several UK facilities to be reared further until deployment into sea cages.

Lumpfish research at CSAR is focussing on overcoming production bottlenecks and on improving sustainability and welfare at all stages during the production cycle (Fig. 5). These have included research on the reproductive biology of lumpfish, egg degumming, larviculture, health, welfare, and genetics.

Reproduction

We are examining variation in gamete quality and its relation to fertilization rates and hatching success. Work is also being carried out on sperm storage and the use of milt extenders. The aim is to optimize production through broodstock selection and reduce the number of males used in production. For example, though the use of milt extender we reduced the number of males needed by 80% and were able to keep milt active for over 1 week with no reduction in sperm viability or fertilization success. Egg pigmentation is also a focus of research, as this has been found to vary greatly between individuals and may have an effect on fertilization rates, egg development, and larval survival.

Egg Degumming

Lumpfish eggs are naturally sticky and one of the difficulties during egg incubation is that dead eggs deteriorate quickly and impair the survival of adjacent ones in the clump. By degumming the eggs, oxygenation could be increased and it may be possible to reduce the risk of infectious diseases spreading, and would also allow a more accurate count of eggs. A range of degumming approaches have been trialled at CSAR with alcalase (an enzyme) showing the best results, with no detrimental effect on egg viability (Fig. 6).

Figure 6
Left: naturally sticky lumpfish eggs in a clump. Middle: 'mini hopper' setup used in degumming trials. Right: a sample of eggs degummed with alcalase showing developing embryos.

Determinants of Variation in Weaning Survival

Weaning trials have shown high variation in survival among families with a critical period after approximately 30 days post hatch. Research into the effect of different diets on larval survival examined the effects of seven different weaning diets, namely (1) high *Artemia* ration (60 *Artemia*/larvae); (2) low *Artemia* ration (30 *Artemia*/larvae); (3) Dry feed in excess (5% BW); (4) Dry feed low ration (2.5% BW); (5) *Artemia* and dry feed (50:50) in excess; (6) *Artemia* and dry feed (50:50) low ration; (7) *Artemia* and dry feed (50:50) in excess and no weaning after 4 weeks.

Mortality rates were relatively low for the first three weeks before increasing rapidly in the low ration diets and in the dry feed to excess diet (diet 3). After the critical time for survival passed, mortality decreased and levelled off. Based on these results, live feeding was extended for seven weeks in 2016, resulting in 85% survival from hatching to post weaning.

Feed Enrichment

Research into feed enrichment examined the merits of two different commercial diets, as well as the addition of vitamin C. Three diets were examined, namely Control = 2.5% A1 Otohime and ori-gro enriched *Artemia*; Treatment 1 = 2.5% Inve Start-S and ori-gro enriched *Artemia*; Treatment 2 = 2.5% A1 Otohime and vitamin C enriched *Artemia*, and Treatment 3 = 2.5% Inve Start-S and vitamin C enriched *Artemia*.

As with previous trials, survival varied highly among families but a critical time for survival was observed at approximately 300 degree days (30 days post

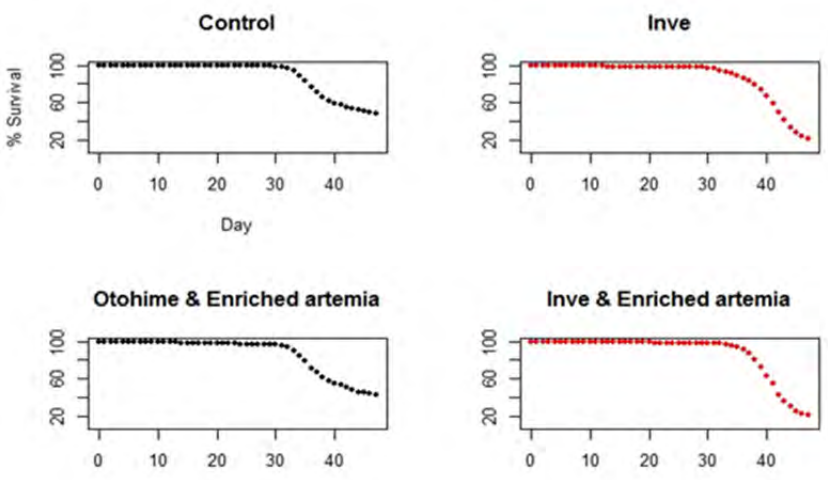

Figure 7
Survival of lumpfish larvae on different diets showing critical time for survival at c. 30 dph.

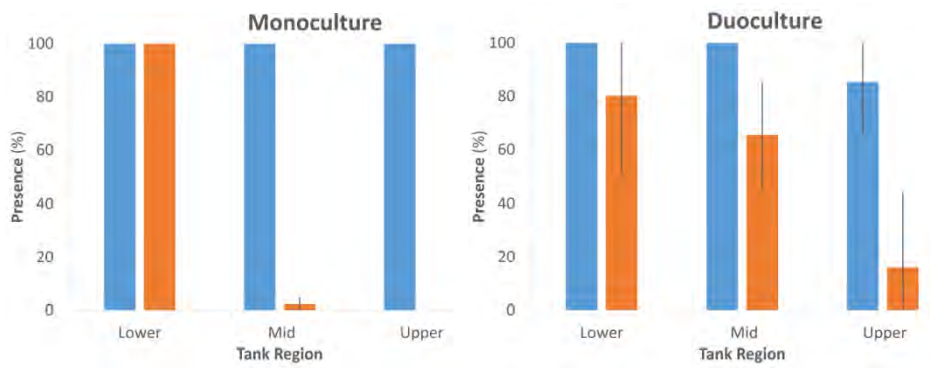

Figure 8
Tank distribution of lumpfish and seabass in monoculture and duo-culture. The presence of lumpfish in duo-culture increases habitat use by seabass. Lumpfish are shown in blue, seabass in orange.

hatch). Otohime performed better than the Inve diet but the addition of vitamin C did not increase larval survival (Fig. 7).

Lumpfish Behaviour in Duo-culture

As lumpfish are deployed in sea cages with other fish (salmon and often other cleaner fish) research at CSAR is being carried out to examine the costs and benefits of duo-culture. Some of the questions we are trying to answer include: (1) Does duo-culture improve production, and (2) does it influence behaviour? Our pilot results (using seabass) indicate that the presence of lumpfish increases the habitat use of the other species (Fig. 8).

Colour and Substrate Preferences

Lumpfish larvae have been found to prefer dark backgrounds to white backgrounds and fine to rough substrates (Fig. 9).

Similar results have been found with larger juveniles and dark structures are now incorporated into tanks to provide enrichment (Fig. 10). The use of structures in the tanks did not improve growth but may reduce aggression and improve welfare.

Health Management

At CSAR, a veterinary surgeon and a welfare officer ensure that lumpfish (as well as other fish) maintain the highest possible welfare standards. Screening for pathogens and visual checks are carried out routinely. In addition, endoscopy techniques are used to aid in clinical work, improve diagnosis, and aid in broodstock management (Fig. 11).

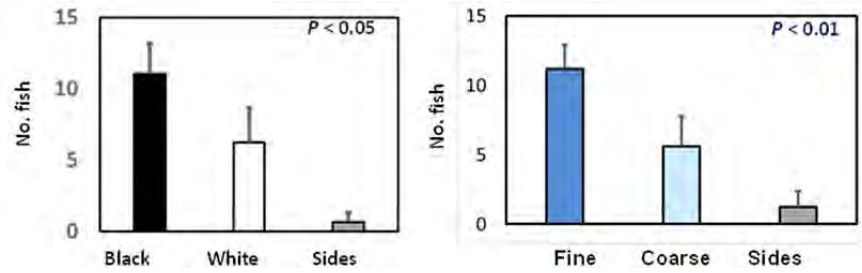

Figure 9
Colour and substrate preferences of lumpfish larvae in culture.

Figure 10
Lumpfish juveniles aggregate around black structures.

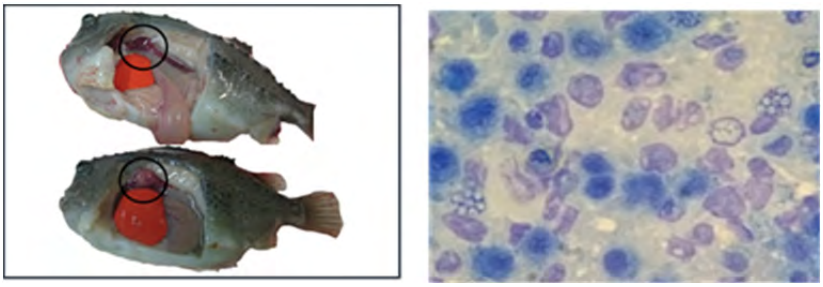

Figure 11
Left: comparison between the kidneys of a healthy fish (top) and a fish infected with Microsporidia (bottom). Right: microsporidia spores in lumpfish lymphocytes.

Outlook

As with other novel species in aquaculture, the past few years have been a valuable learning experience; much has been learned but much remains to be done. Improved standard operating procedures have led to greater control and consistency in the production of lumpfish. Each passing year has seen improvements in survival and sustainability and in 2016 survival from hatch to deployment was 60% better than when we started. Knowledge sharing will allow the incipient lumpfish industry to continue moving forward. But it is also essential that research continues to inform the development of the lumpfish aquaculture industry, so that improvements can go even further. Research will allow the industry to confidently make changes to production techniques based on robust data and testable methodologies.

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INNOVATION AND THE CAPACITY TO MANAGE CLIMATE-RELATED RISKS IN INLAND COMMERCIAL FISH AQUACULTURE IN THAILAND

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Introduction

Figure 1
Red hybrid tilapia in open-top cages in a reservoir in Northern Thailand.

Aquaculture accounts for around 40% of the fisheries production of Thailand, and about 40% of this is freshwater (DOF, 2013). Aquaculture production in 2010 was valued at around 3 billion USD and generated the equivalent of around 360,000 full-time jobs. In Northern Thailand, tilapia and a few other species are reared in earthen ponds as well as cages in rivers and reservoirs (Fig. 1). Extreme weather events and climate have significant impacts on farm profits, in particular, floods and droughts, but also heat waves (Lebel et al., 2015b; Lebel et al., 2016a). Poor water quality and disease outbreaks have significant impacts on production, suggesting that changes towards more sustainable farm practices and water management will be needed for successful adaptation to climate change.

Over the past four years, the AQUADAPT project has been carrying out research for development of the aquaculture sector in Northern Thailand, focusing on improving the management of climate-related risks. The working assumption is that improving capacities to manage risks under current climate are important for sustainability today, and should contribute significantly to adaptation to longer-term changes in

climate.

The specific objective of the presentation made at the Aquaculture Association of Canada meeting in St. John's was to assess the implications of innovation for capacities to manage climate-related risks in inland aquaculture in Thailand. Innovation was defined broadly as new ideas or applications. The analysis was based on sets of quantitative surveys of pond and cage fish farmers, in-depth interviews with key informants in the aquaculture sector, and a series of roundtables with key stakeholders (farmers, officials, firms, and researchers).

Innovation and Adaptation

The key finding was that not only technical but also managerial, social, and institutional innovations are likely to be needed as well in order to adapt to climate change. While there was substantial evidence of recent technical innovations having contributed to better management of climate-related risks at the individual farm level (Table 1), evidence of managerial innovations at the farm level, or institutional innovations at collective levels were much rarer and mostly in the form of strategies and suggestions not yet implemented (Fig. 2).

Table 1. Examples of technical, informational and institutional innovations important to the management of climate-related risks.

