

**Growing Quality Seafood  
Through Innovation**

**Aquaculture Canada**

**2008**



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**Proceedings of contributed papers**

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**C.I. Hendry**  
editor



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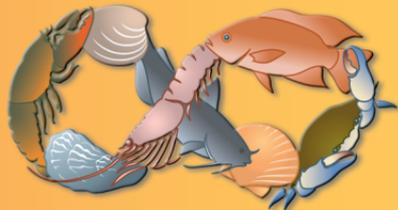


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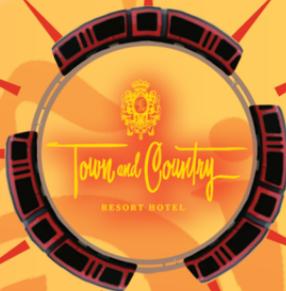


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Aquaculture Canada<sup>OM</sup> 2008 – Proceedings of the Contributed Papers of the 25<sup>th</sup> Annual Meeting of the Aquaculture Association of Canada, Saint John, NB, May 10-14, 2008.

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# Aquaculture Canada<sup>OM</sup> 2008

May 11-14, 2008  
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Proceedings of Contributed Papers

President's Report . . . . .	5
<i>Alistair Struthers, AAC President 2007-2008</i>	
Aquaculture Canada <sup>OM</sup> 2008 Lifetime Achievement Award . . . . .	6
AC08 Sponsors and Contributors . . . . .	8
AAC 2007-2008 Board of Directors . . . . .	10

## **Contributed Papers**

Egg Abnormality and Embryonic Development in Atlantic Cod, <i>Gadus morhua</i> L. . . . .	11
<i>TS Avery, SS Killen, and TR Hollinger</i>	
Atlantic Canada Aquaculture Industry Research and Development Network – Driving Research . . . . .	14
<i>Atlantic Canada Aquaculture Industry Research and Development Network (ACAIRDN)</i>	
Little Bugs with Smaller Bugs: Preliminary Studies on the Role of Sea Lice as a Vector of Bacterial Pathogens . . . . .	20
<i>DE Barker, LM Braden, MP Coombs, and B Boyce</i>	
Characterization of the Spatial Pattern of Benthic Sulfide Levels at Salmon Farms in Southwestern New Brunswick, Bay of Fundy . . . . .	24
<i>BD Chang, FH Page, RJ Losier, EP McCurdy, and KG MacKeigan</i>	
Anaerobic Digestion of Aquaculture Waste . . . . .	30
<i>S Fraser, MF Couturier, J Conroy, KS Singh, and A Desbarats</i>	
Phosphorus Leaching During the Hydrolysis of Fish Waste Solids . . . . .	33
<i>J Conroy and MF Couturier</i>	
Preservation of Lipid Content in Microalgae Concentrates from Ultrafiltration Process . . . . .	36
<i>D Diouf, R Tremblay, R Fournier, and F Pernet</i>	
Challenges to Applying Eco-based Research – Analysis of a Two-Year Research Program in the Broughton Archipelago, British Columbia . . . . .	39
<i>J O’Riordan</i>	
The Assessment of Impacts on the Benthic Environment from Suspended Oyster Aquaculture in Baynes Sound, British Columbia, Canada . . . . .	42
<i>WA Heath, S Carroll, R Devos, and B Provan</i>	
How Does the Spotted Wolffish ( <i>Anarhichas minor</i> ) Adjust to Constant and Fluctuating Oxygen Concentrations? . . . . .	46
<i>F Larouche, NR Le François, SG Lamarre, and D Chabot</i>	
Canadian Trout Industry: Competitive Advantage and Strategic Options . . . . .	49
<i>R Nabi</i>	
Effects of Poultry Oil as a Replacement to Fish Oil in Atlantic Salmon ( <i>Salmo salar</i> ) Diets . . . . .	52
<i>D Deslauriers and K Rideout</i>	

Modelling the Transport and Deposition of Particulate Effluent from Fish Farms in Southwestern New Brunswick, Bay of Fundy . . . . . 55  
*FH Page, RJ Losier, BD Chang, and EP McCurdy*

Building a Collaborative Research Program. . . . . 60  
*P Parker*

Virulence of Infectious Salmon Anaemia Virus (ISAV) Isolates in Atlantic Salmon (*Salmo salar* L.) in the Bay of Fundy: A Review . . . . . 63  
*RJ Ritchie and N Gagné*

Protein Hydrolysates and Trypsin Inhibitor Enhanced Digestive Capacities, Growth and Survival of Newly Hatched Spotted Wolffish . . . . . 67  
*A Savoie, NR Le François, SG Lamarre, PU Blier, L Beaulieu, and C Cahu*

Effect of Rearing Density on Growth and Plasma Ion Levels of Juvenile Spotted Wolffish (*Anarhichas minor*): Preliminary Results . . . . . 70  
*S Tremblay-Bourgeois, NR Le François, RL Roy, TJ Benfey, and AK Imsland*

Aquaculture Collaborative Research and Development Program (ACRDP): History and Future Initiatives . . . . . 73  
*CL Whelan and GJ Parsons*

Aqua-L . . . . . 75

AC08 Committees . . . . . 76

## AAC President's Message



Welcome to the Aquaculture Canada<sup>OM</sup> 2008 Proceedings. Each year I look forward to the publication of the Proceedings of Aquaculture Canada with much anticipation, as it is my favourite issue of the AAC publications. I hope that this will serve as a fond reminder for those who attended and as a good overview for those of you who were unfortunately unable to join us. Our 25<sup>th</sup> anniversary meeting was a great success, being held in the heart of Atlantic Canada's salmon aquaculture industry and successfully joining forces with AquaFair 2008.

The theme of the meeting, *Growing Quality Seafood through Innovation*, was very nicely reflected in the breadth, depth and quality of the papers and posters presented during the meeting. Hopefully this publication will serve as a reflection of the meeting. Co-hosting the meeting with AquaFair resulted in the addition of industry-focused sessions and workshops, thus bringing more industry relevance to the meeting. Student participation was again excellent this year. I hope that the meetings of the Aquaculture Association of Canada will continue to serve as a bridge between industry, students and all those involved in the dynamic Canadian aquaculture industry.

Organizing a meeting the size and caliber of Aquaculture Canada<sup>OM</sup> is no small task, and I would like to thank Joanne Burry (Conference Organizer), Cyr Couturier (Program Chair) and Tim Jackson (Local Organizing Committee Chair) along with their teams for the tremendous amount of work that went into Aquaculture Canada<sup>OM</sup> 2008. In addition, I would also like to thank Chris Hendry for his efforts in putting together and editing these proceedings. Lastly, I gratefully acknowledge the generous support of the Province of New Brunswick, in addition to our co-hosts, the New Brunswick Salmon Growers Association and the Professional Shellfish Growers Association of New Brunswick. The combined efforts of all helped to make Aquaculture Canada<sup>OM</sup> 2008 a resounding success.

Our 25<sup>th</sup> anniversary meeting provided a great opportunity for many industry and AAC veterans to reminisce and share their experiences. It's from these experiences that we will learn and carry the Canadian aquaculture industry forward. I eagerly look forward to what the next 25 years hold in store for all of us!

Sincerely,

Alistair Struthers  
AAC President (2007-2008) and AC08 Chair

## AAC Lifetime Achievement Award

### Yves Bastien



Yves Bastien retired from the Federal Public Service in April 2007 after a 30-year career in the provincial and federal public services, and 24 years in aquaculture. He started his aquaculture career with the Québec Department of Agriculture Fisheries and Food. Over a 13-year period he occupied 3 aquaculture positions: Aquaculture Industrial Development Officer, Coordinator for technical assistance to fisheries and aquaculture, and Mariculture Coordinator. His main function during this period was to organize and deliver extension services and technology transfer activities to the shellfish farming sector. He was then seconded to the private sector, where as Director General, he designed, established and managed a semi-private mariculture investment fund called SODIM (Société de développement de l'industrie Maricole). In 1999 he became Canada's first Commissioner for Aquaculture Development, a position that he held until 2004. Reporting to the Minister of Fisheries and Oceans, he was tasked with championing the development of aquaculture in Canada, particularly within the federal government. Key achievements included the creation of the Program for Sustainable Aquaculture (\$75 M over 5 years), a funding program to foster partnership and cooperation within the aquaculture industry, and 3 major reports with recommendations to the government of Canada: (1) *Legislative and Regulatory Review of Aquaculture in Canada*, (2) *Achieving the Vision*, and (3) *Recommendations for Change*. At the end of his mandate as Commissioner he was offered the opportunity to implement his recommendations within the federal government and accepted the position of Executive Director, Aquaculture Management with DFO. During this period he managed DFO's policy and regulatory responsibilities regarding aquaculture. He was co-chair of the Aquaculture Task Group (ATG) of the Canadian Council of Fisheries and Aquaculture Ministers (CCFAM), played a key role in the creation of the National Aquatic Animal Health Program (NAAHP) and laid the groundwork for the federal investment announced in the last budget. During his career he was a member of several organizations including Aquanet, the Canadian Aquaculture Industry Alliance, the World Aquaculture Society, Advisory Board of the Atlantic Veterinary College and the Aquaculture Association of Canada (of which he was twice elected President). In 2004 he received the Herb Dhaliwal Sustainable Aquaculture Award.



Yves Bastien (left) receives his Lifetime Achievement Award from Alistair Struthers, AAC President (2007-2008).

## AAC Prix honorifique pour contributions

### Yves Bastien

Yves Bastien a pris sa retraite du gouvernement fédéral en avril 2007 après 30 années de carrière au sein de la fonction publique provinciale et fédérale et 24 années en aquaculture.

Il a débuté sa carrière en aquaculture au Ministère de l'agriculture, des pêcheries et de l'alimentation du Québec où il a occupé 3 positions sur une période de 13 ans : Agent de développement industriel en aquaculture, Coordonnateur à l'aide technique aux pêches et à l'aquaculture et Coordonnateur à la mariculture. Durant cette période, sa principale fonction consistait à organiser et à livrer des services d'aide technique et de transfert de technologie au secteur de la conchyliculture. Il a ensuite été prêté au secteur privé où, à titre de Directeur général, il a conçu, mis en place et géré un fonds semi privé d'investissement en mariculture nommé SODIM (Société de développement de l'industrie maricole). On lui a ensuite offert de devenir le premier Commissaire canadien au développement de l'aquaculture, une position qu'il a occupé de 1999 à 2004. Se rapportant au ministre des pêches et des océans, il devait se faire le champion du développement de l'aquaculture au Canada, particulièrement au sein du gouvernement fédéral. Entre autres réalisations, il a joué un rôle clé dans la création du Programme pour l'aquaculture durable (\$ 75 M sur 5 ans), il a conçu et géré un programme d'aide financière au partenariat et à la coopération au sein de l'industrie aquicole et a publié 3 rapports contenant des recommandations au gouvernement fédéral : (1) Revue légale et réglementaire de l'aquaculture au Canada (2) Concrétiser la vision (3) Recommandations pour un changement. À la fin de son mandat de Commissaire, on lui a offert de mettre en oeuvre ses recommandations au sein du gouvernement et il a accepté la position de Directeur exécutif de la gestion de l'aquaculture au MPO. Au cours de cette période, il a géré les responsabilités du MPO en matière de politiques et de réglementation en aquaculture. Il a été co-président du Groupe de travail en aquaculture (GTA) du Conseil canadien des ministres des pêches et de l'aquaculture (CCMPA), a joué un rôle clé dans la création du Programme national sur la santé des animaux aquatiques (PNSAA) et a préparé le terrain pour l'investissement fédéral annoncé lors du dernier budget. Au cours de sa carrière, il a été membre de nombreuses organisations incluant Aquanet, l'Alliance de l'industrie canadienne de l'aquaculture, la World Aquaculture Society, le Conseil consultatif du Collège vétérinaire de l'Atlantique et l'Association Aquacole du Canada, organisation pour laquelle il a été élu président à 2 reprises. En 2004 il a reçu le prix d'aquaculture durable Herb Dhaliwal.