Higher Risk Conditions	Conventional management	Innovative management	Benefits from adoption
Ponds with high nutrient inputs and phytoplankton blooms	No aeration in ponds	Aeration at key times of day with monitoring of DO	Reduce risks of low DO episodes that cause fish deaths
Sites at risk from high flows in wet season	Flimsy floating river cages	Stronger cages, better moorings, flow deflectors	Reduce flow speeds and net deformation that cause fish deaths
Rapidly changing climate and water conditions	Early warnings rely on mass media weather forecasts	Social media (chat) on river & canal conditions	Information place and culture specific, timely
Sites vulnerable to dry season scarcity and low water quality	Individual farms store water with no coordination with other water users	Groups lobby for shared infrastructure and manage water resources	Reduce risks of scarcity and low quality water
Sites vulnerable to extreme weather events that can lead to mass mortality	Limited compensation or *ad hoc* assistance	Mutual or index-based insurance	Risk shared in way that reward good risk reduction practices

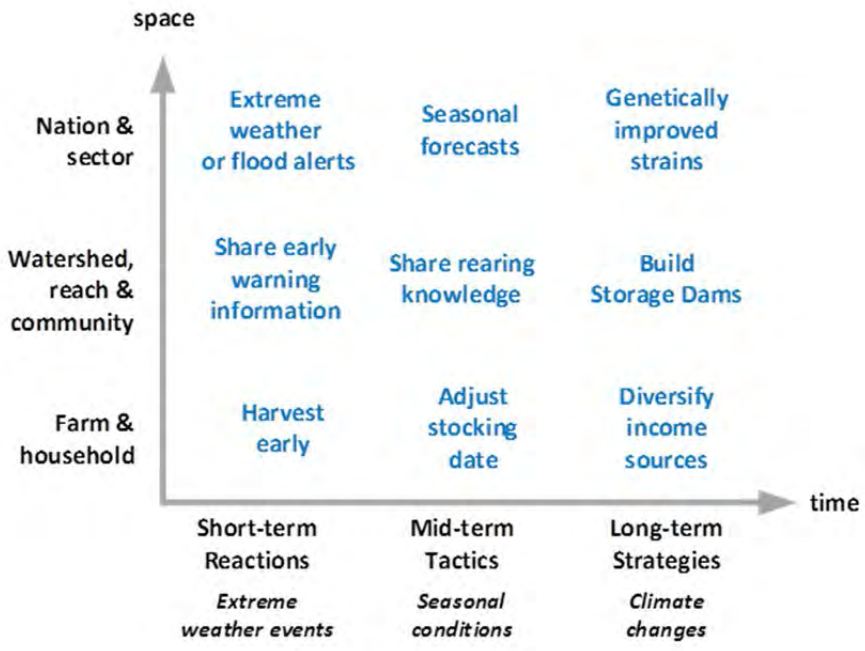

Figure 2
Red hybrid tilapia in open-top cages in a reservoir in Northern Thailand.

Figure 3
AQUADAPT Thailand's Facebook page (600+ Likes) has been a valuable platform to disseminate research findings, share industry news, and engage stakeholders.

Farmers did, however, rank maintaining good social relations with other fish farmers and officials as important to management of risks, and in some places have formed cooperatives or fish farmers' groups, suggesting a willingness to engage more in collective action (Lebel et al., 2015a; Lebel et al., 2016a). There was also a strong interest in insurance, although it was recognized that this would take a lot of effort and lobbying to get implemented. Finally, fish farmers increasingly use social media tools to communicate and exchange information underlining the likely importance of these channels for improving access to and uptake of innovations (Fig. 3).

Attention to perceptions and individual differences adds nuance to these generalizations. Fish farmers vary widely in their willingness to accept and take risks (Lebel and Lebel, 2016). Women are slightly more concerned with climate-related risks than men, and are more likely to think risk-management actions are important. Women are also more likely to prepare beforehand, when there is time to do so, such as in the case of slow-onset drought (Fig. 4). Perceptions of risk are influenced by recent experience (Lebel et al., 2015c), and risk decisions by perceptions of expected gains and losses and

emotional responses to risk situations (Lebel and Lebel, 2016). In experimental studies with fish farmers, we've shown that it is difficult to estimate the level of flood risk, and thus make the best investment decisions, when flood risks are high or increasing (Lebel et al., 2016b). Taken together, these findings underline the importance of innovations in communications so as to better fit messaging to individual risk preferences as well as the variation in risks of extreme weather events or water conditions among locations, seasons, and climates.

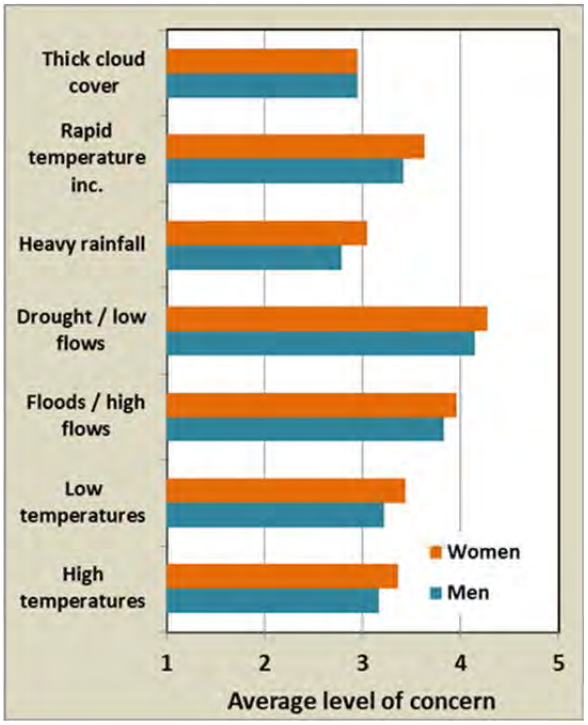

Figure 4
The perceptions and responses to climate-related risks of women and men differ slightly.

Policy and Practice

Where does policy fit into all of this? First, innovation in risk management is needed at multiple time and space scales for successful adaptation to be realized (Fig. 2). Managing risks from drought on large farms, for example, may involve maintaining ponds for water storage while operation of larger irrigation infrastructure may also be important in critical periods (Fig. 5). One of the key challenges in the Thai context is to have aquaculture recognized as a stakeholder in water management.

Figure 5
Options to store water in ponds on a farm to help cope with a longer than usual dry season are much greater for large farms with lots of land and good access to water.

Second, even if most adaptation actions are undertaken by individuals and private firms, public policy is critical as an enabler of better practices – whether it is through voluntary standards, regulations, or information. Zoning policies of the department of fisheries could potentially play a greater role in the future, guiding new production into suitable areas given changes in climate (Uppanunchai et al., 2016). Policies on trade and investment, because of their influence on markets and the aquaculture value chains, are also critical, but their implications for adaptation are not yet well understood.

Third, improving sustainability is often a no- or low-regret option with respect to climate change adaptation. But not much is known about the synergies and trade-offs between improving management of climate-related risks and the impacts on ecosystems, water quality, or fish health. Joint analysis of the effects of changes in climate, water demand, and fish markets is needed and underway. Preliminary findings underline the importance of robust strategies – those which perform well under a wide range of possible conditions.

In conclusion, innovations important to adaptation are multi-scale and are not just about making technical changes at the farm level. The value of innovation is context-dependent and adaptation strategies should be as robust as possible, adding up to a 'no regrets' to risk reduction. While these findings are based on work on commercial aquaculture in Thailand, they have wider significance for our understanding of the different roles of innovation required for successful adaptation to climate change across the aquaculture sector.

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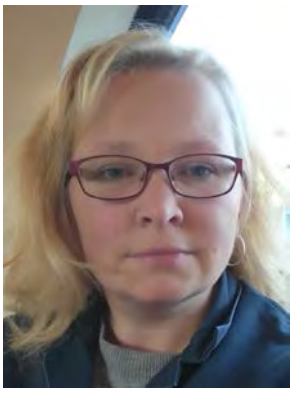

CLEANER FISH RESEARCH AND PRODUCTION IN NEWFOUNDLAND

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Abstract

Sea-lice control is one of the top research and development priorities for Atlantic Canada's finfish aquaculture sector. Potential losses in market value and resistance concerns related to prolonged reliance on any single therapeutant are key drivers that have prompted interest in the potential utilization of local cleaner fish species such as lumpfish and cunner. A multifaceted research team is conducting research dedicated to developing a "new tool" for industry to use to mitigate and control sea lice in Atlantic salmon. Cleaner fish are fish that provide a service to other species by removing ectoparasites. The feeding behaviour of the cleaner fish is harnessed to create a natural defense for the farm. Fish health and welfare are top priorities for Cold Ocean Salmon Ltd., a subsidiary of Cooke Aquaculture Inc. As farmers, they want to take an integrated pest-management approach to minimizing the impact of parasites, like sea lice, on their animals. Their in-house science and farming teams are extremely pleased to be working with world-class experts at the Memorial University, Department of Ocean Science to solve real world farming challenges. This paper will highlight some innovative methods and technology acquired over the past few years to produce cleaner fish, which are being tested in a real-world environment in Atlantic Canada.

Introduction

The ongoing battle to combat sea lice (*Lepeophtheirus salmonis*) remains one of the major issues facing salmon farmers today. The parasite poses a significant

threat to salmon aquaculture operations worldwide and, as the salmon-farming industry continues to grow and production increases, the sea-lice situation is likely to get worse. While there are a number of preventative and post-infestation treatments available, including therapeutic and nontherapeutic methods, the emerging development of resistance to these drugs increases the necessity to develop new and alternate strategies to combat the problem. The excessive use of chemical therapeutants also comes with environmental concerns as well as a lack of public acceptance of chemical use in food production.

Currently, numerous salmon farms throughout Europe are using cleaner fish as part of an integrated pest management strategy as a non-pharmaceutical approach to controlling sea lice. Sea lice have been controlled successfully by using cleaner fish from the wrasse family, namely goldsinny (*Ctenolabrus rupestris*) and ballan wrasse (*Labrus bergylta*), over the past number of years. While wrasse are an effective tool as a cleaner fish species, they do have some limitations that have been identified, such as cold-water temperature sensitivity and a long, expensive, larval period. As a result, recent research has focused on the development and use of lumpfish (*Cyclopterus lumpus*) as an alternate or additional species.

Two species that are being investigated as part of the project in Newfoundland are the cunner (*Tautogolabrus adspersus*), which is a member of the wrasse family and has similarities the to both the goldsinny (size and spawning behaviour) and ballan (juvenile behaviour), and the lumpfish, which has favourable characteristics such as a greater tolerance to cold-water temperatures and a relatively short larval period.

Cleaner Fish Production

The success of this strategy depends upon the availability of high-quality cleaner fish in high numbers. Salmon farms could possibly require upwards of 10% of the salmon population per cage site. This means that a site containing 500,000 salmon could require up to 50,000 cleaner fish of appropriate size. Salmon farms may also require a year-round supply of these fish, meaning that it would be very difficult to meet the demand by wild capture. Culturing cleaner fish will enable a continuous supply of different sizes as demand requires. Cultured cleaner fish also have the added advantage of health screening and vaccination prior to deployment to reduce any risk associated with disease transfer. Cunner egg production at the Joe Brown Aquatic Research Building (JBARB) can be obtained through two separate groups of broodstock. One group is under a two-

month advanced photoperiod and typically begins to release eggs in early March. The second group includes broodstock that are under an ambient photoperiod, including wild captured as well as a group of broodstock retained from the 2013 year-class production season. These ambient spawners usually begin producing eggs in late April. Cunner broodstock at JBARB produced 165 L of viable eggs during the 2016 season. Assuming high fertilization and hatch rates, this would produce approximately 250 million larvae available to stock larval tanks.

During the 2016 spawning season, the 2014 YC lumpfish began releasing egg batches passively into the tank in early April. These batches of eggs were either not fertilized or had variable fertilization rates. In an attempt to increase fertilization rates we began strip spawning these broodstock. Strip spawning proved to be quite successful in that we were able to obtain good batch sizes, and fertilization rates were consistently high. During the 2016 season, we strip spawned 20 females and 11 males from the 2014 year-class broodstock and created 20 different crosses from these fish generating 4 kilograms of fertilized eggs. We also stripped eggs from one 2016 wild-captured female and created a hybrid cross using milt from a 2014 year-class male.

This season, we were also successful in collecting wild egg masses. Six wild egg masses were collected weighing approximately 3.0 kg in total.

Wild-captured broodstock collected in late May/early June began releasing fertilized egg batches directly in the tank throughout the month of June. They released 22 egg masses weighing approximately 22.7 kilograms in total. There are approximately 100,000 eggs per kilogram of fertilized lumpfish eggs. Assuming a fertilization rate of only 70% and a good hatch rate, the total mass of eggs collected during the 2016 season has the capability of generating approximately two million larvae.

Lumpfish Temperature vs. Growth Trial

Lumpfish juveniles have a very high growth rate and as a result can very quickly exceed optimum stocking densities in available on-growing tanks. Therefore, space can be a major limiting factor of the holding capacity of juveniles. An additional constraint that must be dealt with is that our lumpfish broodstock typically spawn in late spring/early summer, with optimum time to transfer these cleaner fish to the sea cage being the spring of the year. This means that juvenile lumpfish will go through a ten-month growth period in holding tanks prior to transfer. As a result, these fish are often larger than the size considered

optimal for use (25 grams). In an attempt to deal with this issue we conducted temperature trials throughout the winter of 2015/2016 using lumpfish juveniles from the 2015 year class to determine if it was possible to stop or slow the juvenile growth through the winter months without compromising health.

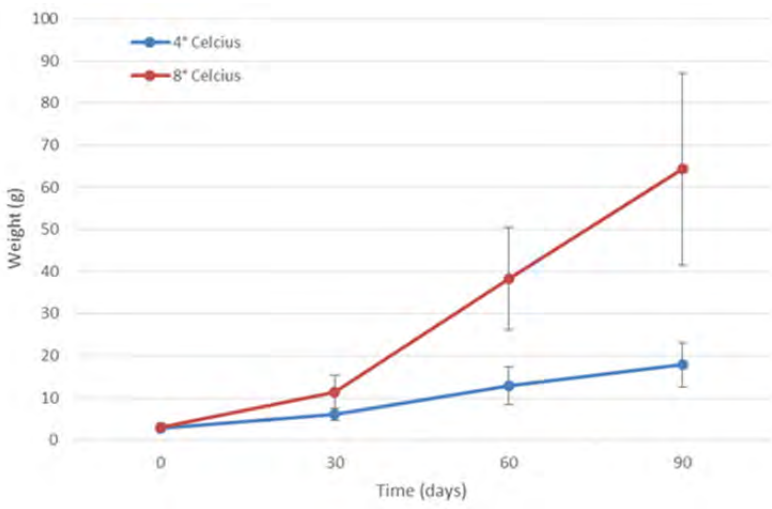

Figure 1
Average weights ± standard deviation of lumpfish juveniles reared for a period of 90 days at two different temperatures.

The trial ran for 90 days in duration. Four tanks were stocked with 200 lumpfish juveniles with an initial average weight of 2.9 ± 1.8 g. Two tanks of fish were held at 4°C, and two tanks of fish were held at 8°C. Each tank was fed 1% body weight per day using a belt feeder. Throughout the trial 10 fish were weighed and measured monthly from each tank (20 per treatment) with 25 fish per tank weight sampled at the end of the trial. Results indicated that the growth of the juvenile lumpfish was lower in the tanks maintained at 4°C versus 8°C. At the end of the trial, juveniles weighed 64.3 and 17.9 g, respectively (Fig. 1).

From this trial, it was determined that by lowering the water temperature we were able to slow the growth rate of the juvenile lumpfish down considerably, which helps reduce the space required to hold these animals. This also indicates that we can reduce the heating of water required to raise these fish without any compromise to their health and save on heating costs.

Cleaner Fish Deployment

In August and September of 2015, the first large-scale trial of cleaner fish was initiated; 3300 lumpfish and 26,000 cunners were deployed to sea cages. The 2013 year-class cunners (20 - 40 g avg.) and 2014 year-class lumpfish (184 g avg.) were deployed in August and September 2015. The transport truck (Fig. 2) was equipped with five 5000-L transport tanks. Transport stocking densities for lumpfish were 33 kg m^{-3} and cunners 16 kg m^{-3}. The transport temperature was maintained at 10 - 11°C and oxygen levels were maintained at 140% saturation. The total transport time

Figure 2
Loading transport truck for cleaner-fish deployment.

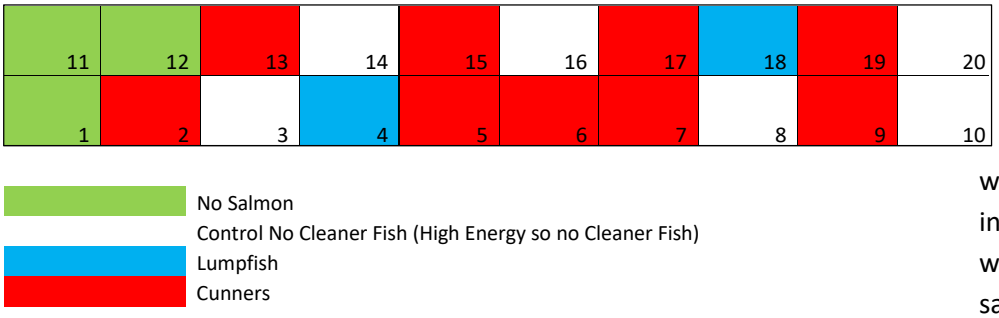

Figure 3
Farm schematic identifying cleaner fish (lumpfish and cunners) and control cages for 2015 deployment.

was twenty hours including the loading and unloading of the fish. These cleaner fish were used in a trial that included twenty cages. Nine were co-stocked with salmon and cunners, two with lumpfish and salmon, six were stocked with just salmon and three were left empty (Fig. 3). The cages were stocked with a ratio of 9% cunners to salmon and 5% lumpfish to salmon.

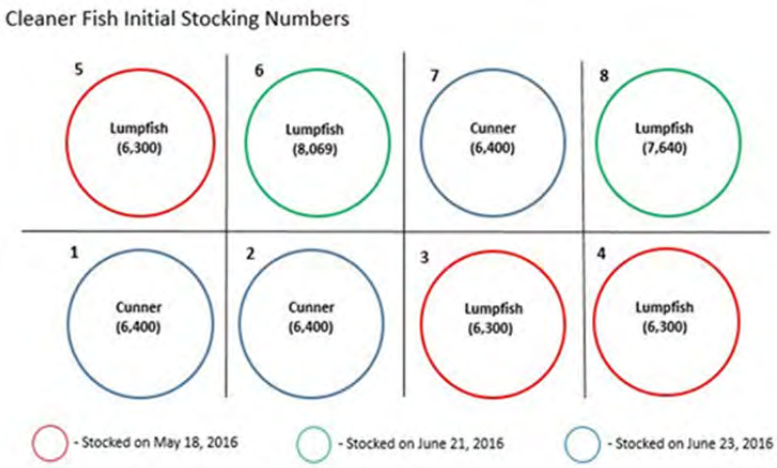

Figure 4
Farm schematic Farm schematic identifying cleaner fish (lumpfish and cunners) for 2016 deployment.

A second group of cleaner fish were deployed in May and June of 2016; 34,609 lumpfish and 19,000 cunners were divided between 8 cages, five were co-stocked with lumpfish and salmon and 3 were co-stocked with cunners and salmon (Fig. 4).

Shelters or artificial hides were added to the sea cages prior to deployment. These hides provide a calm environment or sheltered area away from the salmon for the cleaner fish. Through diver observations, it was noted that long strands of plastic (Fig. 5) acting as artificial kelp was sufficient as a hide for the cunner but was not suitable for the lumpfish since the material was too flimsy and not deep enough in the cage. Therefore, the cage site crew introduced barrels (Fig. 6) which had a hard surface/substrate which enabled the lumpfish to attach. These hides were sunk to a depth of 15 - 20 m below the surface.

Figure 5
Hides used in sea cages for cunner.

Technical staff monitored the sea-lice counts at each site two times per week throughout the year. Lice counts included all stages such as chalimus, mobiles, adults, and gravid. These sea-lice counts were then used to decide whether a site required treatment to reduce sea-lice numbers. There were three sites used for comparison purposes in this trial, and the results thus far have shown that, of the three sites used, the site using cleaner fish (site C) has not required any further treatments to keep the sea-lice counts at an acceptable level. The other

two sites (sites A & B) have required some form of treatment, either a bath treatment or an infeed treatment (Figs. 7 & 8).

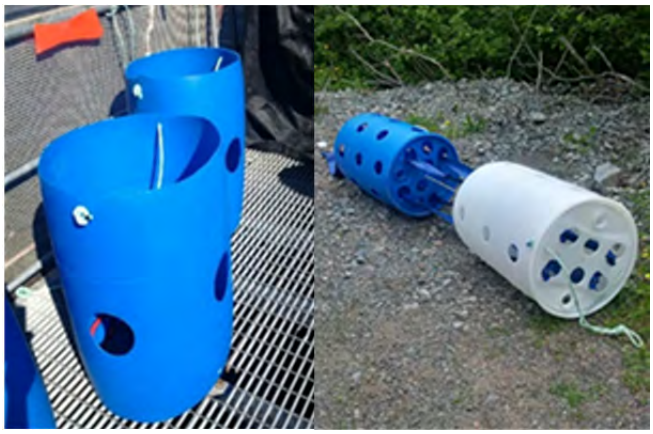

Figure 6
Barrel hides used in sea cages for lumpfish.

Continuing Research

Moving forward, there are some key areas that will require further research and development during the upcoming seasons. There will be further work completed to improve upon our cage-site strategies to determine optimal deployment protocols, including further work to improve upon cleaner-fish hides. One type of hide that will be investigated will be a strip of solid plastic material (10 inches in height) that will span the width of the cage and will be submerged at a low depth. Further research will be carried out on broodstock management, including diet development and photoperiod manipulation. Photoperiod manipulation is important if we are to be able to achieve a broodstock that produces eggs at optimal times of the year to provide cleaner fish of appropriate size. Finally, further work will also need to be carried out on improving fish-health strategies, including vaccine development.

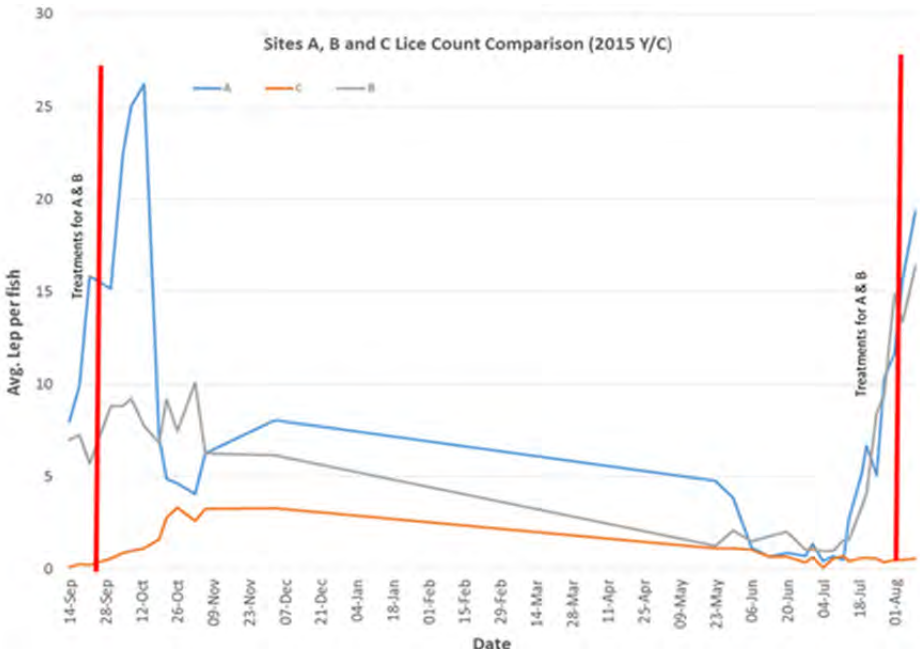

Figure 7
A comparison of sea lice counts present on three different sites from the 2015 deployment. Sites A and B are not stocked with cleaner fish while site C is.

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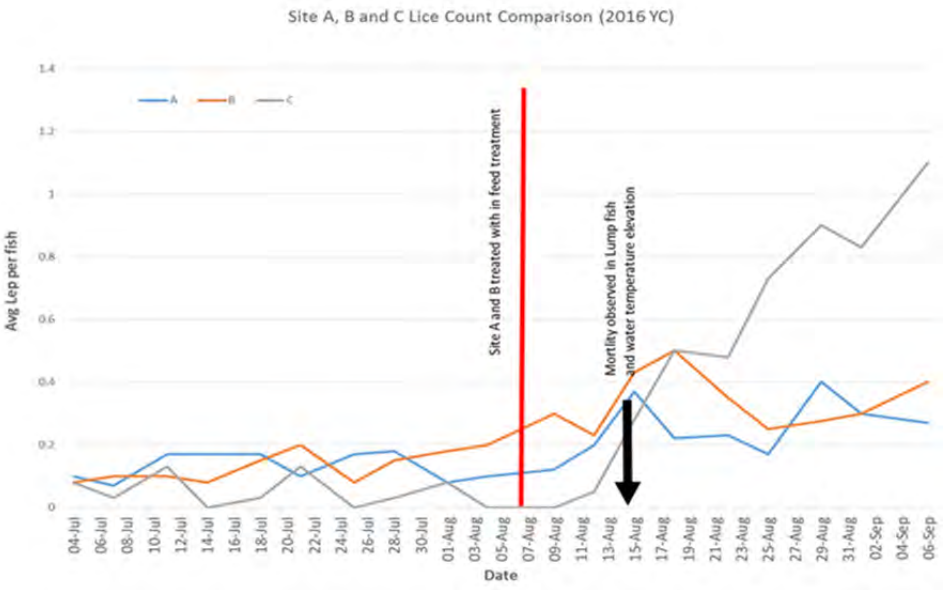

Figure 8
A comparison of sea lice counts present on three different sites from the 2016 deployment. Sites A and B are not stocked with cleaner fish while site C is.