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DFO - Biological Station, 531 Brandy Cove Road, St. Andrews, NB E5B 2L9  
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## **Administrative Office:**

Susan Waddy, Association Office Manager

Catrina Wong, Administrative Assistant

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Aquaculture Association of Canada, 16 Lobster Lane, St. Andrews, NB, Canada E5B 3T6

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# Egg Abnormality and Embryonic Development in Atlantic Cod, *Gadus morhua* L.

Trevor S Avery<sup>1\*</sup>, Shaun S Killen<sup>2</sup>, and Tamara R Hollinger<sup>1</sup>

<sup>1</sup> Department of Biology, Acadia University, Wolfville, NS, Canada, Tel: 902-585-1873, (\*Corresponding e-mail: trevor.avery@acadiau.ca)

<sup>2</sup> Institut des Sciences de l'Evolution de Montpellier, Station Méditerranéenne de l'Environnement Littoral, Université Montpellier II, 1 Quai de La Daurade, F-34200 Sète, France, Email: shaun.killen@univ-montp2.fr

A reliable method for assessing the viability of fertilized embryos early in development would be beneficial for the aquaculture industry, allowing egg batches with a high probability of low hatching success to be discarded before costly resources are devoted to their culture. During the last decade, the observation of cellular morphology during embryogenesis has received attention as a potential early indicator of embryo quality. However, most often, abnormally cleaving eggs are assessed *en masse*, although noticeable, individual differences in cleavage patterns are generally present. We separated six batches of Atlantic cod, *Gadus morhua*, eggs into normal and abnormal cleavage patterns, reared them individually in a temperature-controlled room, and recorded mortality rate each day until hatch. Seven abnormal cleavage patterns were readily distinguishable and all showed moderate variability in egg mortality. No significant differences were found between any abnormal cleavage patterns, but overall, abnormal eggs had significantly lower hatching success than normally cleaving eggs.



Trevor Avery

## Introduction

Egg quality among batches in serial spawners is highly variable<sup>(1,2)</sup>, and is thought to be influenced by many factors including broodstock diet, stress, genetics, and water quality. A reliable method for assessing the viability of fertilized embryos early in development would be beneficial for the aquaculture industry, allowing batches of eggs indicating high probability of low hatching success to be discarded before costly resources are devoted to their culture.

During the last decade or so, the observation of cellular morphology during embryogenesis has received attention as a potential early indicator of embryo quality. The transparency of most marine fish eggs allows for easy observation of cleavage abnormalities including asymmetrically arranged blastomeres, differences in blastomere size, or poor adhesion between adjacent blastomeres. Abnormal blastomere cleavage during embryogenesis has been correlated with low hatching success in serial-spawning, marine fish such as Atlantic cod *Gadus morhua*<sup>(3,4)</sup>, yellowtail flounder *Limanda ferruginea*<sup>(5)</sup>, haddock *Melanogrammus aeglefinus*<sup>(6)</sup>, and Atlantic halibut *Hippoglossus hippoglossus*<sup>(7)</sup>. The postulate is that malformations of undifferentiated blastomeres during early embryogenesis adversely affect subsequent development, thus leading to egg death before hatching.

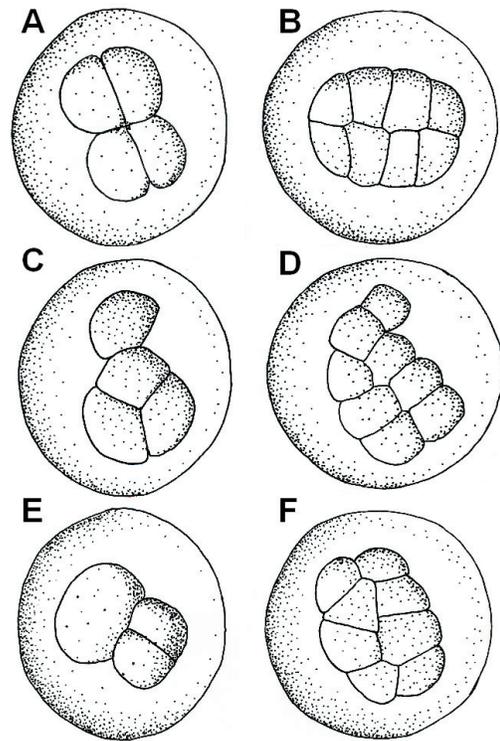
Researchers have characterized cleavage abnormalities into groups (e.g., poor differentiation of margins, poor cellular adhesion, the existence of asymmetry<sup>(2,6,7)</sup>), but none have examined specific cellular patterns of the morula, or categorized patterns for severity of their effect on embryogenesis. More importantly, few have tracked the development of eggs with abnormal cleavage patterns on an individual egg basis. We suggest that some patterns of abnormalities will be more serious or occur at critical periods of development and may cause immediate death, and others are less critical or occur at non-critical development periods and are 'corrected', thus allowing subsequent development to proceed normally. A clear understanding and quantification of the effects of abnormal cleavage patterns on hatching success is necessary. However, the assessment of all abnormal embryo patterns combined confounds estimates of embryonic mortality; e.g., more 'severe' patterns of abnormality may be responsible for a large proportion of the observed mortality, while less 'severe' abnormalities may have little effect on embryogenesis and subsequent hatching success.

## Materials and Methods

Eggs were collected from an Atlantic cod, *Gadus morhua* L., broodstock maintained at the Ocean Sciences Centre, Logy Bay, Newfoundland, Canada and housed in the Joe Brown Aquatic Research Building (JBARB). Males and females were kept in tanks supplied with degassed, filtered seawater. After each daily spawning event, buoyant eggs were collected from an automatic egg collector and examined under a dissecting microscope to determine their stage of development. If a batch had sufficient numbers of fertilized (2-cell stage), developing eggs, they were transported im-

Figure 1.

4-cell and 8-cell morula patterns of normal (A and B), and abnormal (C-F) Atlantic cod eggs. C-D represent a 'separated' pattern where one or more blastomeres are attached to only one other blastomere, and E-F a 'triple' pattern displaying an incomplete separation during cell division.



mediately to a cold room for sorting at the 4-cell stage. Ninety-six fertilized eggs (48 normal and 48 abnormal consisting of several abnormal patterns) were selected from each of six batches. Each egg was transferred with a modified plastic pipette tip into a separate well of a spectrophotometric plate (~1 ml in volume). The wells were filled with seawater containing 0.1 g/L Streptomycin sulphate and 0.06 g/L Penicillin G to reduce bacterial contamination, and were maintained in the cold room at 6°C through the experiment. Half of the water in each well was changed daily using a glass pipette without contacting the developing embryo.

Abnormal eggs were classified based on the nature of the morula arrangement at the 4-cell stage of development. The abnormal patterns generally differed from each other on the basis of the parameters described by Shields et al.<sup>(7)</sup> and subsequently modified by others, namely blastomere size, adhesions, margins, and inclusions, but where Shields et al.<sup>(7)</sup> assigned embryos a qualitative score describing the degree to which they appeared 'normal', we assigned descriptive names to all observed abnormal cleavage patterns that resulted from variation in one or more of these parameters similar to Penney et al.<sup>(2)</sup>, Avery and Brown<sup>(5)</sup>, and Rideout et al.<sup>(6)</sup>. Irregular cleavage patterns were observed until the 16-cell stage, after which it was nearly impossible to discern if the pattern was different from normally cleaving eggs. Each egg was observed every 6 to 8 hours until mortality or hatching (hatching generally occurred around 14 days post-fertilization or 84 deg days). Once the terminal condition of each egg was recorded, it was removed from the plate and the well water removed to prevent possible bacterial cross contamination from dead eggs or larvae.

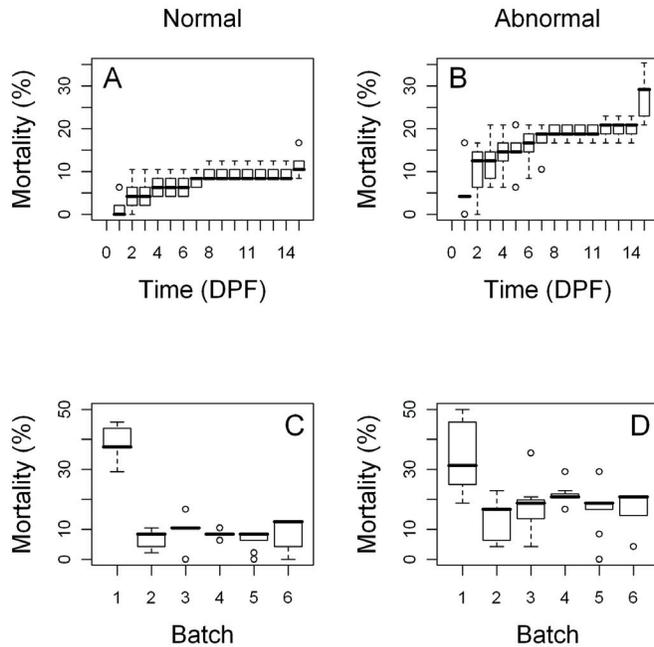
Hatching success (100 - cumulative mortality as percent) at day 14 (weighted overall mean day of hatching) was analyzed with paired Student's t-tests between batches to determine differences between normal and abnormal (overall and each pattern) eggs. A Holm correction was applied to the resulting p-values to maintain a familywise error rate of  $\alpha = 0.05$ <sup>(8)</sup>. All analyses were completed in R<sup>(9)</sup>.

## Results and Discussion

Normally cleaving eggs (normal eggs) had relatively consistent blastomere sizes with complete margins, and were symmetrical (Fig. 1A). Seven abnormal patterns were apparent at the 4-cell stage: pie (morula radially symmetric, but with wedge-shaped blastomeres), offset (similar to normal, but asymmetrical with margins shifted or misaligned), separated (Fig. 1C), donut (symmetrical, incomplete margin(s) with definite hole between blastomeres), unequal (symmetrical, but two blastomeres larger), triple (Fig. 1E; incomplete margin when dividing from the 2-cell stage [or beyond]), and jumbled (severely asymmetrical, differences in blastomere sizes and/or incomplete margins). A standard classification system for abnormal cleavage patterns would be beneficial if patterns were common among species.

Only the first batch contained the jumbled pattern, albeit in low quantities, and this batch had the highest mortality rates overall (Fig. 2C) and for each day. Since this batch also exhibited additional indicators of poor quality (some translucent or cloudy eggs, reduced egg buoyancy, higher proportion of unfertilized eggs) suggestive of some other effective agent (possible bacterial infection, increased temperature shock or mechanical stress, or sitting in the egg collector too long before transport and sorting), it was discarded from further analysis. The jumbled pattern contains several errors, so more severe morula deformities may indicate poorer quality egg batches. Categorizing abnormal patterns as more or less severe, or relating abnormal patterns to drivers would be useful for aquaculture operations.

Abnormalities consistently continued through the 16-cell stage (Fig. 1A-F), but by the 32-cell stage, some abnormal eggs were not distinguishable from normal eggs. In more severe cases, however, such as eggs displaying the jumbled pattern or eggs with separated blastomeres, some


**Figure 2.**

Daily and batch mortalities for normally (A and C) and abnormally (B and D) cleaving Atlantic cod eggs. Day 15 includes larval mortalities that occurred after the weighted mean hatch day 14. Batch 1 was not included in A and B.

blastomeres remained on the periphery of the morula, suggesting that some errors persist and cannot be corrected. By the 64-cell stage, most eggs looked the same. We suggest that error correction during embryogenesis may occur, since low hatching success was not prevalent in any one pattern.

Mortality rates were initially high (days 1-8; ~48 degree days), but reached an asymptote at around day 9 (Fig. 1A-B). In yellowtail flounder, *Limanda ferrugineus*, the rates were highest from day 1-3 (~27 degree days)<sup>(5)</sup>, but these differences could be species specific. Comparing stages of embryogenesis to the characteristics of the mortality curves for various species is an obvious next step to determine if mortality is related to development stage. The increase in mortality on day 15 is due to both egg and larval mortalities.