Innovation (CCFI), Research and Development Corporation (RDC) and the Province of Newfoundland and Labrador for providing the funding for this work. We acknowledge Mr. Richard Prickett (RSP Services) for providing valuable advice and guidance throughout and Cold Ocean Salmon and Grieg NL for participating as industry partners.

SELECTIVE BREEDING PROGRAM FOR SEA LICE, *Lepeophtheirus Salmonis* (KRØYER 1838), RESISTANCE AT THE USDA'S NATIONAL COLD WATER MARINE AQUACULTURE CENTER

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Abstract

Sea lice are likely the most economically costly pathogen that has faced the salmon farming industry over the past 40 years. Recent economic estimates put the annual cost of sea lice at 742 million USD in 2012. With the rise of resistance to multiple drugs used to treat sea lice, there has been a significant shift in sea-lice management away from dependence on drugs and towards an approach utilizing multiple non-drug and drug-based control methods, such as selective breeding. The USDA has begun a comprehensive program to test 100-120 families per year from their pedigreed North American stocks and incorporate sea-lice resistance into their existing Atlantic salmon broodstock program. Initial testing revealed a highly variable susceptibility between families, with family average lice counts ranging from 0.0229 to 0.1853 lice cm^{-2}. Conservative estimates calculated a heritability of 0.19. This data is being combined with a low density marker genotyping approach to improve within-family broodstock selection.

Introduction

Sea lice (*Lepeophtheirus salmonis* Krøyer 1838) are one of the most significant pathogens facing the global salmon farming industry. Recent estimates put the global cost of sea-lice management at 742 million USD in 2012 (Roth, 2015), up from an estimate of 480 million USD in 2006 (Costello, 2009). The rise in costs is attributed to changing treatment methods. One of the underlying factors in changes to lice-treatment methods has been the development of resistance to a range of existing chemotherapeutants used to manage sea lice (Aaen et al., 2015; Roth, 2015; McNair, 2015). The development of resistance has led to an increased interest in non-chemotherapeutant means of controlling sea lice. There have been a variety of methods examined or commercially developed including the use of cleaner fish, mussels, snorkel cages, plankton nets, hot water, lasers, and selective breeding to try and control sea-lice populations on farmed fish (Stien et al., 2016; Gharbi et al., 2015; Imsland et al., 2014; Bartsch et al., 2013; Stien et al., 2012; Gjerde et al., 2011; Molloy et al., 2011). In response to industry requests, the USDA ARS National Cold Water Marine Aquaculture Center (NCWMAC) is incorporating selection for sea-lice resistance into its existing, broodstock, development program.

Materials and Methods

The NCWMAC produces approximately 100 - 150 pedigreed families of North-American-origin Atlantic salmon every year. Families for breeding are selected from the top 20% of the previous generation based on an evaluation of full siblings reared under commercial conditions. The selected fish are further divided into high, medium, and low breeding value groups. Typically one male is mated to two females of a comparable breeding value group. Starting in 2015 with 2013 - 2014 year class of fish, families are being subjected to a standardized challenge protocol in order to evaluate their resistance to sea-lice infection.

Sea-lice challenges are carried out in a small recirculating system with twelve 1 m^3 tanks. Each tank is stocked with one fish from each of the families to be evaluated (n = 120 in 2015, n = 98 in 2016). The system is maintained at 11°C and the fish are maintained on their normal ration of a commercial diet (Bio Oregon, Westbrook, ME).

Lice for the challenges are hatched at the University of Maine's sea-lice hatchery at 33 ± 3 ppt salinity and 10.5 ± 1°C. All egg strings are collected from local salmon farms and then transported to the University. Upon arrival, the egg strings are removed from the ovigerous females and placed collectively into a

single, 4”-diameter, hatching pot. Unhatched egg strings are moved to a new pot and the development of the larval lice are monitored daily. Copepodids that are between 2 and 4 days old are then used for experimental infections.

A standardized bath challenge model using 100 copepodids per fish is used for each infection. Copepodids are counted and placed into glass jars for transportation to the NCWMAC. The transport jars are placed in the system water and the lice are allowed to acclimate for a minimum of 30 minutes prior to use in the infection. In the infection tank, the water level is dropped to 1/3 of the normal level using a standardized drain pipe and water inflow is shut off. Supplemental oxygen is added as needed to maintain dissolved oxygen levels between 80 and 120% of saturation. Acclimated lice are added to the tank and allowed to infect the fish for four hours before normal water flow is restored. Any unattached copepodids are removed from the system by a 60-µm drum filter. The discharge from this filter is then held in a static sump where freshwater is added to bring the salinity below 10 ppt and it is then held for a minimum of 12 hours (Bricknell et al., 2006).

Lice are allowed to develop on the fish for 10 - 13 days post infection before the fish are euthanized with an overdose of Tricaine-S (Western Chemical, Ferndale, WA) at 250 mg L^{-1} for 10 minutes after all operculum movement has stopped. All fish are euthanized before the lice develop to the mobile pre-adult stage. The lice are then stained on the fish by placing the fish in 12 L of freshwater with 10 mL of Neutral Red stock solution (0.01 g mL^{-1}, 72% total dye content, Fisher Scientific, Fairlawn, NJ) for 15 - 20 minutes. Fish are individually bagged and placed on ice until the lice are counted under a dissecting scope and they are weighed and fork length measured.

Lice susceptibility was defined for each fish based on calculated lice density (Gjerde et al., 2011) and the number of lice per cm^2 of fish surface area. Lice density was defined as Lice Density = Lice Count / Fish Weight^(2/3) where lice count is the number of lice counted on that individual fish and weight is the weight of the fish in grams (Gjerde et al., 2011). Lice per surface area was defined as Lice Count / Surface Area. The surface area of each fish was estimated based on the equation: Surface Area = 14.53 * Weight^0.6044 where surface area is in cm^2 and weight is the weight of the fish (Unpublished Fredericks and Bricknell). The average lice density and lice per cm^2 were calculated for each family. The families were also ranked for each trait for best, lowest average, to worst. Estimated breeding values for each family were generated using the program Multiple Trait Derivative Free REML (MTDFREL).

Results

In 2015 (2013/2014 YC), a total of 1001 fish were infected and counted from 120 different families. In 2016 (2014/2015 YC), a total of 1350 fish were infected and counted from 98 different families. In 2016, the infections resulted in a higher average number of lice per fish, 52.4 compared to an average of 21.1 lice per fish in 2015 (Table 1). Other infection parameters for the two years are reported in Table 1.

Table 1. Summary of various infection parameters. Infections done on 2013/2014 YC are listed under 2015 and those from 2014/2015 YC are under 2016. Parameters given in bold are averages with the minimum and maximum values given in parenthesis.

	2015	2016
Infection Dates	June 26 – Nov 8	June 15 – Aug 22
Tanks Infected	9	15
Ave # fish per Family	**8.3** (5 – 10)	**13.8** (10 – 15)
Ave Weight	295.1	210.3
Ave # Lice per fish	**21.1** (8.1 – 60)	**52.4** (32.4 – 114.6)
Lice per cm2	0.05	0.22
Lice Density	0.52	1.55

Comparison of family averages and rankings showed little difference lice density and lice per cm^2. For example in the 2013/2014 YC fish, the same 10 families were in the top 10 regardless of which metric was used to rank the families. In both years, similar patterns were seen in the overall distribution on sea lice resistance between all of the families examined (Fig. 1).

Discussion

Overall, the model presented of challenging multiple small batches of fish has resulted in successfully generating estimated breeding values for use in the USDA's Atlantic salmon breeding program. The challenge model represents an alternative to mass challenge of large numbers of fish all at once and provides a viable alternative in situations where the resources needed to conduct and count 1000 - 2000 fish at once are lacking. When challenging all of the fish at once, either 20 - 30 people are needed to count all of the fish within two days or fish need to be frozen and then thawed and counted over a long period of time. The small batch method allows for a much smaller number of people to actually count the fish. In our case, it allows for 2 - 4 people to count 100 - 120 fish over

3 days with all counts being made on fish freshly euthanized that day. However, the trade off in reduced mass counting is the time needed to conduct multiple challenges, which can take several months (Table 1).

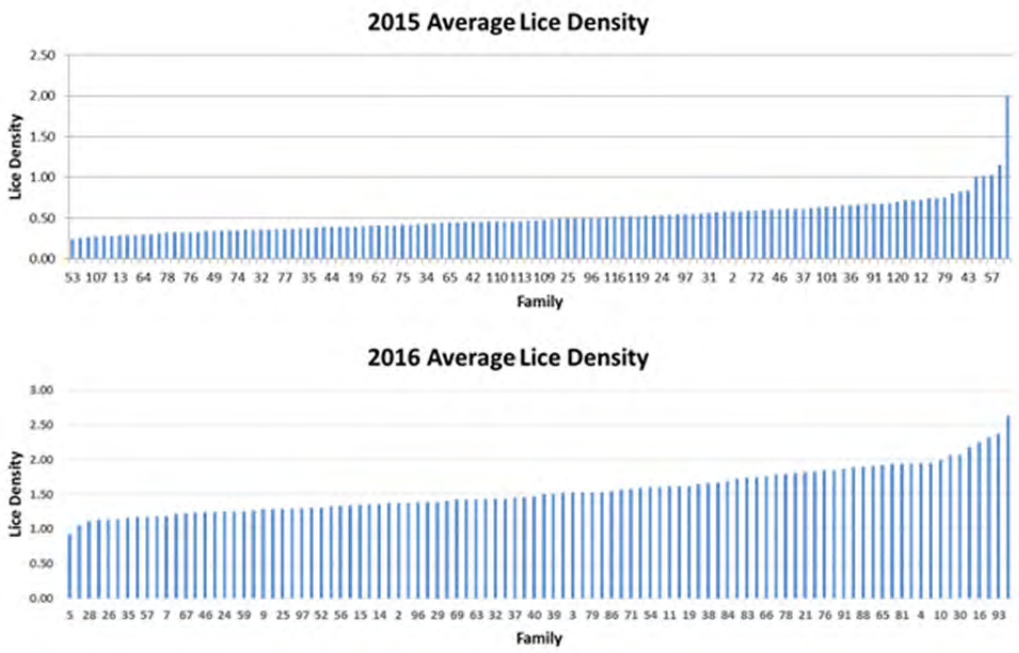

Figure 1
Average lice density by family for YC 2013/2014 tested in 2015 and YC 2014/2015 tested in 2016. Family numbers are unique to each year and are not representative of the same family between year classes.

The USDA will continue to assess the resistance to sea-lice settlement in future year classes and will incorporate this into the overall selection model. Further work is being conducted to establish genetic breeding values in addition to the current phenotypic values and to look for potential genetic markers for sea-lice resistance. Future project goals include evaluation of offspring from the selection program to document improved resistance.

Acknowledgements

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A REVIEW OF CLEANER-FISH PRODUCTION IN THE UK

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Abstract

Infestations of the sea louse (*Lepeophtheirus salmonis*) are the main health issue in the Scottish salmon industry, costing millions of pounds in terms of mortalities, lost growth, and treatment. In recent years, cleaner-fish have become more popular as part of the control of lice and include several members of the wrasse family particularly ballan wrasse (*Labrus bergylta*), goldsinny (*Ctenolabrus rupestris*) and the lumpfish (*Cyclopterus lumpus*). Ballan wrasse are caught in the wild as well as produced at three hatcheries in Scotland. Goldsinny wrasse (and other lesser used species) is only caught in the wild. While no lumpfish juveniles are caught in the wild, eggs from wild broodstock caught in the UK, Norway, and Iceland are used to produce lumpfish juveniles in several hatcheries. Ballan wrasse production has proved to be far more challenging than lumpfish production and most of the costs of production have been financed by the main salmon producers themselves. Use of autogenous vaccines to protect cleaner fish from bacterial diseases both in the hatchery and post deployment are preferred to commercial vaccines and there are several research programmes underway looking at hatchery protocols and the deployment of cleaner fish particularly at Stirling and Swansea Universities.

Introduction

The Scottish salmon farming industry produced over 160,000 t of Atlantic salmon in 2014 (FEAP, 2015). The biggest health issue is infestations by the sea-louse, (*Lepeophtheirus salmonis*), which causes loss of growth, disfiguration, and mortality through secondary infections. Traditionally, the use of therapeutics such as organophosphate pesticides administered orally or by baths has been the main method for controlling this problem, but due to increasing resistance to these chemicals by the sea lice, combined with a need to reduce costs and

avoid potentially environmentally damaging treatments, the industry has been looking at alternative methods of control.

Mechanical methods such as thermal de-licers, lasers, pressure washers, and brush systems are being widely tested with variable results. One biological method that was previously tried with mixed results in the early 1990's was the use of cleaner-fish species such as the wrasses (family Labridae). These early trials failed mainly because of the availability of new chemical treatments and a lack of knowledge regarding the management of the cleaner fish in the salmon cages. However, with few other alternatives available, the salmon producers have now been forced to re-examine the use of cleaner fish and recent efforts have proved much more successful.