Mean survival was greater in normal eggs ( $90.4 \pm 1.86\%$ ) than abnormal eggs ( $80.0 \pm 2.38\%$ ) when all abnormal egg patterns were combined ( $p = 0.022$ ). Avery and Brown<sup>(5)</sup> showed higher overall mortality rates (lower survival) for abnormal versus normal eggs of yellowtail when comparing mortality curves directly. No significant differences in hatching success were found among the abnormal patterns nor between any one abnormal pattern and normal eggs ( $p > 0.341$  for all comparisons). Some patterns had highly variable mortalities among batches, and/or low sample sizes which may mask any true differences. Excluding batch 1, egg batches showed relatively consistent mortalities, with abnormal eggs consistently greater and with higher variability than normal eggs (Figure 1C-D).

## Acknowledgements

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# Atlantic Canada Aquaculture Industry Research and Development Network – Driving Research

## Atlantic Canada Aquaculture Industry Research and Development Network (ACAIRDN)

The Atlantic Canada Aquaculture Industry Research and Development Network (ACAIRDN) is a unified voice for the Atlantic Canadian Aquaculture Industry in matters of R&D, providing leadership, coordination and communication for the direct benefit of the industry. The Network first began in 2002, with the placement of Research and Development Coordinators (RDCs) at each of the major aquaculture industry associations in Atlantic Canada. Since the inception of ACAIRDN, this model has been emulated by other associations across Canada which have hired RDCs.

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## Goal of the ACAIRDN Research Workshop

The overall purpose of ACAIRDN Research Workshops is to focus on industry R&D priorities and on ways of developing closer linkages and compatibility between researchers, academics and funding programs to better assist industry in meeting their challenges.

The goal of this particular workshop is to provide an overview of the key research activities being undertaken by the aquaculture industry. The workshop also provided an opportunity to promote joint undertakings within Canadian aquaculture associations, such as the ACAIRDN R&D Priorities Matrix and collaborative research initiatives. In addition, ACAIRDN includes a presentation from a resource agency, providing an opportunity for industry and researchers to learn more about particular funding programs that may be of use in aquaculture research.

A key component of all ACAIRDN workshops is a discussion period, however, due to time constraints, it was agreed to distribute a short series of questions regarding R&D collaborations to workshop participants.

This session was held during Aquaculture Association of Canada's annual meeting (Aquaculture Canada<sup>OM</sup> 2008), held in Saint John, New Brunswick, allowing the RDCs to reach out to well respected researchers from across Canada.

## SLICE Update: 2006-2008

### Kathy Dalton, New Brunswick Department of Agriculture and Aquaculture

SLICE is a registered product in all major aquaculture producing countries throughout the world, with the exception of the USA and Canada. Registration of SLICE in Canada and the US has been ongoing for several years and is not likely to be registered here for at least another 3 years. SLICE is available for producers under the Emergency Drug Release (EDR) program in Canada.

Other jurisdictions worldwide have set the minimum residue limit for SLICE at 100 ppb with a withdrawal time of 0 days (Norway/Chile) to 2 days (UK). The USA has not established a minimum residue level (MRL) for SLICE but has placed a 60 day withdrawal time under their INAD policy (equivalent to Canada's EDR). The Veterinary Drug Directorate (VDD) had set the new MRL for SLICE at 42 ppb with an associated withdrawal time of 68 days. Both Health Canada and CFIA are prepared to look at the data provided from this multi-year study and use it in their deliberations on reviewing the withdrawal period and minimum residue level.

This presentation gave an overview of results found to date (from 2006-2007) and expected results from the in-tank study being performed at the Atlantic Veterinary College (2008-2009).

This project is of great importance to the salmon industry as a whole as Health Canada will not make any changes to current 68 day withdrawal period without having field data collected and supporting data from a controlled in-tank study.

Kathy Dalton has an honours degree in Marine Biology from The University of New Brunswick in Saint John, NB. She has been working in and around the aquaculture industry for approximately the last 12 years on both the East and West coasts of Canada.

*Kathy is an Aquaculture Fish Health Specialist with the Sustainable Aquaculture and Fish Health Branch of NB DAA. She has been working with the Department since 2005 and focuses on issues related to fish health, disease management, environmental management and wild and farmed fish interactions.*

## Research Activities at the Professional Shellfish Growers Association of New Brunswick

**Kevin Burke, Professional Shellfish Growers Association of New Brunswick**

Founded in 1997, the New Brunswick Professional Shellfish Growers Association (NBPSGA) has its head office in Shippagan, in northeastern New Brunswick. The Association currently has 57 members, including shellfish producers, institutes, enterprises, and students. Oysters (*Crassostrea virginica*) and mussels (*Mytilus edulis*) constitute our primary products.

The occurrence of marine birds (e.g., cormorants, gulls) on aquaculture structures can affect the salubrity of oysters. A project aimed at developing a mechanism to modify the structure of the Oyster Gro® cages was undertaken in 2007. A small plastic, triangular-shaped structure with a jagged edge at the top was added to the cages. These new structures discourage birds from using the cages as a loafing site.

Undergoing a developmental phase, the oyster industry in New Brunswick has a remarkable potential for growth. Therefore, available space for new leases is becoming limited in a number of bays and the stocking densities will likely increase in the next few years. Consequently, the Association wishes to support a project to explore this problem. It could be argued that the overstocking of oysters represents an unlikely scenario since it would negatively affect bivalve productivity (animal growth) and hence farm profitability. The aim of the project is to identify the farming intensity that does not have a negative impact on either the oyster growth or the benthic habitat.

The goal of shellfish growers is to sell their oysters as rapidly as possible. In order to have a rapid growth rate, it is important for the producers to know the most appropriate way(s) for their oysters to be distributed in the water column. It is important to evaluate factors such as line spacing and or bag spacing on both the horizontal and the vertical axes. In order to optimize the market growth in New Brunswick, the NBPSGA wants to undertake the project of studying the growth of oysters by investigating different distribution patterns of the oysters in the water column.

Other projects in collaboration with the Department of Fisheries and Oceans, Department of Agriculture and Aquaculture, Coastal Zone Research Institute (Shippagan, NB) and consulting firms will also be undertaken in the future. A follow-up of the utilization of aquaculture software by shellfish growers and the evaluation of the Code of Practice are also part of our work plan. The construction of the NBPSGA website is on-going with the official launch due to take place on July 18th during the Aquaculture & Fisheries Day.

*Kevin Burke graduated from the Biology MSc. Program at the Université de Moncton in Moncton, New Brunswick. Following his degree, he has worked with different organizations, including short-term contracts with the Université de Moncton (New Brunswick Aquarium & Marine Centre in Shippagan), and the Pokemouche Watershed, before being hired as R&D coordinator in January 2008 by the New Brunswick Professional Shellfish Growers Association (NBPSGA). The mandate of this association is to promote the interests of the members and to represent industry in public and governmental agencies. Kevin Burke will coordinate R&D for the Association which will involve a number of studies that will help to maximize the potential of this promising field for the future and self-sufficiency of New Brunswick.*

## Industry-Driven Aquaculture R&D

**Peter Warris, PEI Aquaculture Alliance**

This presentation reviewed the current PEI aquaculture industry R&D priorities, broken down by sector. It then focuses on key projects with particular focus of how industry priorities were translated into research activities and how a collaborative approach helped successfully manage them. Finally there is a brief review of some future research initiatives.

*Peter is the Research and Development Coordinator (RDC) for the PEI Aquaculture Alliance, and one of four members of the Atlantic Canada Aquaculture Industry Research and Development Network. Peter has a B.Sc. in Biological Sciences from the University of Plymouth and a M.Sc. in Shellfish Biology, Fisheries and Aquaculture from the School of Ocean Sciences, University of Wales.*

## Update on Ongoing and Upcoming R&D Projects in Newfoundland and Labrador

**Darrell Green, Newfoundland Aquaculture Industry Association**

The Research and Development Coordinator (RDC) plays a key role within the Newfoundland Aquaculture Industry Association in helping stimulate and support research initiatives. This presentation describes how R & D priorities are determined and gives an update on some of the research projects which are currently ongoing in Newfoundland and Labrador. It also describes a number of possible future projects and the key collaborations within Atlantic Canada which can enable the success of these initiatives.

Research and Development priorities for NAIA are determined through holding sector meetings (shellfish and finfish) at least yearly. These meetings also allow for the discussion of industry issues which may not necessarily be added to the list of R&D priorities. Another important element in the development of R&D priorities is site visits, where the RDC met with site managers, site workers and/or business owners to discuss their successes, their challenges and their goals. The list of priorities and issues, developed through these activities, is then added to the ACAIRDN priorities matrix.

Some of the current and future R&D projects at NAIA include:

### **Shellfish**

- 1. Seed Supply - access to new seed sites and protecting “good quality” existing sites
- 2. Aquatic Invasive Species - Public Education
- 3. Marketing - Atlantic-wide, marketing, Increasing consumption and market price
- 4. Processing Investment - Ice slurry technology, gentle debussing, etc.
- 5. Infrastructure Improvements
- 6. Lobster fishery and mussel farming interactions - fish harvesters – concerns over decreasing landings in Notre Dame Bay
- 7. Tunicate mitigation - gastropod predators of tunicates

### **Finfish**

- 1. Biosecurity plan for the Coast of Bays Region - wharves, potential BMAs, etc.
- 2. Environmental data on Fortune Bay / Bay d’Espoir - SmartBay concept ([www.smartbay.ca](http://www.smartbay.ca))
- 3. On-land net cleaning – feasibility of developing this service
- 4. Waste management - morts, offal, plastic bags, etc.
- 5. Public education - value and sustainability of aquaculture
- 6. Cod Genome Project - identify genetic markers relating to industry-relevant characteristics for use in broodstock selection

*Darrell has a B.Sc. in Biology from Memorial University and a Graduate Diploma in Aquaculture from the Fisheries and Marine Institute of Memorial University. Since 1997 he has worked in the aquaculture industry, starting off with short contracts at NAIA and the Canadian Centre for Fisheries Innovation (CCFI) and then as a fish farm manager in Ontario. He then spent seven years at the Ocean Sciences Centre working on aquaculture research projects involving cod, halibut and blue mussels, before joining the team at NAIA in January 2007. In his current capacity as R&D Coordinator he helps to initiate and coordinate aquaculture R&D in Newfoundland while acting to maintain communication of aquaculture R&D issues between industry, government and academic researchers throughout Atlantic Canada.*

## **IPSFAD Overview**

**Eric Boucher, Interprovincial Partnership for Sustainable Freshwater Aquaculture Development**

IPSFAD, Interprovincial Partnership for Sustainable Freshwater Aquaculture Development, is national in scope and brings together several internationally-recognized experts into a collaborative framework of industry, academic and government interests. It is a unique opportunity to pool expertise and resources and to focus them around fostering the sustainable development of the freshwater aquaculture sector. It differs from previous initiatives revolving around the aquaculture file in that IPSFAD has spent time developing a specific mandate for the freshwater sector and it has

been structured to be industry-driven with regional representation on its Board of Directors. All regional and provincial freshwater aquaculture associations are members of IPSFAD Board.

**Mission Statement:**

To promote sustainable development of freshwater aquaculture in Canada.

**Objectives :**

- 1. Create consensus regarding applied research, development and commercialization (RDC) priorities identified by industry stakeholders.
- 2. Promote applied research, development and commercialization projects and assemble required research and/or technology transfer expertise for execution.
- 3. Foster the establishment of necessary synergies among various players while avoiding duplication of work and making optimal use of resources.
- 4. Organize and seek funding for projects that result directly from priorities identified by industry stakeholders.