Although several wrasse species are being used, the main cleaner-fish species preferred by the industry is ballan wrasse (*Labrus bergylta*), which is both hardy and efficient at cleaning lice at UK ambient temperatures higher than 10°C. Hatchery production has proved to be difficult, however, and the industry has had to rely on wild caught juveniles. Lumpfish (*Cyclopteris lumpus*) are much easier to cultivate and work well at low temperatures while goldsinny (*Ctenolabrus rupestris*), which makes up most of the wild catch, is particularly good at cleaning lice on salmon smolts.

Cleaner-fish Production

Assuming a stocking level of about 5 - 10% of the salmon population, there is a theoretical requirement for about 3 - 5 million cleaner fish in the UK. In 2016, the author estimates that about 1 - 2 million wild wrasse will be caught, 250,000 ballan wrasse produced in three hatcheries, and 2.5 million lumpfish produced in eight hatcheries.

Wild Wrasse

These are caught inshore using traps and pots in order to avoid damaging the fish. Most wrasse are caught in Scotland but, due to the increasing demand, more fish are being caught in other parts of the UK. Preferred fish size is 12 - 20 cm in length in order to avoid escapes from the cages. The fishing season is all year but, due to weather conditions, is generally limited to May - November.

Fishermen usually hold the fish in small, keep nets or tanks until the customer's transporter collects them. Alternatively, fish are held for longer periods in larger

systems and allowed to recover before being transported to Scotland. This can greatly improve the survival (from 50% to 95%).

Goldsinny tend to dominate the catches with smaller amounts of rock cook (*Centrolabrus exoletus*), corkwing (*Crenilabrus bailloni*), and cuckoo wrasse (L*abrus mixtus*). Ballan wrasse, the most prized species, are often removed from the catch and sold separately. Such is the demand for this species that prices rose to C$30 per fish in 2016.

Cultured Cleaner Fish

1. Dorset Cleanerfish Ltd. Lumpfish
2. Swansea University. Lumpfish
3. Ocean Matters Ltd. Lumpfish
4. Machrihanish. Ballan wrasse
5. Otterferry. Ballan wrasse and Lumpfish
6. FAI, Ardtoe. Ballan wrasse and Lumpfish R&D
7. Aultbea Benchmark. Lumpfish
8. Broonies Tang Benchmark. Lumpfish
9. Hjatland (Grieg). Lumpfish

Figure 1
Cleaner-fish Hatcheries in the UK 2016.

There is increasing pressure for hatchery-produced cleaner fish not only to meet demand but to provide an all year round supply of graded disease free (vaccinated) fish. Production of ballan wrasse started at Ardtoe and at the old cod hatchery in Machrihanish in 2011 but proved to be more problematic than anticipated. Following the example of the Norwegian industry, production of lumpfish started in 2013 at Weymouth mainly because of a good local supply of lumpfish eggs. In 2016, there were nine hatcheries producing cleaner fish, 3 producing ballan wrasse, and 8 producing lumpfish (Fig. 1). Production for 2015 with estimates for 2016 - 2017 is given in Table 1 (lumpfish) and Table 2 (ballan wrasse).

Table 3 compares the main differences in hatchery protocols between the 2 species. Although both are substrate spawners, ballan wrasse lay eggs on mats whereas lumpfish are generally stripped and the egg mass incubated in upwelling incubators. Lumpfish take considerably longer to hatch (280 degree days vs. 100 degree days) and produce larger larvae (5 mm vs 3.6 mm). Ballan wrasse larvae require a long rotifer and *Artemia* phase while lumpfish can be weaned directly onto artificial feed. Growth of ballan wrasse is considerably slower than lumpfish which typically take 5 - 6 months to reach deployable size (15 - 20 g) compared to 18+ months for ballan wrasse (40 - 60 g).

Table 1. UK lumpfish production 2015-2017

Company	Location	2015	2016	2017
Dorset Cleaner-fish	Portland, Dorset	280,000	250,000	700,000
Swansea University	Swansea, S.Wales	158,000	150,000	150,000
Ocean Matters	Anglesey, N. Wales	-	500,000	1,000,000
Otterferry Seafish	Loch Fyne, Scotland	230,000	600,000	750,000
Benchmark	Ardtoe, Scotland	25,000	25,000	25,000
Benchmark	Aultbea, Scotland	-	300,000	1,000,000
Benchmark	Broonies Tang, Shetland	-	300,000	800,000
Hjatland	NAFC, Shetland	-	400,000	400,000
	TOTALS	**693,000**	**2,525,000**	**4,825,000**

Table 2. UK ballan wrasse production 2015-2017

Company	Location	2015	2016	2017
Macwrasse	Machrihanish, Scotland	72,000	200,000	300,000
Otterferry Seafish	Loch Fyne, Scotland	25,000	50,000	200,000
Benchmark	Ardtoe, Scotland	5000	0	30,000
	TOTALS	**102,000**	**250,000**	**530,000**

These differences are reflected in the costs of production, which are typically C$1 - 2 per 15 g fish (unvaccinated) for lumpfish but as much as 20x this for ballan wrasse. The quid pro quo is lumpfish are often useful for only one season due to its high growth rate but ballan wrasse can potentially be re-used 2 - 4 times.

Table 3. Hatchery characteristics of cleaner-fish species in the UK

Criteria	Ballan Wrasse	Lumpfish
Spawning behaviour	Substrate spawner, difficult to strip	Substrate spawner, easy to strip
Spawning season	May - June	January - May
Incubation period	100 deg days	280 deg days
Size of larvae at hatch	3.6 mm	5 mm
Rotifers	Day 4 - 20	N/A
Artemia	Day 15 - 50	Day 4 - 21 (optional)
Weaning	Day 30 - 60	Day 4+
Average survival	1 - 20%	50%+
Deployable size	40 – 60 g	15 - 20 g

Research and Development

The production of cleaner fish and its deployment has stimulated several research projects between industry and the scientific institutes. Among the current projects are:

- Scottish Aquaculture Innovation Centre (SAIC) funded project – Hatchery protocols and cage management of ballan wrasse. Participants include Stirling University, Marine Harvest (Scotland) Ltd., Scottish Salmon Company, and Biomar

- Scottish Aquaculture Innovation Centre (SAIC) funded project – Captive breeding programme and cage management including feeds and disease control of lumpfish. Participants include Stirling University, Marine Harvest (Scotland) Ltd., Scottish Salmon Company, Otterferry Seafish Ltd., Biomar, and Pharmaq

- Swansea University and Marine Harvest (Scotland) Ltd. are sponsoring a PhD project looking at egg degumming, genetic screening of broodstock, and rearing protocols for lumpfish.

Bottlenecks to Production and Future Goals

Production of ballan wrasse has proved to be far more problematical than lumpfish production. Some of the main issues in ballan wrasse production include disease control (particularly against atypical furunculosis), swim bladder over-inflation (probable symptom of stress), feed formulation (wrasse are fussy eaters and are susceptible to dietary deficiencies e.g., selenium), slow growth, and deformities. Lumpfish issues include poor milt production from male fish, sucker deformities preventing fish from settling, fin erosion, and sensitivity to bacterial infections post deployment.

Disease issues in both species are being addressed by the use of autogenous vaccines against atypical furunculosis, vibriosis, and pasteurellosis with encouraging results. This is an important part of preparing fish for deployment as well as improving management of cleaner fish in the cages by using optimally designed hides and feeding strategies.

In the hatchery, there is a need to produce eggs throughout the year, which has been achieved for ballan wrasse through the manipulation of photoperiod and temperature. Lumpfish egg supplies are still dependent on wild sourced broodstock and out-of-season egg supplies have to be imported from Iceland

and Norway where fish spawn for longer periods. Genetic selection programmes are also starting in the case of lumpfish where selection for lice-eating ability and slower growth will be the required traits. Finally increasing ballan wrasse production and reducing production costs is of great importance as summer temperatures in the UK will not be optimal for lumpfish.

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PHOTOPERIOD AND TEMPERATURE CHANGE ON THE GONADAL DEVELOPMENT AND MATURATION OF LUMPFISH *Cyclopterus lumpus* L.

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Abstract

Cultured lumpfish adults (360 with 1:1 sex ratio) were transferred to four 1500-L tanks in late October 2015. Two of these tanks were kept in short day light (SDL; 8L:16D) and other two were kept in continuous day light (CDL) for three months. The temperature in all four tanks was kept ambient. After 3 months, photoperiod for both of the SDL tanks were changed to continuous light and one of the SDL tank provided ambient water temperature while the other was provided ambient + 3°C. Both CDL tanks were kept at continuous light but the temperature of one CDL tank was kept at ambient while the other was kept at ambient + 3°C. Five males and females were terminally sampled at the start and every three weeks thereafter from each photoperiod group and blood and gonadal samples were taken. Length and body, gonad, and liver weights were also recorded. Blood plasma were analysed for sex steroids. Our results showed that increased temperature had a positive effect on the final gonadal maturation of lumpfish females along with photoperiod.

Introduction

Seasonal changes in photoperiod and temperature influence the gonadal maturation of many animals including fish (Bromage et al., 2001). Photoperiod is regarded as the key environmental factor for manipulating puberty and gonadal development in fish species living at moderate to high latitudes, securing spawning in the appropriate season with the most favourable conditions for the

offspring (Taranger et al., 2010). Increasing spring temperature triggers and synchronizes the spawning in most temperate and cold-water finfish species (Brown et al., 2007).

The ongoing battle to combat sea lice remains one of the major issues facing the salmon farmers today. Due to the negative effects of current methods for combatting salmon lice (direct costs, negative public opinion, environmental contamination, and creation of resistant lice strains); there is a need for developing new and alternative methods to solve the problem (Imsland et al., 2014). Symbiotic biological control agents such as wrasse and lumpfish species are being used to control the salmon lice in sea cages in the Atlantic Ocean of many countries, including Norway. While both species have their advantages and disadvantages, lumpfish are preferred due to their effective feeding of salmon lice at low temperatures (Imsland et al., 2014). With increasing demand for cleaner fish and the need for smaller size lumpfish at the right time, knowledge on basic lumpfish reproductive physiology is needed. Captive commercial production of lumpfish has been attempted since 2013 but no information on reproductive biology and physiology is available. Thus, a better understanding of the reproductive biology and physiology of broodfish is necessary for lumpfish domestication.

To understand the role of photoperiod and temperature on gonadal maturation and spawning, we manipulated the photoperiod and temperature 5 months prior to spawning to monitor the gonadal development and sex steroid hormones.

Materials and Methods

360 cultured lumpfish adults of 18 months old were transferred to four 1500-L tanks (90 fish per tank) comprising 1:1 ratio of males (480 g – 1609 g; average 844 g) and females (663 g – 2881 g; average 1541 g) in October 2015. Temperature was kept ambient (4 - 9°C) in all four tanks. Two of these tanks were kept in short day light (SDL; 8L:16D) and other two were kept in continuous day light (CDL) for three months. After 3 months, photoperiod for both of the SDL tanks were changed to continuous light and one of the SDL tank was provided ambient water temperature (SDL0T) while the other was provided ambient + 3°C (SDL3T). Both CDL tanks were kept at continuous light but the temperature of one CDL tank was kept at ambient temperature (CDL0T) while the other was kept at ambient + 3°C (CDL3T). Initial sampling was done in January 2016 after the temperature and photoperiod changes and every three weeks thereafter (Fig. 1). During each sampling, five males and five females from

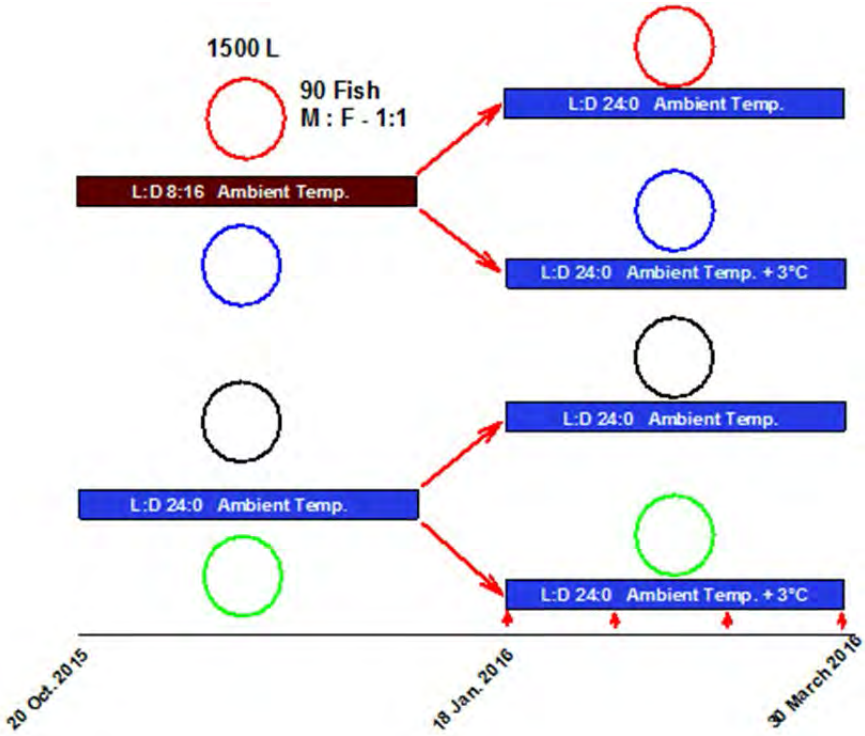

Figure 1
Schematic illustration of the experimental set-up. The red arrows indicate sampling points.

each tank were killed and blood and gonadal samples were taken. Length and body, gonad, and liver weights were also recorded. Gonadosomatic index (GSI) was calculated from body and gonad weight ((GW/BW)*100). Blood plasma was analysed for sex steroids using RadioImmunoAssay (RIA) according to Tveiten et al. (2010). Analysis of variance was used to identify differences in GSI and sex steroids among individual groups ($p < 0.05$).