**IPSFAD Current Industry Action Plan 2007-2009 activities:**

- Symposium on Developments in Freshwater Aquaculture in Canada, held in Gatineau in 2007
- Phosphorus content / feed labelling
- Regional Workshop and Industry Action Plan III 2007/2009
- 2nd National Freshwater Symposium / AAC 2007 Edmonton
- NSERC strategic workshop
- Canadian Experimental Aqua-Farm

*M. Eric Boucher graduated with a Bachelor's degree in Biology from UQAR in Rimouski and, in 1997, obtained his Master's degree in Science with specialization in Applied Aquaculture at UBC in British Columbia. He worked to refine a prototype of a biomass system for cage culture, the VICASS (Video Image Capturing and Sizing System). He was then hired by Sigma Technologies to assist in the commercial production of the VICASS system. M. Boucher has joined, in 2003, the founders of Interprovincial Partnership for Sustainable Freshwater Aquaculture Development (IPSFAD) as project coordinator. Since then, several projects with quick commercial applicable results were carried out under his supervision. The impacts of some of the research are already tangible within the aquaculture industry and within its quest of sustainable development. M. Boucher is also currently the executive secretary for the IPSFAD.*

**Everything We Want to Do is Illegal – Anarchy in the Shellfish Farming Industry**

**Dave McCallum, BC Shellfish Growers Association**

Well, not everything is illegal of course, but at times it feels like we are pushing limits! The BCSGA recognizes the importance of academic projects and data collection to answer certain research questions, but often this information does not trickle-down to deliver a bottom-line benefit to industry. There is certainly plenty of industry-related research, but until recently, we have noted a lack of development and commercialization. New federal government commitments to industrial innovation are particularly timely. The BCSGA is now organized with priority projects and we represent an industry hungry for applied innovations. This presentation discussed (1) a brief background on the industry; (2) a synopsis of current BCSGA R&D Priorities; and (3) three specific industry-conceptualized and driven projects that will advance the BC shellfish farming industry and ultimately improve the farmers' bottom line.

*David McCallum is the Research & Development Coordinator for the BC Shellfish Growers Association (since May 2006). He completed a Masters Degree (UVic Geography) and has a background in social issues associated with coastal development and activity. In addition to being a conduit for BC shellfish industry communications (Tidelines Newsletter and BCSGA website editor), his multiple responsibilities at the BCSGA span from facilitating the BC shellfish Aquatic Animal Health Program, through to developing and managing the BC shellfish industry Environmental Stewardship Initiative and other Innovation projects.*

## Looking Forward: British Columbia Salmon Farmers Association Research Strategy

**Norman Penton, BC Salmon Farmers Association**

The British Columbia Salmon Farmers Association (BCSFA) established its research and development priorities in 2007 but required further work to see that these priorities were addressed. A workshop was held with invitees from both levels of government, researchers, consultants and industry personnel/experts. The goal of the workshop was to breakdown the priorities into their key elements, assign resources and personnel needed to see them completed. Once this was completed actual research project became apparent. These will be included in a research strategy for the BCSFA which may be promoted to potential funding agencies and researchers.

*Norman Penton, Research and Development Coordinator for the British Columbia Salmon Farmers Association, has been active in the salmon farming industry in BC for the past 5 years. He holds degrees in biology, public policy and aquaculture from St. Francis Xavier University and the Marine Institute of Memorial University. Previous to his current position he worked in the environmental management departments of Heritage Salmon and Mainstream Canada. A native of the east coast of Canada he moved to British Columbia in order to pursue his career in salmon farming.*

## Introduction to Scientific Research and Experimental Development Incentive Program (Canada Revenue Agency)

**Gayle Armstrong, Canada Revenue Agency**

The Scientific Research and Experimental Development (SR&ED) program provides tax incentives to Canadian businesses that conduct SR&ED in Canada. This program is designed to encourage all businesses, including small and start-up companies, to do work that will lead to new or improved technologically advanced products or processes.

The SR&ED program is delivered by the Canada Revenue Agency (CRA).

Website: [www.cra-arc.gc.ca/sred/](http://www.cra-arc.gc.ca/sred/)

*Gayle Armstrong is a Certified Management Accountant (CMA), and is a Financial Reviewer for Scientific Research and Experimental Development, Canada Revenue Agency, based in Saint John, NB.*

## Discussion

The RDCs recognize the importance of meaningful discussion at workshops like this. Unfortunately, due to time constraints, the discussion period was not held. However, an email soliciting input from participants was distributed after the workshop. The RDCs will compile any comments that are received, and will consider any suggestions and comments that are provided by participants.

The following are the discussion points that were sent to workshop participants:

- Do you have any suggestions on how to foster collaboration between industry and researchers?
- In your opinion, what is the best way to communicate the research needs of industry?
- How can industry encourage research initiatives that address their challenges?

Participants were also invited to provide any additional comments or suggestions.

## Other Industry Research-Related Activities

RDCs can also increase collaboration between industry and researchers through several routes. These include the compilation and publication of a consolidated list of industry research and development priorities. The ACAIRDN Funding Matrix is a compilation of priorities from aquaculture associations throughout Atlantic Canada and has been available since 2007. The Matrix will be updated as new priorities are identified, and possibly with priorities from other associations.

The RDCs from Atlantic Canada publish the ACAIRDN Newsletter, which highlights research initiatives within the aquaculture industry in that region. RDCs from other regions also routinely contribute to the Newsletter. The circulation of this newsletter continues to grow, and is distributed

to industry, government, researchers, academia across Canada, and internationally.

ACAIRDN RDCs host Annual Research Workshops, such as this one, as an avenue to promote the research activities underway within the aquaculture industry in Atlantic Canada. In addition, the RDCs are often involved with the development and delivery of issue-specific workshops that target a particular audience.

Individuals interested in learning more about any of these initiatives should contact the RDC in their region, listed below.

## **Aquaculture Research and Development Coordinators**

### ***Newfoundland***

Darrell Green, Newfoundland Aquaculture Industry Association  
Phone: (709) 754-2854, ext 2; E-mail: dgreen@naia.ca; Web: www.naia.ca

### ***Prince Edward Island***

Peter Warris, PEI Aquaculture Alliance  
Phone: (902) 368-2757; E-mail: rd@aquaculturepei.com; Web: www.aquaculturepei.com

### ***Nova Scotia***

Fernando Salazar, Aquaculture Association of Nova Scotia  
Phone: (902) 422-6234; E-mail: fsalazaraans@eastlink.ca; Web: www.aansonline.ca

### ***New Brunswick***

Caroline Graham, New Brunswick Salmon Growers Association  
Phone: (506) 467-7199; E-mail: c.graham@nbsga.com; Web: www.nbsga.com

Kevin Burke, Professional Shellfish Growers Association of New Brunswick  
Phone: (506) 336-4794; E-mail: kevinb@nb.aibn.com; Web: www.acpnb.com

### ***IFSAD***

Eric Boucher  
Phone: (418) 656-2131, ext. 6580; E-mail: Eric.Boucher@fsaa.ulaval.ca; Web: www.ipsfad.ca

### ***British Columbia***

David McCallum, British Columbia Shellfish Growers Association  
Phone: (250) 890-7561; E-mail: david@bcsga.ca; Web: www.bcsga.ca

Norman Penton, British Columbia Salmon Farmers Association  
Phone: (250) 286-1636; E-mail: normanpenton@telus.net; Web: www.salmonfarmers.org

## **Acknowledgements**

The RDCs wish to acknowledge NRC-IRAP for its support of the industry research network, and ACRDP for providing financial assistance for this workshop. In addition, ACAIRDN wishes to thank the organizing committee of Aquaculture Canada 2008 for incorporating this workshop into the Conference schedule.

# Little Bugs with Smaller Bugs: Preliminary Studies on the Role of Sea Lice as a Vector of Bacterial Pathogens

Duane E Barker<sup>1\*</sup>, Laura M Braden<sup>1</sup>, Maria P Coombs<sup>2</sup>, and Brad Boyce<sup>3</sup>

<sup>1</sup> Fisheries & Aquaculture Department, Vancouver Island University, 900 Fifth St., Nanaimo, British Columbia, Canada, V9R 5S5 (\* (250) 753-3245 (ext. 2296) email: Duane.Barker@viu.ca)

<sup>2</sup> Animal Health Branch, Ministry of Agriculture & Lands, Courtenay, B.C., Canada, V9N 5M6

<sup>3</sup> Marine Harvest Canada, #124 - 1334 Island Highway, Campbell River, B.C., Canada, V9W 8C9

The ability of a parasite to carry and transmit other parasites or pathogens, thus serving as a disease vector, is fascinating. Parasite vector-pathogen associations (e.g., malaria from mosquitoes, the plague from fleas), have been well described from humans and domestic animals but have rarely been examined within an aquatic ecosystem. Farmed fish provide a unique host system to study because they represent an artificial aggregation of hosts; however, farmed fish can be treated, thus interrupting the cyclical development of parasite and pathogen. Farmed salmon in British Columbia (BC) provide an almost ideal system to examine because of the abundance of wild and farmed salmon and the potential for reciprocal pathogen exchange. The potential role of sea lice in a vector-pathogen association affecting salmon has not been explored, despite an obvious need. Using standard OIE bacteriological screening protocols, we sampled the external carapace and internal viscera of motile sea lice collected from farmed Atlantic salmon from May 2007 to April 2008 in BC. Our preliminary results include the first isolation of three pathogenic bacteria (*Tenacibaculum maritimum*, *Pseudomonas fluorescens* and *Vibrio* spp.) from sea lice and their salmon hosts. Spatiotemporal variation among bacteria prevalence was evident from external (58-100%) and internal (12.5-100%) samples. From such intriguing preliminary results, we propose to examine and describe this association within an ecological context to identify significant geographic, seasonal and biological influences. In other words, where (geographically) and when (seasonally), could sea lice carry important salmon pathogens?

## Introduction

Ectoparasites can act as vectors of pathogens, as illustrated by *Plasmodium* malaria carried by *Anopheles* mosquitoes, *Yersinia* plague carried by *Ctenocephalides* fleas and *Babesia* redwater fever carried by *Boophilus* ticks. Novel studies<sup>(1-3)</sup> examined the influence of ecological factors on such vector-pathogen relationships in an effort to mitigate and control pathogens of humans and domestic animals. In such studies, the relative influences of vector biology and abiotic factors on overall pathogen abundance are analysed, modelled and tested. To date, such vector-pathogen analyses have not been well documented within an aquatic setting. Farmed fish provide a unique host system to study vector-pathogen relationships because these fish represent an abnormal aggregation of hosts which influences parasite and pathogen transmission. However, farmed fish can be treated, thus interrupting the cyclical events of parasite and pathogen development. Farmed salmon in British Columbia (BC), Canada, provide an almost ideal system because of the abundance of wild and farmed salmon species in the region and the possibility of reciprocal pathogen exchange. Once farmed salmon have been treated for any parasites or pathogens, the seasonal abundance of wild salmon hosts in the area can serve as an alternate route for pathogen transmission. Two copepod species, *Lepeophtheirus salmonis* and *Caligus clemensi* are commonly reported from Pacific salmon (*Oncorhynchus* spp.)<sup>(4-8)</sup>. Curiously, the role of sea lice as vectors of bacterial pathogens to both wild and farmed salmon has not been studied.