Results

All female lumpfish with above 10% GSI had well developed oocytes. Female lumpfish that were subjected to SDL had significantly higher GSI ($p < 0.005$) and blood plasma testosterone levels ($p < 0.031$) than the female lumpfish that were subjected to CDL during the first three months from October to January (Table 1). When the photoperiod was switched to continuous and both SDL and CDL fish groups were given either ambient or ambient + 3°C temperature, the female lumpfish kept at SDL3T showed significant increase in GSI compared to the fish kept at CDL0T and CDL3T at week 3 and 6 of experimental period. Between the two SDL temperature groups, females kept at ambient + 3°C had significantly higher GSI than the females kept at the ambient temperature group (Table 1). At week 3, females from SDL3T had significantly higher testosterone (T) levels than the females from all other three treatments (Table 1). In SDL3T group, 80% of the females were matured and releasing eggs while only 20% of the females were matured in SDL0T and CDL3T. No females from CDL0T were matured. There was no significant difference in the Estradiol (E2) concentration among females from the four different groups (Table 1). E2 levels increased in the two high temperature treatments until week 6 and decreased thereafter (Table 1).

All male lumpfish with 2.25% of above GSI had well developed spermatogonia. There was no significant difference in GSI among male lumpfish from all four groups (Table 1). Male lumpfish from CDL0T had significantly lower T levels

compared to male lumpfish from SDL3T at week 3 and 6 (Table 1). Males from SDL3T also had significantly higher 11-Ketotestosterone (11-KT) levels compared to males from the other three groups at week 6. 11-KT levels in males from both SDL groups increased initially but decreased after week 6 (Table 1).

Table 1. Effects of photoperiod and temperature on GSI, testosterone and estradiol (E2) of female lumpfish and GSI, testosterone and 11-Ketotestosterone (11-KT) of male lumpfish at 0, 3, 6, and 9 of the experimental period. Values are mean ± SD. Different alphabets in each column for each of the 4 sampling periods indicate significant difference at *p* < 0.05.

		Female			Male		
Week	Treatment	GSI	Testosterone	E2	GSI	Testosterone	11-KT
0	SDL	4.50 ± 1.48[a]	0.8 ± 0.39[a]	1.09 ± 0.75	1.59 ± 2.16	2.64 ± 2.87	1.12 ± 1.05
	CDL	1.85 ± 0.38[b]	0.31 ± 0.15[b]	0.37 ± 0.29	1.79 ± 1.79	0.55 ± 0.34	0.38 ± 0.14
3	SDL3T	8.86 ± 5.71[a]	2.77 ± 3.12[a]	1.91 ± 1.38	3.50 ± 2.88	21.44 ± 18.24[a]	4.84 ± 3.07
	SDL0T	4.51 ± 3.97[ab]	0.65 ± 0.42[b]	1.16 ± 1.27	3.08 ± 2.83	12.14 ± 9.68[ab]	4.94 ± 3.97
	CDL3T	4.08 ± 2.13[b]	0.39 ± 0.26[b]	0.84 ± 0.76	4.35 ± 3.43	11.61 ± 17.69[ab]	2.61 ± 2.82
	CDL0T	3.08 ± 3.07[b]	0.44 ± 0.53[b]	0.76 ± 1.17	3.12 ± 3.28	2.91 ± 3.17[b]	1.46 ± 1.41
6	SDL3T	16.88 ± 9.53[a]	11.87 ± 18.13	2.42 ± 2.51	3.24 ± 1.61	47.12 ± 22.42[a]	20.87 ± 14.97[a]
	SDL0T	5.93 ± 10.72[b]	6.75 ± 14.48	0.38 ± 0.34	4.51 ± 2.69	23.00 ± 13.84[ab]	9.34 ± 5.94[b]
	CDL3T	7.19 ± 9.31[b]	2.79 ± 5.17	2.14 ± 2.68	3.85 ± 4.11	3.99 ± 7.54[b]	1.42 ± 2.32[b]
	CDL0T	1.59 ± 0.63[b]	0.34 ± 0.23	0.28 ± 0.13	3.92 ± 2.93	11.38 ± 11.66[b]	3.02 ± 3.14[b]
9	SDL3T	5.12 ± 7.45	0.47 ± 0.37	0.62 ± 0.58	1.75 ± 1.38	10.84 ± 9.73	3.42 ± 2.48
	SDL0T	1.22 ± 0.12	0.28 ± 0.09	0.26 ± 0.03	1.90 ± 2.27	6.75 ± 9.87	3.17 ± 4.39
	CDL3T	2.38 ± 2.07	0.27 ± 0.16	0.56 ± 0.47	2.32 ± 2.88	10.93 ± 23.48	2.18 ± 3.91
	CDL0T	1.42 ± 0.37	0.20 ± 0.00	0.21 ± 0.01	1.64 ± 1.99	4.90 ± 9.29	2.51 ± 4.37

Discussion

GSI of the female lumpfish increased simultaneously with ovarian development. Female lumpfish seemed to become mature about 6 weeks after the temperature increase. They responded to the photoperiod and temperature change positively. When comparing SDL3T and SDL0T, it is evident that the temperature increase had a positive effect on gonadal maturation and ovulation. In contrast, male lumpfish did not show any response to temperature or photoperiod change. The majority of males from all four treatments were matured and releasing milt at week 3 and 6. In high latitude fishes, photoperiod has been suggested to affect the early gonadal development (vitellogenesis) while temperature modulates the final oocyte maturation and ovulation

(Pankhurst & Porter, 2003). While extreme water temperatures during cytoplasmic growth and pre-vitellogenesis have been shown to affect the gonadal development, moderate temperature increase during final gonadal maturation are shown to advance the spawning with no deleterious effects (Dorts et al., 2012).

Our results showed that temporal changes in plasma T and E2 concentrations were in close association with the ovarian development of the lumpfish, which is in agreement with other teleost fishes (Kagawa et al., 1981). T and E2 concentrations correlated well with GSI values up to c. 10%. For higher GSI values, T and E2 did not correlate with GSI indicating a shift in sex steroid synthesis. This is supported by the observed change in the E2:T ratio from about 2.0 for GSIs of 5 - 10% to about 0.5 for those with higher GSIs than c. 15%. A reduction in E2 plasma concentration as well as a reduced E2:T ratio is often found in the period prior to ovulation and spawning (Nagahama, 1994). A significant reduction in GSI as well as sex steroids at the last sampling may also indicate that egg deposition had been initiated.

In lumpfish males, T and 11-KT was found at relatively high concentrations during testis growth, which is in accordance with observations of other teleost fishes (Nagahama, 1994). However, in males, T and 11-KT was not related to GSI, but the observation that 11-KT was significantly influenced by temperature indicates a direct effect of temperature on sex steroid synthesis and/or metabolism in this species (Borg, 1994).

Our results demonstrated that an increase in temperature had a significant effect on final gonadal maturation and ovulation of female lumpfish. We recommend use of both photoperiod and temperature manipulation to control spawning in captive lumpfish broodstock.

Acknowledgements

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IS THE INVASIVE GREEN CRAB RESPONSIBLE FOR LOBSTER DECLINE IN NEWFOUNDLAND – IS A HATCHERY NEEDED?

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Abstract

The green crab (*Carcinus maenas*) has been classified as one of the top 100 world's worst invaders because it can have devastating effects on fisheries and aquaculture operations. Since the invasion of the green crab to Newfoundland in 2007, American lobster (*Homarus americanus*) landings in Placentia Bay have decreased substantially. The aim of this project was to determine if green crabs were the cause of this collapse by investigating the behavioural interactions between crabs and lobsters, in terms of food acquisition and catchability. Experiments were conducted at two temperatures (4°C, 12°C) and with four densities (0/1/5/25) of green crabs interacting with an individual lobster. As the density of crabs increased, individual lobsters consumed less food. Lab and field experiments also showed the presence of green crabs inside a trap will actively deter a lobster from entering. As the green crab continues to spread around Newfoundland, it could have the potential to disrupt the lobster fishery and other fisheries. The Fish Food & Allied Workers Union had proposed to start a lobster hatchery in order to replenish stocks in Newfoundland; this research will help to inform policy and determine if such an operation is necessary or feasible.

Introduction

The American lobster, *Homarus americanus*, is the most commercially important decapod species to Atlantic Canada, on average generating 75,000 t yr^{-1} which equates to 680 million CAD. Since the invasion of the green crab, *Carcinus maenas*, to Newfoundland waters in 2007, lobster landings in Placentia Bay have decreased by 34.2% (DFO, 2015). The habitat ranges of *H. americanus* and *C. maenas* overlap in some areas (Williams et al., 2006) and the presence of the green crab may disrupt the native crustacean species due to increased

competition around a food source (Williams et al., 2006). The green crab has been categorized as one of the top 100 worst world invaders (Invasive Species Specialist Group, 2016) due to its potential to decimate valuable eelgrass beds that are vital nursing grounds for many commercially important invertebrate and fish species to the fishing and aquaculture industries. Previous studies have found that adult green crabs eat juvenile lobsters (Rossong et al., 2006) and, in Newfoundland waters, the habitat and diet of lobster and green crab overlap (Williams et al., 2006), increasing competition for food resources for lobsters. From 2009 - 2010, research into the viability of a lobster hatchery was conducted by the Canadian Centre for Fisheries Innovation (CCFI), the Fish, Food and Allied Workers Union (FFAW-Unifor), and the Marine Institute of Memorial University (MI) (MI, 2016) but was abandoned due a lack of continuous funding. Research conducted for this project aims to quantify the behavioural interactions between the American lobster and the green crab to provide more evidence of the impact the presence of green crab has on the lobster. Local harvesters in Placentia Bay have stated that there are high densities of green crab in the area and that lobster traps are rapidly filling up with crabs, which consume the bait (personal communication). Previous studies on competition between lobsters and green crabs have just pitted one individual against another. However, in the natural environment green crabs form dense aggregations, particularly around a food source, so it is important that experiments are conducted to observe how crab density affects lobster food acquisition.

Methods

Food acquisition – Individual lobsters put against four different densities of green crab (0/1/5/25) in each feeding trial (n = 15):

Each trial lasted three hours and was video recorded to determine behavioural interactions - time taken for the first species to reach food, species displaced from food, food handling time (lobsters only), and any “non-passive” interactions between the two species. The food source used was a mix of 75 g blended mackerel, 5 g of gelatin and 0.45 g glass Ballotini beads. After each feeding trial, individuals were put into a LIXI fluoroscope to quantify the amount of food eaten. Another set of trials of 150 crabs against an individual lobster were also conducted (n = 10) to determine how long the average amount of crabs found in a baited trap took to consume the entire food source.

Catchability – Individual lobsters put against three different positions of green crab (IN/OUT/ ABSENT) around a baited trap in a tank environment (n = 10).

Twelve-hour trials were conducted to quantify behavioural interactions - time for each species to approach trap/attempt trap, number of approaches/attempts/escapes (lobsters only), time inside trap (lobsters only). An "approach" was determined by the individual physically touching the trap in the trial and an "attempt" was quantified by the animal attempting to enter the trap by using the entry gaps.

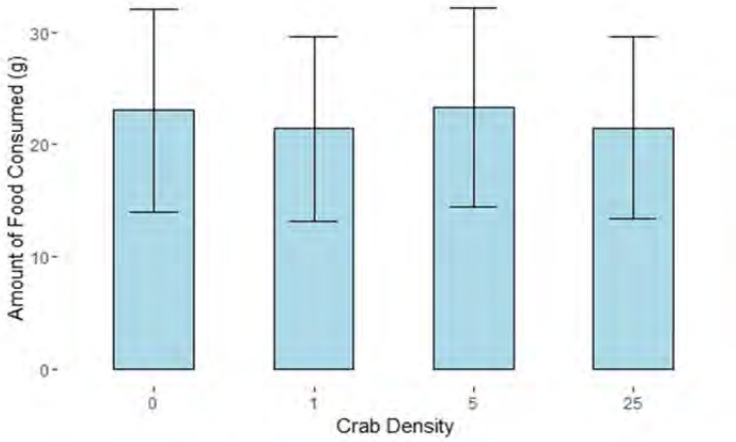

Figure 1
Amount of food consumed in lobsters with different crab density.

Results

There was no significant effect of crab density (0/1/5/25) on lobster food consumption at 4°C (Fig. 1); however, it is expected to be significant when repeated at 12°C, as animals will be more active due to higher metabolic demand. Lobsters did not feed against 150 crabs, as crabs consumed the entire food source in 8 minutes on average (Fig. 2). There was no significance between the number of approaches a lobster made to a baited trap (ANCOVA, F = 0.01, p = 0.936) (Fig. 3), or the number of attempts to enter the trap with crab position (ANCOVA, F = 0.086, p = 0.818); however, an evident trend can be noted with lobster attempts (Fig.4). On average, lobsters made the most attempts to enter the trap when crabs were absent from the experimental tank environment, and made the least amount of attempts when crabs were present outside of the trap. It is also expected to see a significant result when these trials are repeated at 12°C, and with more replicates.

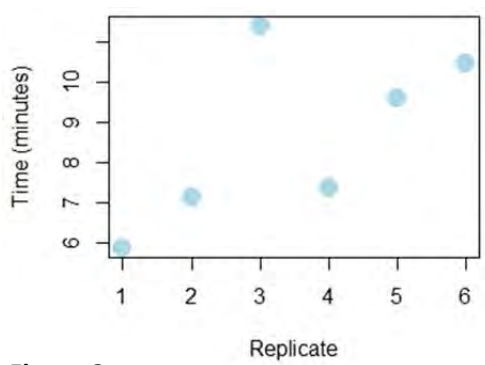

Figure 2
Preliminary data for the time it took 150 crabs to consume food source.

Discussion

The presence of the invasive green crab negatively affects lobster landings as lobsters are actively deterred from entering traps. It is expected that at warmer temperatures, crabs will have a significant effect on lobster food acquisition by increasing interspecific competition for the food source and therefore decreasing the amount of food a lobster will consume. When 150 crabs were present, no lobsters fed in any trial, as crabs consumed the entire food source in 8 minutes and outcompeted the lobster. As the literature shows green crabs and lobsters overlap in diets (Williams et al., 2006), the need for more research into

interspecific competition in Newfoundland is high. With the increase in green crab populations in Placentia Bay, and the recent spread to Fortune Bay (DFO, 2015), the wild Newfoundland lobster populations could continue to decrease. Although there was a lack of significance, a trend occurred in the green crab position around a baited trap, as it was also found to affect lobster behaviour. When crabs were positioned outside of the trap, able to move around the trap and tank freely, lobsters attempted to enter the trap the least. In the related literature, Miller (1990) found a relationship between the frequency of crabs entering a trap and the presence of crabs already in a trap. Crabs may be "intimidated" by the presence of other crabs either via odour, sound, or threatening posture, and this may be true for lobsters. Previous studies conducted on green crab predation on juvenile lobsters have shown to be of the most concern to the lobster fishery in Newfoundland. Rossong et al. published research in 2006 to show that juvenile lobsters (28 - 57 mm carapace width) were being consumed by adult green crabs (53 - 76 mm carapace width) and could have contributed to the decline of lobsters in the area. This research is the most profound to support the reintroduction of the lobster hatchery to Placentia Bay that was abandoned due to lack of funding. The previous hatchery reared lobster larvae with a 30% survival rate, and larvae were released at stage IV (15 mm total length). However, due to high green crab predation on juveniles, we would advise the hatchery to rear the lobsters for longer, until they are at sub-adult stage (57 mm+) so they are able to defend themselves against green crabs. To conclude, this research provides evidence to show that the presence of green crabs is a detriment to the lobster fishery in Newfoundland and, to increase wild stocks, it would be highly beneficial to reintroduce a commercial lobster hatchery to replenish the population.

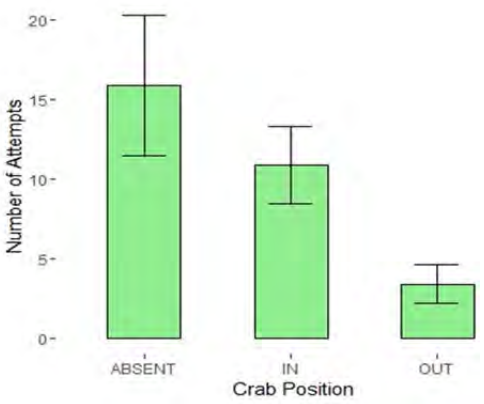

Figure 3
Number of attempts to enter a baited trap made by a lobster dependent on crab position.

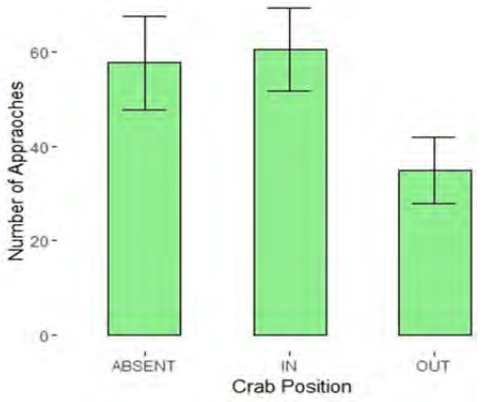

Figure 4
Number of approaches to a baited trap made by a lobster dependent on crab position.

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THE HEALTH AND VACCINATION OF LUMPFISH IN NORWAY

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Introduction

Pharmaq is currently the only global pharmaceutical company solely focused on aquaculture. In 2015, Pharmaq joined with Zoetis, a leading animal health company, as a separate business unit within the company with a goal of addressing growing demand for customized aquaculture products. Salmonids remain the core area of focus; however, expansion into new regions includes such species as lumpfish. Presently, Norway is home to 27 lumpfish hatcheries, and production of the species has increased from 3.75 million (2014) to an estimated 15 million in just two years.

Lumpfish are becoming more widely used as cleaner fish by the aquaculture industry in Norway and other countries (UK, Ireland, Iceland, and Faroe Islands) and also is being tested in Canada. This is due to their natural behaviour of grazing on Atlantic salmon infested with salmon lice. Lumpfish are thus contributing to keeping the salmon lice situation under control.

Lumpfish are also susceptible to infections, and we will provide an overview of detections of *Listonella anguillarum*, atypical *Aeromonas salmonicida*, *Pasteurella* sp, *Tenacibaculum* sp., and *Pseudomonas anguilliseptica* as well as an overview of other disease agents and pathogens screened for that could potentially be transferred to salmon.

Commercial-scale vaccination of lumpfish has been conducted for some years, and the vaccines are under continuous evolution. Experiences from vaccination of lumpfish will be presented.

Disease Problems

Bacterial infections represent the largest challenge in lumpfish production. In 2015, over 75% of diagnostic testing on lumpfish was attributed to bacterial species including *Vibrio ordalii*, *Vibrio anguillarum*, *Pasteurella* sp., and atypical furunculosis (*Aeromonas salmonicidae*) (Bornø et. al, 2015).

Atypical furunculosis is common in all farmed marine species, including lumpfish, and presents in both the acute and chronic form. Vaccination has resulted in good protection against this pathogen.

Typical furunculosis is not widespread, and has only been observed in 6 farms in one region of Norway (late 2015 and in 2016). The disease was found to be in lumpfish but not in salmon in the same farms, although the region does have furunculosis within the wild salmon population.

Although phylogenetically related, *Pasteurella* sp. isolated from lumpfish is distinct from *Pasteurella* sp. identified from salmon internally, small granulomas are located within the kidney, and may be difficult to distinguish from atypical furunculosis. Apparently healthy fish can act as carriers, resulting in repeat outbreaks. *Pasteurella* sp. has been reported in several regions of Norway, including within broodstock populations.

Vaccine Development and Vaccination Technique

Development of lumpfish vaccines began in 2011. Initial efforts focused first on applying existing cod vaccines and then on vaccines developed for trout and salmon. Ultimately, vaccines adapted for lumpfish were produced in 2014, and new versions are still being developed.

Vaccination is achieved predominantly (90% in 2015) through injection, and to a smaller extent by dip/bath vaccine. Recommended size for vaccination is 8 g, with the average lumpfish being vaccinated at 10.63 g. The injection site has been identified as midway between the vent and the posterior portion of the sucker cup. The needle should be positioned at a 90° angle, with no side deviation. Approximately 1/3 of farms are using anaesthetic prior to vaccination. Vaccination quality results have been high, with 87% of injections being classified as optimal and a miss injection rate of only 3%. Applied correctly, very low (< 1%) mortality has been reported in healthy fish populations.

Immune response in lumpfish is visible by 6 weeks post vaccination. It has therefore become common practice in Norway to transfer fish 5 weeks post vaccination, regardless of temperature. Thus far, no cases of *L. anguillarum* have been reported while following this timeline. Work is ongoing to evaluate the immune responses as well as sizes at vaccination.

Figure 1
Sea lice found in a lumpfish's stomach.

Future Work

Going forward, research will focus on anaesthesia trials and their role in stress response. Trials modelling bath and cohabitation challenge method development are ongoing, as well as vaccine trials testing dip vaccine against *V. anguillarum*. Finally, continuation of the basic immunology research with the University of Bergen is presently ongoing.

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SPATIAL PLANNING WITH AQUACULTURE: THE CURRENT STATE AND THE NEED FOR PRACTICAL TOOLS

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Worldwide, the increase of human pressures on coastal and offshore ecosystems together with the increased risk of adverse effects on marine ecosystem health fueled the development and implementation of integrated management approaches such as marine spatial planning (MSP) (Katsanevakis et al., 2011; Collie et al., 2013; Stelzenmüller et al., 2013). For any ecosystem-based MSP process to be sustainable, all current and future human activities together with their associated pressures on key ecosystem components have to be included. As today, in many planning processes existing human activities such as fisheries or aquaculture have not been considered (Stelzenmüller et al., in press; Stelzenmüller et al., 2016). In an effort to make an integrated assessment of spatial planning operational and to aid spatial planning with aquaculture, the Thünen Institute, Institute for Sea Fisheries, is currently developing a spatial explicit cost-benefit analysis tool (GIS Add-in). This development takes place in the course of the EU project AquaSpace (http://www.aquaspace-h2020.eu/) which aims to provide increased space of high water quality for aquaculture by adopting the Ecosystem Approach to Aquaculture (EAA).

By definition, the CBA tool is meant to allow for a spatial representation of costs and benefits of a proposed aquaculture site in a multi-use context. The CBA tool comprises functions that enable the user to assess the spatial explicit performance of inter-sectorial, environmental, economic, and socio-cultural indicators for different aquaculture planning scenarios. Thus, cost indicators reflect for instance the spatial conflict potential between human uses, benthic nutrient load of planned aquaculture, habitat vulnerabilities or combined environmental effects of a proposed aquaculture activity, direct and indirect economic costs, or visual impacts. In contrast, indicators reflecting benefits of a planned aquaculture site comprise total expected revenues or synergy potential with other sectors (Gimpel et al., in prep.-a).

The CBA tool is equipped with an end-user driven interface and an interactive menu. It visualizes areas of constraint (e.g., priority shipping lanes) and potential

synergy (i.e., co-location), defined by an interaction matrix which can be modified according to the user needs. Further, the tool enables the user to explore a range of options to identify potential sites and assess the costs and benefits of several scenarios at once. Tool outputs comprise detailed reports and graphical outputs which should facilitate planning trade-off discussions hence allowing key stakeholders (e.g., industry, marine planners, licensing authorities) to take more informed, evidence-based decisions on proposed aquaculture developments and the associated risks and opportunities.