Sea lice development is positively correlated with salinity and temperature, based on lab<sup>(9-10)</sup> and field studies in BC<sup>(4-8)</sup> and the Atlantic<sup>(11-13)</sup>. In these field studies, seasonal and annual fluctuations in abiotic (salinity, temperature) and biotic (host migration) influences correlated with seasonal and annual variations in abundance of lice on wild and farmed salmon. Similarly, there exist seasonal and annual fluctuations in the occurrence of various bacterial diseases (e.g., furunculosis, myxobacteriosis) on farmed salmon in BC<sup>(14)</sup>. On a related note, sea lice (*Lepeophtheirus*) may serve as vectors for viral, ISA<sup>(15)</sup> and bacterial, *Aeromonas salmonicida*<sup>16</sup> pathogens of farmed salmon. However, those studies<sup>(15-16)</sup> documented pathogen isolation but did not demonstrate transmission. Thus, spatiotemporal variability in the abundance of vector (sea lice) and pathogen (bacteria) and the existence of a possible vector-pathogen relationship has been demonstrated, but no studies have examined the ecological role of this relationship, despite an obvious need. Any information pertaining to the identification of sea lice as carriers of bacterial diseases and their geographic and seasonal occurrence is critical to the salmon farming industry in terms of future and ex-



Duane Barker

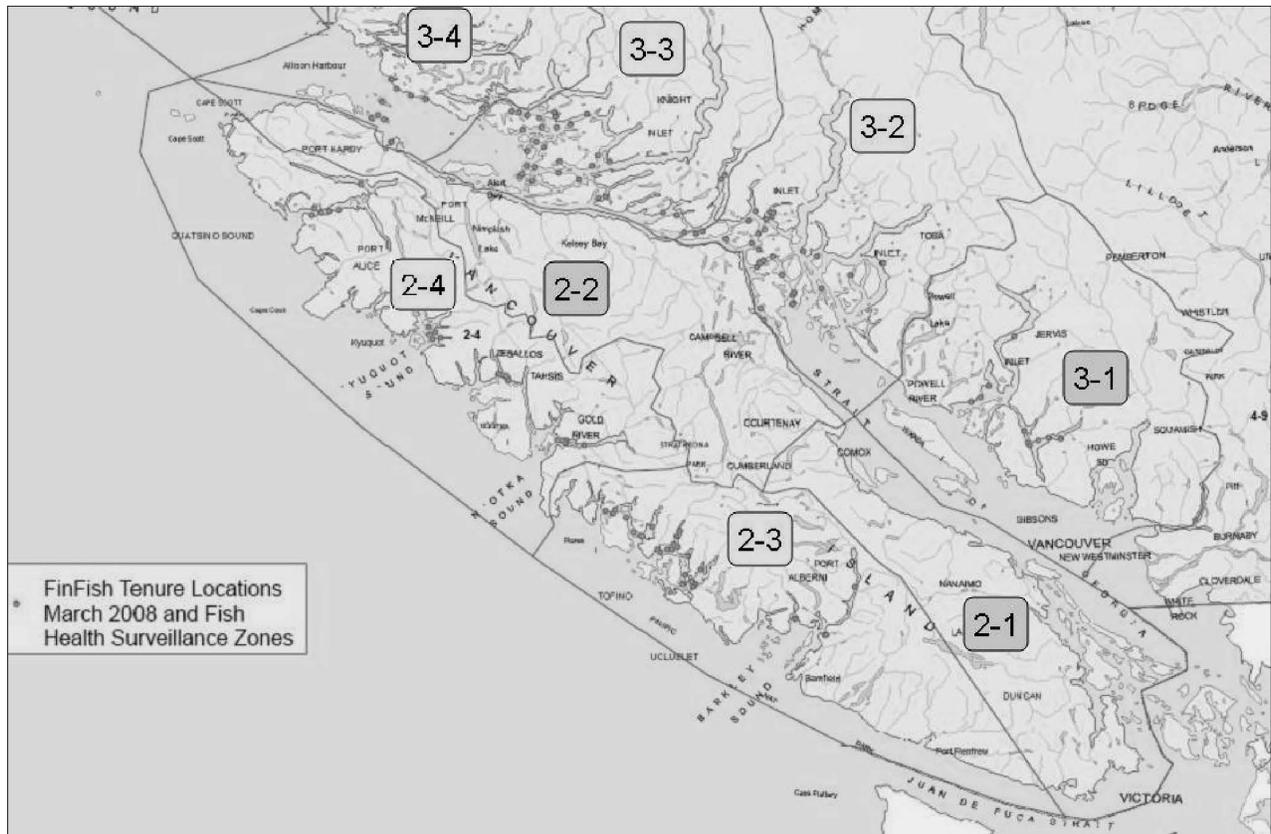
isting site placement. In this paper, we report the preliminary results of our long-term study of this vector-pathogen relationship. Here, we document the first isolation of *Tenacibaculum maritimum* (= *Flexibacter maritimus*), *Pseudomonas fluorescens* and *Vibrio* spp. from sea lice, *Lepeophtheirus salmonis*, parasitizing farmed Atlantic salmon, *Salmo salar*, in BC, Canada.

### Materials and Methods

All sea lice (*L. salmonis* and *C. clemensi*) were collected by our research collaborators from Marine Harvest Canada (B. Boyce) and BC MAL (M. Coombs). Our goal was to monthly sample sea lice (min = 15, max = 40) infecting at least 20 farmed Atlantic salmon (*Salmo salar*) during May 2007 to April 2008, from five of the BC MAL fish health surveillance zones surrounding the coastline of east and west Vancouver Island<sup>14</sup> (Fig. 1). These zones include regions of active Atlantic (> 80%) and Chinook, *Oncorhynchus tshawytscha* (< 15%), salmon farming and are areas abundant with wild Pacific salmon.

For bacterial screening, we sampled only the mobile, actively feeding phases (preadult and adult males, preadult and adult females and gravid females). Lice were aseptically removed from fish and placed in vials (1 vial per host) of filtered, autoclaved seawater (ASW) and stored for a maximum of 24 h (on ice) prior to screening. From each louse, we sampled the exoskeleton by using one sterile cotton-tipped swab from the dorsal and ventral surfaces of the cephalothorax. Next, each specimen was disinfected with 95% ethanol for 10 s and rinsed in ASW prior to collecting an internal sample. As a verification of external disinfection, we collected a second external swab from the lice for plate inoculation. Internal samples were obtained by aseptically opening the ventral surface of cephalothorax and abdomen and making one swab through the internal viscera using a sterile, disposable, plastic inoculating loop. We also sampled 0.5 ml of the ASW that contained the sampled lice. All swabbed samples were inoculated on a variety of nutrient media for 5-7 days: Marine Agar (MA), Tyes Agar (TA) + 3% sea salt (both at 22°C) and Blood Agar (BA) + 3% sea salt, Brain Heart Infusion (BHI) agar + 3% sea salt (both at 12°C). From these primary cultures, pure sub-cultures were isolated, Gram stained, then identified by simultaneously using a variety of differential media and assays: Oxidative-Fermentative (O-F) media, motility media, Cytochrome-Oxidase, API-20E & 20NE and vibriostatic (0/129<sub>150</sub>) sensitivity discs. The data presented represent *L. salmonis* only, due to limited numbers of *C. clemensi*.

**Figure 1.** Vancouver Island, British Columbia, illustrating the BC Ministry of Agriculture & Lands (BC MAL) fish health surveillance zones with sites of active salmon farms. Zones sampled: 2.3, 2.4, 3.2, 3.3 and 3.4. (Source: BC MAL 2008)



## Results and Discussion

Because of very low lice numbers ( $< 1.2$  lice per fish<sup>(17)</sup> and mostly immature) on the farmed salmon, we only obtained sufficient numbers of lice to sample during the months of May, June, August, December and April. However, our data indicate the first published isolation of *Tenacibaculum maritimum* (= *Flexibacter maritimus*), *Pseudomonas fluorescens* and *Vibrio* spp. from sea lice, *L. salmonis*, parasitizing farmed Atlantic salmon in BC. Saptiotemporal variation in the abundance of bacteria from both external (58-100%) and internal (12.5-100%) samples of sea lice (Table 1) was evident. In addition, there was a trend of increased bacterial prevalence with increased seawater temperature, with the highest prevalences of bacteria occurring in June and August. *Tenacibaculum* and *Pseudomonas* were the most common species isolated from external and internal samples from all sampled zones; whereas, *Vibrio* was found in only one of the five sampled zones and during only one month (August 2007; Table 1).

The bacterial prevalence values were quite surprising, given that there were very low lice levels on the salmon during each sampling period. *Tenacibaculum* grew well on the TA + 3% sea salt and the MA, exhibiting the typical flat, yellow colonies and filamentous cell characteristics, but had erratic to low growth on the BA and BHI media. This species is common in marine environments, exhibiting a wide geographic distribution, variable strains and opportunistic pathogenicity<sup>18-19</sup>, thus its isolation was not unexpected. *Tenacibaculum* has been locally reported from farmed salmon in BC as a causative agent of fin and mouth rot<sup>(14,20)</sup>. In our April 2008 sample, we also isolated *Tenacibaculum* from the same salmon host that we obtained our lice.

Although less common, *Pseudomonas* and *Vibrio* both grew well on the MA and BA + sea salt plates at both temperatures. *Pseudomonas* is described as a secondary invader, also associated with fin rot and variable pathogenicity among salmonids<sup>(18,21)</sup>. Oddly *P. fluorescens* is often associated with infections in freshwater; however, one of us (M. Coombs) had also isolated *P. fluorescens* one month earlier from the same collection of farmed Atlantic salmon from which we obtained our sea lice. Although this only provides anecdotal evidence, it justifies investigating whether this transmission pathway is significant.

Our isolates of *Vibrio* were confusing in that our miniaturized tests (API 20E, 20NE) resulted in different species (*V. alginolyticus* and *V. vulnificus*) from the same sub-cultures. Moreover, on repeated assay tests we obtained different results. *Vibrio vulnificus* has traditionally been categorized as a bacterial pathogen of eels, but has been reported from other marine fishes, including salmonids<sup>(18-19)</sup>. Without a genetic screening technique such as PCR, we were unable to discern what species of *Vibrio* was/were present given the single occurrence in our samples.

Our isolation of *Tenacibaculum*, *Pseudomonas* and *Vibrio* from the stomach contents of sea lice parasitizing farmed salmon represents the first published report and is an important finding in itself. However, detection alone does not provide any information on pathogen transmission. Therefore, we have begun an NSERC-funded multi-year study to explore the potential role of sea lice as vectors of these (and other potential species), by first experimentally testing transmission (fulfilling Koch's postulates), then determining the influence of seasonal and geographic factors on this pathway.

**Table 1.**  
Monthly prevalence (%) of *Tenacibaculum maritimum*, *Pseudomonas fluorescens* and *Vibrio* spp. from external (EX.) and internal (INT.) samples of sea lice, *Lepeophtheirus salmonis*, parasitizing farmed Atlantic salmon in BC, Canada during 2007 and 2008.

Bacteria Month	<i>T. maritimum</i>		<i>P. fluorescens</i>		<i>Vibrio</i> spp. <sup>1</sup>	
	EX.	INT.	EX.	INT.	EX.	INT.
May 2007 (Z-2.3, n = 30)	100%	26.9%	58.3%	73.1%	-	-
(Z-3.2, n = 40)	100%	12.5%	58.3%	73.1%	-	-
June 2007(Z-2.4, n = 30)	100%	100%	100%	87%	-	-
(Z-3.3, n = 40)	100%	94%	100%	94%	-	-
(Z-3.4, n = 15)	100%	20%	100%	20%	-	-
August 2007 (Z-2.3, n = 30)	100%	35%	100%	15%	100%	25%
December 2007(Z-3.3, n=30)	100%	27%	-	-	-	-
April 2008 (Z-3.3, n = 24)	100%	33%	50%	29%	-	-

**Note:** Samples sizes of lice in parentheses after each BC MAL surveillance zone.

<sup>1</sup>*Vibrio* species mixed and identified as *V. alginolyticus* and *V. vulnificus*

## Acknowledgements

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# Characterization of the Spatial Pattern of Benthic Sulfide Levels at Salmon Farms in Southwestern New Brunswick, Bay of Fundy

BD Chang\*, FH Page, RJ Losier, EP McCurdy, and KG MacKeigan

*Fisheries and Oceans Canada, Biological Station, 531 Brandy Cove Road, St. Andrews, NB, E5B 2L9 Canada*

In this study we conducted spatially intensive sediment sulfide sampling surveys at six salmon farms in southwestern New Brunswick, Bay of Fundy. We found that the sulfide distribution under farms was spatially and temporally patchy. The highest sulfide levels were generally found near cages, but sulfide levels were low near most cages. At two farms, relatively high levels extended outside the cage array. The number of sample locations in the Environmental Monitoring Program's mandatory Tier 1 sampling (2-8 locations per farm, annually in late summer to early fall) is insufficient to describe the spatial heterogeneity. At most farms, the mean sulfide levels from samples collected within the cage array in the spatially intensive sampling surveys were quite different than those obtained in the EMP. This may have been due to differences in the dates between our sampling and the EMP monitoring at some sites and/or small-scale spatial heterogeneity of the sulfide distribution under farms. The Tier 2 monitoring program, used since 2006 (required at farms where Tier 1 results averaged  $>3000 \mu\text{m}$  sulfide), more adequately describes the sulfide distribution under the cage array at a horizontal resolution of about 100 m, but it does not include sample locations beyond the cage array. Data from an enhanced Tier 1 study (5 sample dates in 15 months) indicated considerable variability in sulfide levels both within and between dates.