Its socio-economic dimension will increase the acceptance of these new developments by local communities and society-at-large (Ramos et al., 2014; Stelzenmüller et al., in press). Environmental assessments will contribute to the implementation of the Integrated Maritime Strategy and its environmental pillar, the EU Marine Strategy Framework Directive (Gimpel et al., 2013; Stelzenmüller et al., 2014; Gimpel et al., in prep.-a). Integrating indicators supporting the assessment of inter-sectorial effects enables authorities to account for the principles of good MSP practice as required by the EU Marine Spatial Planning Directive (Gimpel et al., in prep.-a). Ultimately, this integrated assessment approach could support the licensing process and facilitate investments (Stelzenmüller et al., in press). The tool is currently applied in European case studies (Mallorca, Bay of Biscay, Germany, Scotland) operating at different spatial scales.

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PROPHYLACTIC EFFECT OF *Hasela ostrearia* CULTURE SUPERNATANT CONTAINING THE PIGMENT MARENNINE AGAINST THE PATHOGENIC BACTERIA *Vibrio splendidus* IN BIVALVE HATCHERIES

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Introduction

A primary requisite in any shellfish culture or farming operation is an abundant, reliable, and inexpensive supply of juveniles, commonly called seed (Helm et al., 2004). Hatchery-produced seed is increasingly becoming the standard raw material for aquaculture, a trend that is likely to broaden in the future (FAO, 2011). One of the major difficulties in bivalve hatchery production is repeated bacterial infections, resulting in heavy mortalities and causing major losses and great expenses for shellfish growers. These mortalities are generally related to bacteria from the genera *Vibrio*, *Pseudomonas*, and *Aeromonas*, with members of the genus *Vibrio* being the most frequently observed (Paillard, 2004). The pathogenic bacteria *V. splendidus* is considered a widespread bivalve pathogen, and its pathogenicity has been thoroughly studied (Tanguy et al., 2013).

Placopecten magellanicus and *Mytilus edulis* are good representatives of the most commonly cultured bivalves, the first being reputed to be difficult to produce when compared to mussels or oysters (Helm et al., 2004). In pectinid hatcheries, bacterial infections are known to be the major cause in massive mortality events around the globe, whatever the production system used

(Devauchelle and Mingant, 1991; Riquelme et al., 1995; Nicolas et al., 1996; Andersen et al., 2011). As antibiotic use in hatcheries is now controlled or prohibited in many countries (Arkinbowale et al., 2006), there is a need to identify new molecules with antimicrobial activities. Marennine could be such a molecule. It is a blue-green water-soluble pigment synthesized by the marine pennate diatom *Haslea ostrearia*. Purified marennine has been shown to display antibacterial activities (Gastineau et al., 2012, 2014), inhibiting in vitro the development of pathogenic marine bacteria *Polaribacter irgensii*, *Pseudoalteromonas elyakowii*, and *Vibrio aestuarianus* at concentrations as low as 1 µg mL^{-1} (Gastineau et al., 2012).

The main hypotheses of this work was that the supernatant of *Haslea ostreria* culture containing marennine (blue water, BW) could exert a prophylactic effect in larval rearing of *P. magellanicus* and *M. edulis*, by controlling *V. splendidus* pathogenicity in bacterial challenge tests with these two bivalves. More specifically, the main objectives were to (1) improve larval survival and physiological condition, (2) reduce the total bacterial load, and (3) inhibit the pathogenicity of *V. splendidus* in challenge conditions by using BW with marennine in *P. magellanicus* and *M. edulis* larval rearing.

Material and Methods

Haslea ostrearia was grown in photobioreactors (PBRs) until maximal marennine concentration was reached. Culture supernatant containing the marennine (BW) from each of the PBRs was then concentrated 100 times (from 100 L to 1 L) using two ultrafiltration units (30 and 3 kDa), as described in Pouvreau et al. (2006b).

M. edulis spawning adults were obtained from Havre-aux-maison lagoon. *P. magellanicus* spawning adults were obtained from Baie de Gaspé and Pointe Saint-Pierre. Spawning was induced by thermal shock and fertilized eggs were reared to D-larvae stage. *M. edulis* and *P. magellanicus* larvae were exposed to *Vibrio splendidus* 7SHRW, a wild strain isolated by Mateo et al. (2009). *M. edulis* larvae were cultured at 20˚C in duplicate with four treatments: Control, Marennine, *Vibrio*, and Marennine + *Vibrio*. An initial concentration of 6.6 x 10^5 bacteria mL^{-1} for *V. splendidus* was used and 0.5 mg L^{-1} for marennine. *P. magellanicus* larvae were exposed to *V. splendidus* at an initial density of 1 larva mL^{-1}, 0.1 mg L^{-1} marennine, and an initial bacterial concentration of 5.75 x 10^5 cell mL^{-1}. Three treatments were applied (n = 3): Control, *Vibrio*, and *Vibrio* + Marennine. In both experiments, samples were collected for larvae survival assessments and bacterial analyses after 72 h.

Survival was assessed by visual counts; growth was measured with a digital microscope. Bacterial counts and high nucleic acid (HNA) proportion were determined by flow cytometry. T-tests and ANOVAs were carried out using JMP 9.0.1 (SAS Institute).

Results and Discussion

Treatment had a significant effect on *M. edulis* (F3, 5 = 15.49; p = 0.01), and *P. magellanicus* (F2, 7 = 60.61 p = 0.001) survival in a bacterial challenge context with *Vibrio splendidus* (Fig. 1). Considering that the pathogen caused significant larval mortalities, that the BW marennine treatment reduced (*P. magellanicus*) or inhibited (*M. edulis*) the mortalities, and that the bacterial load and percentage of cells with high nucleic acid content (% HNA used as a proxy of the physiological state of the bacterial population) did not change under the treatment (or only slightly for % HNA in *M. edulis*), the positive effect of BW marennine could result from a reduction in bacterial pathogenicity. In the work of Tardy-Laporte et al. (2012), marennine was found to interact with the LPS in

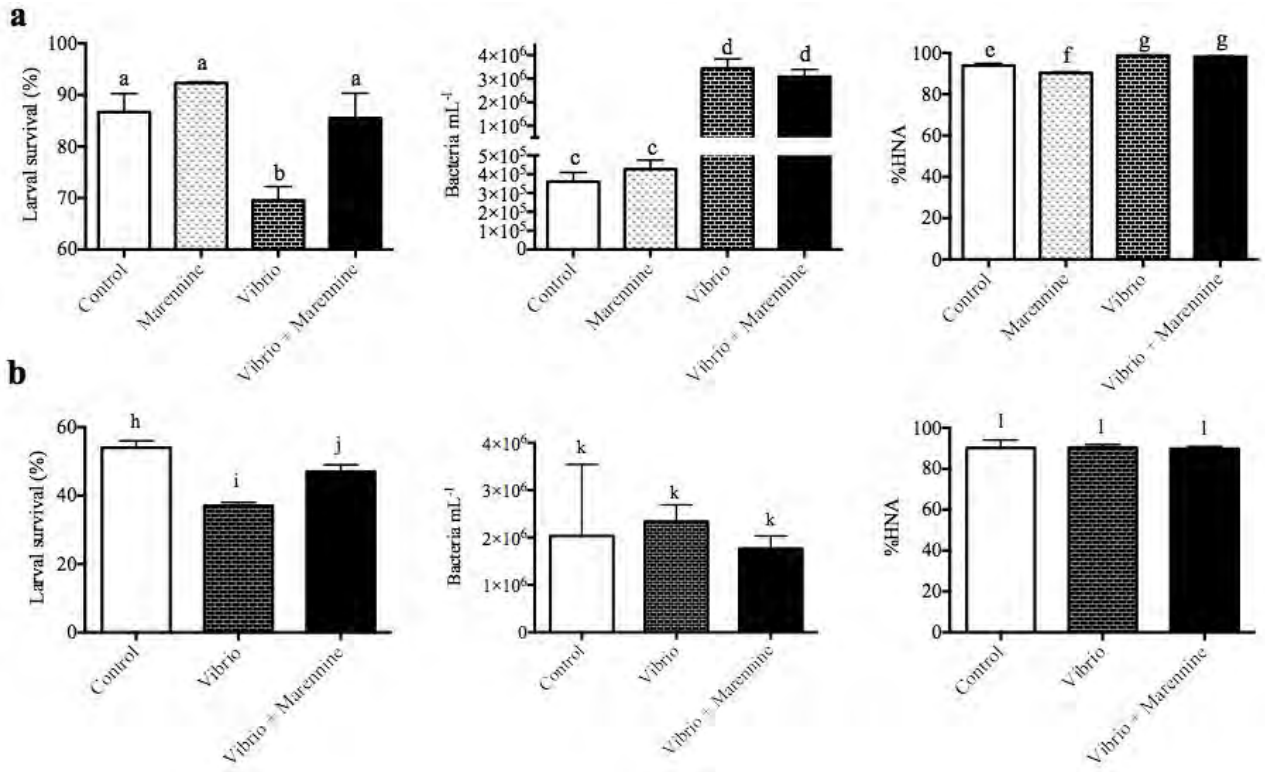

Figure 1
Larval rearing success of *P. magellanicus* (a) and *M. edulis* (b) and bacterial cell concentration (cells mL^{-1}) and high nucleic acid bacteria proportion (% HNA) of larvae culture water for the control without addition, blue-water-containing marennine treatment (0.1 mg L^{-1}), *Vibrio* treatment (105 bacteria mL^{-1} of *V. splendidus*), and the *Vibrio* + BW marennine treatment (0.1 mg L^{-1} of marennine and 105 bacteria mL^{-1} of *V. splendidus*) after 72 h of exposure. Data are means ± SD (n = 2 for *M. edulis* and n = 3 for *P. magellanicus*) and different letters indicate significant differences.

bacterial membranes, lipids related to Gram-negative bacteria toxicity (Beutler, 2004). Also, the outer membrane protein (OMP) OmpU was shown to be a major determinant of *V. splendidus* strain LGP32 virulence, contributing to the host antimicrobial peptide/protein (AMPs) resistance, to the host cell adherence, and to the pathogen recognition (Duperthuy et al., 2010). Thus, we suggest that marennine could interact with the bacterial cell outer structures responsible for *V. splendidus* pathogenicity, but the mechanisms of action need to be demonstrated. As marennine was shown to exhibit antioxidant activity (Pouvreau et al., 2008), it could also provide a protective action to the host against the oxidative stress burst in larvae exposed to pathogenic bacteria (Genard et al., 2013). Marennine mechanism of action could thus be explained by both activities (antimicrobial and antioxidative).

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APPENDIX 1

Past AAC Award Winners

List of Lifetime Achievement Award Recipients

Date Bestowed	Name	Residence
May 30, 2000	Neil Bourne	Nanaimo, British Columbia
May 9, 2001	David Aiken	Saint Andrews, New Brunswick
September 20, 2002	Rene Lavoie	Dartmouth, Nova Scotia
October 31, 2003	Bill Pennell	Nanaimo, British Columbia
October 19, 2004	Ovila Daigle	Richibucto, New Brunswick
July 5, 2005	Louis Deveau	Dartmouth, Nova Scotia
November 21, 2006	Lucien Poirier	Rimouski, Quebec
May 13, 2008	Yves Bastien	Chelsea, Quebec
May 19, 2010	Al Castledine	Victoria, British Columbia
May 2012	Chris Frantsi	Saint Andrews, New Brunswick
June 3, 2014	Cry Couturier	St. John's, Newfoundland & Labrador
June 3, 2014	Santosh Lall	Halifax, Nova Scotia
September 21, 2016	Rod Carney	Saint Andrews, New Brunswick

List of Research Award of Excellence Recipients

Date Bestowed	Name	Residence
May 30, 2000	Santosh Lall	Halifax, Nova Scotia
May 9, 2001	Joseph Brown	St. John's, Newfoundland
September 20, 2002	Joel de la Noue	Laval, Québec
November 1, 2003	Tillmann Benfey	Fredericton, New Brunswick
October 20, 2004	Ed Donaldson	West Vancouver, British Columbia
July 6, 2005	John Castell	Saint Andrews, New Brunswick
November 22, 2006	David A. Higgs	West Vancouver, British Columbia
September 25, 2007	Richard Moccia	Guelph, Ontario
May 12, 2009 (joint)	Thierry Chopin	Saint John, New Brunswick
	Shawn Robinson	Saint Andrews, New Brunswick
May 11, 2011	Debbie Martin-Robichaud	Saint Andrews, New Brunswick
June 4, 2013	Marcel Fréchette	Mont Joli, Québec
June 3, 2014	Fred Page	Saint Andrews, New Brunswick
June 2, 2015	Céline Audet	Rimouski, Québec

We are now accepting nominations for **2017 Research Award of Excellence**. Details on how to nominate someone for this award can be found on our website here http://www.aquacultureassociation.ca/awards/research-award-of-excellence/research-award-of-excellence-nominations/ . The deadline for nominations is **March 17, 2017**.