## Introduction

The first industry-wide monitoring of fish farms in southwestern New Brunswick (SWNB) was conducted in 1991 and 1992, but mandatory annual monitoring of all farms did not start until 1995<sup>(1)</sup>. The monitoring program in 1995-2001 was based on video transects, sediment samples, and diver observations at each farm in late summer to early fall of each year. Each farm was given a qualitative rating of low, moderate, or high impact, based on the sea floor type (erosional vs. depositional), the percent silt/clay in sediment under the farm, the amount of bacterial mat coverage (*Beggiatoa* sp.) on the seafloor, the relative frequency of gas releases (hydrogen sulfide and methane) from the seafloor, and the diversity of benthic infauna and epifauna under the farm.

Research by Hargrave et al.<sup>(2)</sup> and Wildish et al.<sup>(3,4)</sup> led to a monitoring program with a more objective classification system, based on the redox potential and sulfide levels in sediments under cages. Redox potential results were subsequently found to be unreliable<sup>(5)</sup>. Since 2006, the ratings in the annual Environmental Monitoring Program (EMP) have been based on sulfide levels alone<sup>(6)</sup>.

The monitoring conducted prior to 2006 was intended to provide an indication of the general magnitude of organic enrichment, but did not provide an estimate of the area impacted. Starting in 2006, the EMP ratings were linked to the possible need for a Fisheries Act authorization (FAA) where there is the potential for a harmful alteration of fish habitat<sup>(6)</sup>. Sulfide levels  $>4500 \mu\text{M}$  are considered to be causing adverse benthic conditions, and will likely require an FAA. Sulfide levels of  $3000-4500 \mu\text{M}$  are likely causing adverse benthic conditions, and may require an FAA. The FAA grants authority to cause a harmful alteration of fish habitat, but requires the proponent to provide compensation for the degraded habitat. The amount of compensation is based on the area of degraded habitat; therefore, there is the need for an estimate of the spatial extent of impact.

Every farm is required to conduct annual monitoring between 1 August and 31 October (Tier 1). Standard Operating Practices describe the protocols for conducting the monitoring, including where to locate transects, how many transects are required, where to collect sediment samples for each transect, and how to analyze the samples. The Tier 1 monitoring locations in the 2007 protocols<sup>(7)</sup> were largely the same as those used since 2004, with some changes in the exact sample locations. There must be one transect per 100 000 fish, with a minimum of two transects per farm. At each transect, three sediment samples are to be taken at the cage edge, in close proximity to each other (Fig. 1). There are also protocols for collecting samples at farms with more complex currents and at deep water sites<sup>(7)</sup>.

A farm's EMP rating is based on the average sulfide values of all samples taken in the Tier 1 monitoring (Table 1). If the Tier 1 average sulfide level is  $>3000 \mu\text{M}$ , then Tier 2 monitoring must be conducted within 20 d. This Tier 2 monitoring has two purposes: to confirm the Tier 1 results and to



Blythe Chang

**Table 1.**  
Quantitative sediment geochemical ratings used since 2006 (see NBDENV 2006). OBMP = Operational Best Management Practices; FA = Fisheries Act (Canada); DFO = Fisheries and Oceans Canada; NBDENV = New Brunswick Department of Environment.

Site classification	Sediment sulfide level (µM)	Monitoring required and other actions
Oxic A	<750	Tier 1; follow OBMP
Oxic B	750 to 1500	Tier 1; follow OBMP
Hypoxic A	1500 to 3000	Tier 1; adjustments to OBMP
Hypoxic B	3000 to 4500	Tiers 1 & 2; additional OBMP; FA authorization may be required
Hypoxic C	4500 to 6000	Tiers 1, 2 & 3; enhanced OBMP; FA authorization likely required
Anoxic	>6000	Tiers 1, 2 & 3; consult NBDENV & DFO; FA authorization likely required

provide an estimate of the spatial extent of the impact. Tier 3 monitoring (which uses the same protocols as Tier 1) must be conducted in the following spring if the Tier 1 average sulfide level is >4500 µM, and additional Tier 2 monitoring must be conducted within 20 d if the Tier 3 average sulfide level is >3000 µM. Changes to Operational Best Management Practices may also be required, to improve a farm’s rating.

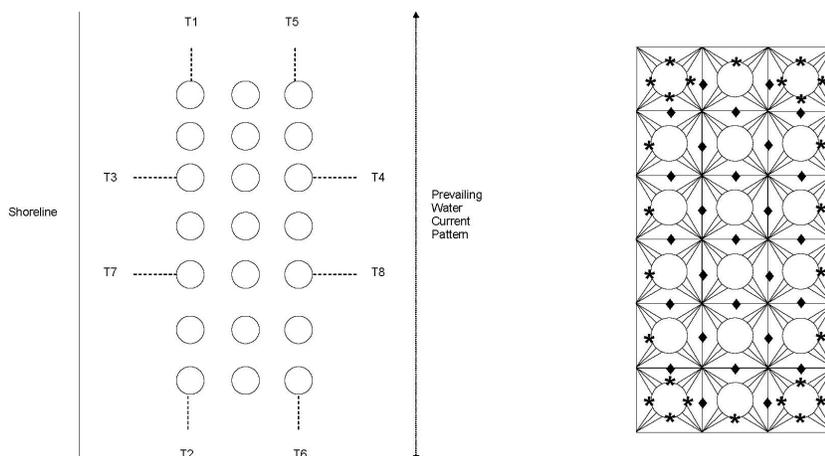
Tier 2 monitoring protocols in 2006 required only 5 sample locations: at the 4 corner cages and near the site centre. The Tier 2 monitoring protocols were changed in 2007 to require considerably more sample locations<sup>(7)</sup>, based on recommendations in Page et al.<sup>(8)</sup>: at 4 locations around each corner cage, at the outside edge of each outer cage in an array, and mid-way between each pair of cages, with triplicate samples at each location (Fig. 1). The intent was to provide an estimate of the area of impact at a spatial resolution scale of about 100 m.

The intent of this project was to conduct spatially intensive monitoring of sulfide levels in sediments under and around some salmon farms in SWNB. This data was used to determine the spatial domain of impact, for comparison with results from models such as DEPOMOD<sup>(9)</sup>; results of the model testing are presented in a separate report<sup>(10)</sup>. The data were also compared to results from the annual EMP conducted at farms. Some preliminary results from this project were previously reported<sup>(11)</sup>.

**Methods**

Sampling was conducted at 6 salmon farms (Sites A-F) between September 2005 and July 2007. Each farm was sampled once in summer (late July-September) and Site A was also sampled in the following spring. All farms had been stocked with Atlantic salmon (*Salmo salar*) smolts one or two years prior to sampling. All farms, except Site B, were actively feeding at the time of sampling; Site B had been harvested approximately 3 months prior to sampling, and had no fish on site. Site E was a farm consisting of two cage clusters; sampling was conducted at only one of the clusters. All farms used circular cages 100 m in circumference, except Site B, which used 70 m cages. The locations of individual cages were estimated, based on sample locations and site plans.

**Figure 1.**  
Protocol for locations of sampling for the Environmental Monitoring Program in 2007. Left: Tiers 1 and 3 (circles represent fish cages; dotted lines are transects; triplicate sediment samples are taken at the cage edge of each transect). Right: Tier 2 (circles represent fish cages; triplicate samples were taken at each location marked by \* and ♦). Reproduced from NBDENV<sup>(7)</sup>.



At each farm, samples were collected at 20-56 locations: within the cage array, at the outer edges of some cages, and at distances of approximately 25, 50, and 100 m from the edge of the cage array. Sample locations were recorded by GPS. In 2005, samples were collected using a 0.096 m<sup>2</sup> grab deployed from the 12.9 m CCGS Pandalus III. In 2006 and 2007, a 0.024 m<sup>2</sup> grab was deployed from the 7.8 m Vector. The grabs were designed with protective covers, to minimize disturbance to the sediment surface layer. From each grab sample, three spatially scattered 5-ml syringe samples of sediment were collected from the top 2 cm of sediment. Samples were stored on ice for transport to the laboratory, where they were stored in a refrigerator. All samples were analyzed for total sulfides within 2 d of sampling, using the method described by Wildish et al.<sup>(3,5)</sup>.

Sulfide values were log-transformed and normalized by subtracting from each value the mean of all values from the same farm, and dividing each difference by the standard deviation of all values from that farm. Because values were normalized to the mean and standard deviation of each farm, normalized values can only be compared within a farm; normalized values cannot be compared between farms (i.e. the normalized values from one farm do not represent the same actual values as the normalized values at another farm). The means of the normalized values for each sample location were mapped using MapInfo Professional (version 8.0) software. Contour plots of the normalized sulfide values were produced using the “triangulation with smoothing” routine within MapInfo Vertical Mapper (version 3.0), using the software’s default values. The mean and individual values were also plotted vs. the distance from the cage array. The edge of the cage array was defined by a line connecting the outside edges of all cages. Sample locations within the cage array or at the edge of the cage array were given a distance value of 0 m.

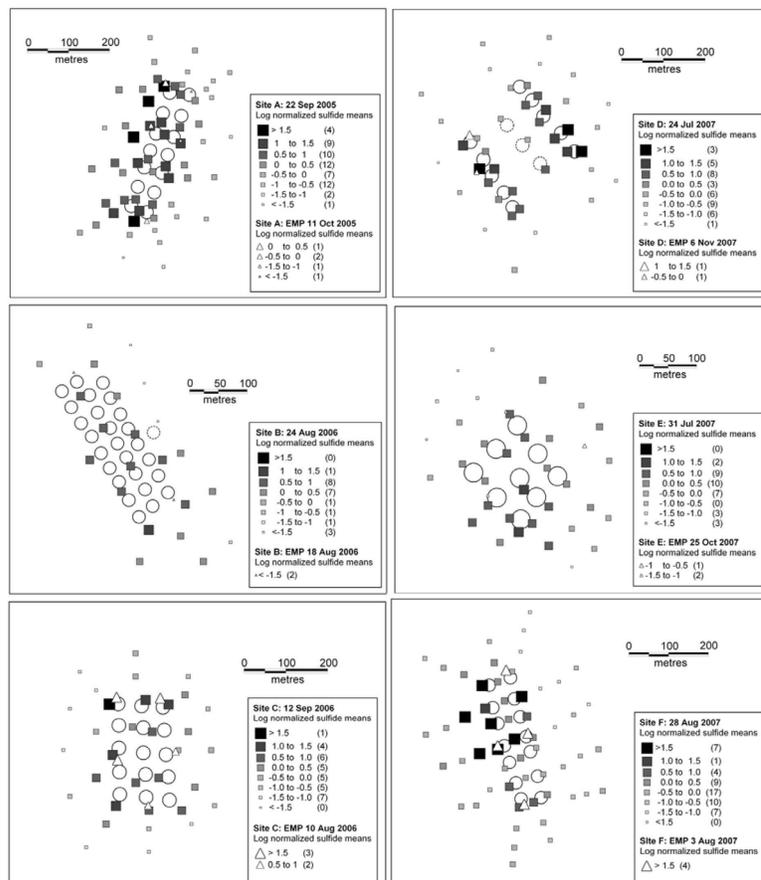
Data were also obtained from an enhanced EMP conducted at Site A. These data were collected by Sweeney International Management Corp., and were based on the Tier 1 protocols for this farm, plus one additional location at the outer edge of a cage, and one location 30 m away from that cage. The enhanced EMP data were collected on 5 dates between July 2005 and October 2006. These data were also log transformed and normalized relative to the means and standard deviations of all samples collected on the 5 dates.

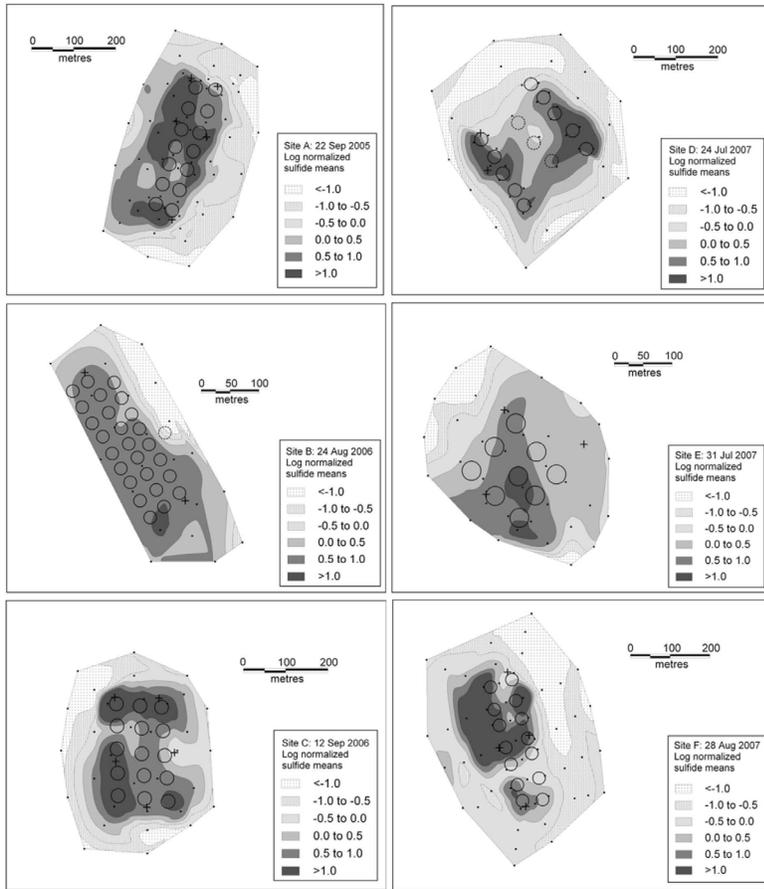
## Results

The results of the summer surveys at all 6 farms are shown in Figures 2-4. Figure 2 shows the sample locations and their mean sulfide values (log transformed and normalized), as well as the data from the EMP Tier 1 monitoring from the same year. Figure 3 shows contour plots derived from the normalized sulfide values. There was high spatial variability within the cage arrays, and the areas of highest sulfide levels occupied relatively small portions of the areas under the cages. At Sites A and F, some higher values occurred outside the cage array. There were fewer samples collected at Site B, especially outside the cage array, due to the presence of rock ledges.

Figure 4 shows the relationship between sulfide values (log transformed and normalized) and the distance from the cage array. Baseline data (from before the farm began operating) were only available for Site A, where the pre-farm sulfide values ranged from 46-265 µM, with a mean of 138 µM

**Figure 2.** Mean sulfide levels (log transformed and normalized within each farm) in summer sampling surveys at 6 salmon farms in SWNB. Black-grey squares represent mean sulfide values for the spatially intensive summer surveys. Open triangles represent mean sulfide values for the EMP Tier 1 monitoring in the same year. Circles represent cages (dotted circles represent locations of cages which had been removed).



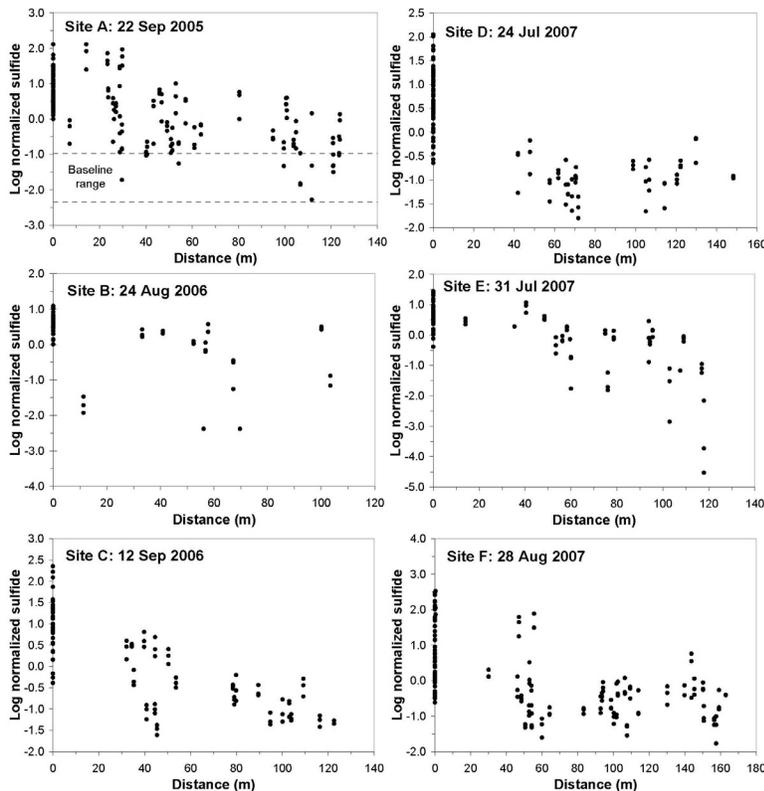


**Figure 3.** Contour plots of mean sulfide values (log transformed and normalized within each farm) in summer sampling surveys at 6 salmon farms in SWNB. Black dots indicate sampling locations for the spatially intensive summer surveys; crosses indicate locations of EMP Tier 1 monitoring. Circles represent cages (dotted circles represent locations of cages which had been removed).

(data collected in February 2001 by Dominator Environmental Diving Services, Saint John, NB). There was a general trend of decreasing sulfide values with increasing distance from the cage array, but there was considerable variability both between and within distances. At Site A, sulfide values were still mostly above the pre-farm baseline values at 125 m (the maximum distance sampled). At Sites C-F, the sulfide values appeared to level off at around 60-100 m. The limited data from Site B did not show clear trends in sulfide levels with distance from cages.

The number of sample locations for the EMP Tier 1 monitoring at these farms ranged from 2-5 locations (6-15 samples). The time difference between the EMP Tier 1 monitoring and our summer surveys ranged from 6-105 d. For Sites A, B, and E, the EMP sulfide values were significantly lower than our summer survey results (comparing samples taken within the cage array; t-test,  $p < 0.05$ ); the EMPs for these sites were conducted 19 d later than our sampling at Site A, 6 d earlier at Site B, and 86 d later at Site E. For Sites C and F, the EMP values were significantly higher than our summer survey results ( $p < 0.05$ ); the EMP at Site C was conducted 33 d earlier than our sampling, while at Site F the EMP was 25 d earlier. For Site D, the summer survey and EMP results were not significantly different ( $p > 0.05$ ); the EMP at this site was conducted 105 d later than our sampling.

Figure 5 shows the spring 2006 survey results for Site A. Sulfide values within the cage array in May 2006 were significantly lower than in September 2005 (t-test,  $p < 0.05$ ). In May 2006, most values had decreased to within the baseline range at about 90 m from the cage array. Both sampling dates showed high degrees of spatial variability, but the patches of high sulfides were larger in September 2005, and the locations of



**Figure 4.** Individual and mean sulfide levels (log transformed and normalized within each farm) vs. distance from cage area in spatially intensive summer sampling surveys at 6 salmon farms in SWNB. The dashed lines in the Site A graph represent the range of values from baseline samples taken before the farm began operations.

**Figure 5.**  
**Results from spatially intensive benthic sulfide sampling at Site A on 24 May 2006. Top: mean values (log transformed and normalized) for each sample location (black-grey squares); also shown are mean values for enhanced EMP monitoring on 14 June 2006 (open red squares). Middle: contour plot of sulfide values (black dots are sample locations in spatially intensive survey; crosses are enhanced EMP monitoring locations). Bottom: graph of sulfide values vs. distance from cage array.**

the patches changed somewhat between the two dates: both dates showed high sulfide values near the top of the cage array and in the right-central area, but the high sulfide patch at the bottom of the site in September 2005 was not seen in May 2006. The results of the enhanced EMP monitoring event on 14 June 2006 were not significantly different from the 24 May 2006 intensive survey results (comparing results from within the cage array; t-test,  $p > 0.05$ ).

The enhanced EMP conducted at Site A in 2005-2006 (Fig. 6) showed considerable variability in sulfide values both within and between dates. At most sample locations, sulfide values were higher in summer (June and July), compared to spring (March) or fall (October). However, there were some exceptions: locations T5 and 'C8 + 30 m' had lower mean values in July 2005 than in October 2005, and location T1 had a lower mean value in June 2006 than in March and October 2006.

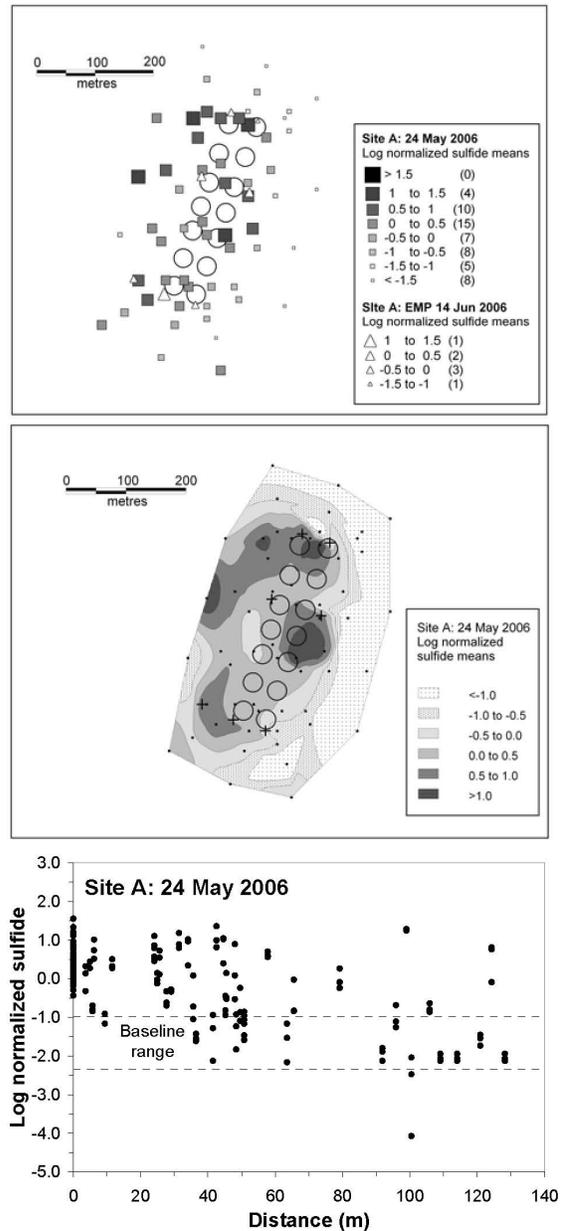
**Discussion**

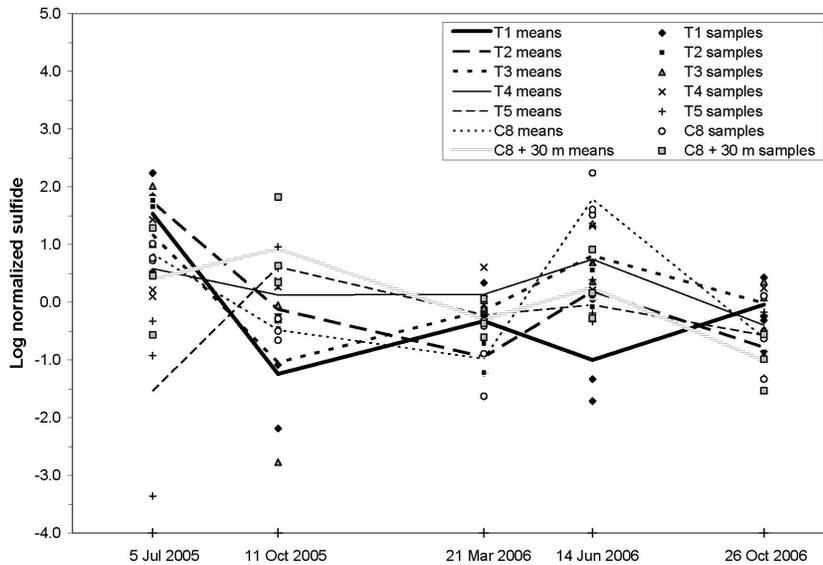
Currently, a farm's environmental rating is based on the average sulfide values for all samples collected in the EMP Tier 1 monitoring. The results of our spatially intensive sampling show that the distribution of sulfide values under salmon farms is highly variable. The areas with high sulfide values cover only a small portion of the total area under the farms, but at some farms may extend beyond the cage area. The limited number of sample locations in the Tier 1 EMP monitoring (triplicate samples from 2-5 locations at cage edges at our study sites) is not sufficient to describe this spatial heterogeneity, and therefore may not provide accurate environmental ratings.

There were often large differences between the values from our surveys and the values obtained in the EMP monitoring for the same farm and year. This could partly be due to the low number of EMP samples, which did not adequately capture the spatial heterogeneity (as described above). Another possible reason was the large difference in dates between our surveys and the EMP monitoring at some of the farms.

The Tier 2 monitoring protocols in 2006 had only 5 sample locations per farm, and would not provide an accurate estimation of the spatial extent of benthic impacts. The Tier 2 monitoring locations used since 2007, with considerably more sample locations (similar to the numbers of samples within the cage array in our intensive surveys), should more adequately describe the sulfide distribution within the cage area at a resolution of about 100 m, but would miss any high sulfide areas that are directly under cages or that occur outside the cage array, since there are no Tier 2 sample locations in those locations.

The results obtained in the enhanced EMP monitoring conducted at Site A showed considerable temporal variability in sulfide values. This suggests that the differences between our survey results and the EMP results may be at least partly due to temporal variability. Currently, Tier 1 monitoring can be conducted between 1 August and 31 October. The enhanced EMP data suggest that farms monitored earlier during this period may be more likely to have high values, compared to farms monitored later in the season. In order to further examine this issue, we recommend conducting a research project in which sampling is done on a weekly or daily basis during August-October at a few farms.





**Figure 6.** Individual (points) and mean (lines) sulfide levels for enhanced EMP monitoring conducted at Site A. Values have been log transformed and normalized using the mean and standard deviation of all values (all dates). Locations T1-T5 (EMP Tier 1 locations) and C8 are on the outer edge of the cage array; location 'C8 + 30 m' is located 30 m from C8 (away from the cage array). Data collected by Sweeney International Management Corp.

The dynamics controlling the spatial and temporal variations in sulfide levels in sediments are not well understood. Some of the spatial and temporal heterogeneity within farms may be due to differences in the numbers of fish and the amount of feed added at different cages at the farm. Oceanographic currents and seafloor topography are also likely to be important factors. Further analysis of the EMP results, in relation to production, feeding, and oceanographic data, may help to explain these differences.

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# Anaerobic Digestion of Aquaculture Waste

S Fraser<sup>1</sup>, MF Couturier<sup>1</sup>, J Conroy<sup>1\*</sup>, KS Singh<sup>1</sup>, and A Desbarats<sup>2</sup>

<sup>1</sup>Recirculating Aquaculture Research Group, Department of Chemical Engineering, University of New Brunswick, PO Box 4400, Fredericton, NB,

<sup>2</sup>E3B 5A3, Canada, Tel.: (506) 453-4690, Fax: (506) 453-3591, E-mail: cout@unb.ca

<sup>2</sup>Atlantech Companies, 89 Hillstrom Avenue, Charlottetown, PEI, C1E 8C3, Canada

A pilot-scale, two-phase anaerobic digester was used to treat fish waste from a land-based recirculating aquaculture farm producing salmon smolts. The acidogenesis step occurred in a feed tank at a low pH (5.5-6.5) whereas the methanogenesis step occurred in the anaerobic reactor at a high pH (7.6-8.0). The unusually high pH in the anaerobic reactor was due to the high nitrogen content of the waste solids (3-10 wt%) which got converted to ammonia in the reactor (2000-3000 mg/L). The high pH in the digester forced the precipitation of dissolved phosphorus whose concentration went from 200-800 mg/L in the feed tank to 30 mg/L in the digester effluent. Over 60% of the total chemical oxygen demand (TCOD) fed to the reactor was consistently converted to methane and carbon dioxide, yielding an effluent gas with a CH<sub>4</sub> content between 52 and 63 vol%.

## Introduction

Land-based aquaculture operations generate on a dry basis between 0.2 and 0.3 kg of dry fish manure/kg feed. Disposal of this sludge can be a significant operating expense. Due to the high nitrogen content of the aquaculture waste solids, anaerobic digestion can be inhibited by the formation of ammonia. Freshwater sludge with a total solids (TS) concentration less than 3 wt% have been successfully treated anaerobically<sup>(1-3)</sup> but attempts to treat more concentrated sludge (4-12 wt%) resulted in digester failure<sup>(1,4)</sup>. The strong inhibition observed with the more concentrated sludge was attributed to high ammonium concentrations<sup>(4)</sup>. Anaerobic digestion is an attractive stabilization method for aquaculture waste, however, the operation of anaerobic digesters at sludge concentrations less than 3 wt% may not be economical<sup>(4)</sup>. The purpose of the present study is to determine whether a two-phase anaerobic digester can be used to overcome inhibition problems observed with concentrated aquaculture sludge in simple CSTR digesters.



Jessica Conroy

## Materials and Methods

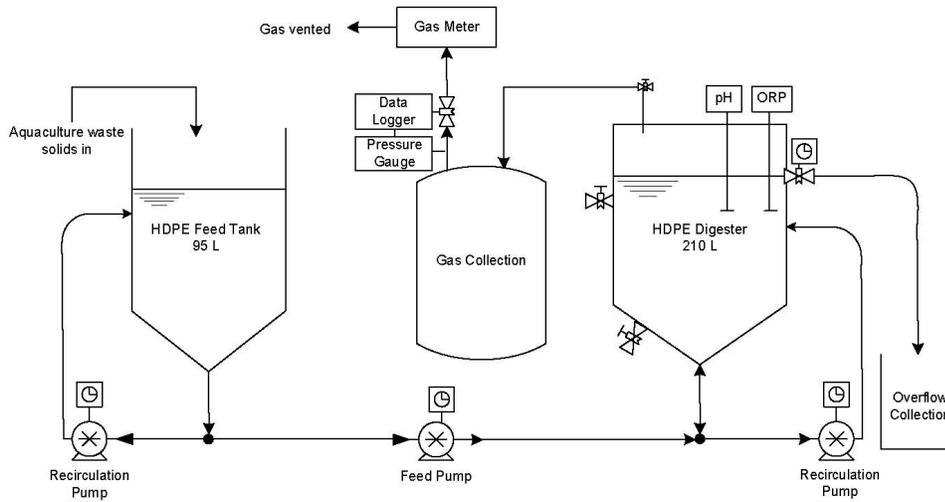
The system consisted of a feed tank for holding the waste solids and an anaerobic digester (Fig. 1). The temperature in the digester was kept constant at 31°C by controlling the power to the electrical heating tape wrapped around the digester with an on-off controller. Probes inserted through the cover of the digester were used to monitor temperature, pH and ORP. The content of the digester tank was mixed using an automated recirculation pump. Biogas produced in the digester accumulated in the headspace of the digester and in a gas collection vessel. Gas pressure within the digester was monitored and recorded every 15s using a data logger. When the pressure reached 2.5 kPa, an automated valve opened allowing gas to vent through a gas meter.

Collected solids were mixed with water to achieve a total solids concentration of 3-10 wt%. This mixture was stored in the feed tank and a portion of this mixture was fed daily into the digester using an automated pump. As solids were fed into the bottom of the digester displaced liquid exited through an automated overflow valve.

Samples were collected twice weekly and were stored in sealed plastic containers, refrigerated overnight and analyzed the next day. Samples were analyzed for TS, volatile solids (VS), TCOD, soluble COD (SCOD), ammonia nitrogen, dissolved phosphorus and volatile fatty acids (VFA). Biogas was collected using a gas storage bag. The daily volume of biogas produced was measured using a gas meter and also calculated from the recorded pressure rise profiles.

## Results and Discussion

For the first 200 days of operation, the ammonia-nitrogen concentration in the reactor remained at about 2200 mgNH<sub>3</sub>-N/L (Fig. 2) and the reactor was very stable as indicated by the low VFA concentrations (~300 mg/L). Approximately 61% of the TCOD and 63% of the VS were removed in the anaerobic digester. At its highest rate, the digester produced 200 L of gas per day at around 58% methane and 38% carbon dioxide.



**Figure 1.**  
Schematic of pilot-scale anaerobic digester system.

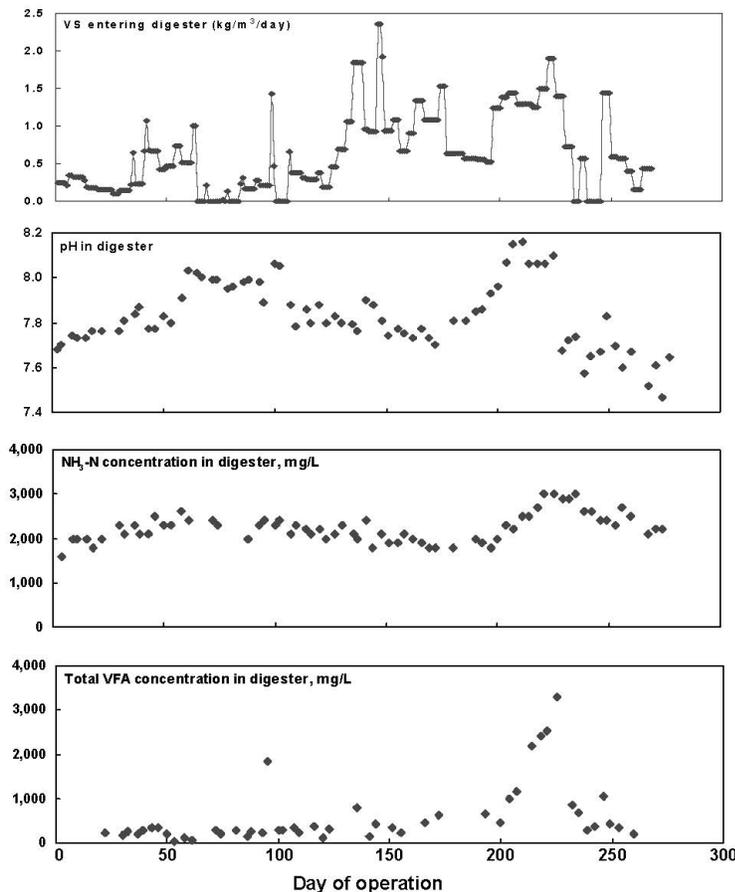
Around day 190, the feed rate was increased from 0.6 to 1.3 kg VS/m<sup>3</sup>/day and was kept at that level for about a month. During that period, the ammonia concentration increased to about 3000 mg NH<sub>3</sub>-N/L and the pH in the reactor rose to 8.2. This created an upset in the reactor and the VFA concentration rose above 3000 mg/L (Fig. 2). Inhibition has been reported to occur at ammonium concentrations of 2000-3000 mg/L and at free ammonia concentrations of about 100 mg/L<sup>(4)</sup>.

The fraction of total ammonia nitrogen (TAN) present as undissociated ammonia is a function of pH and to prevent undissociated ammonia concentrations from reaching toxic levels, it is important

to keep the pH below 8. Reactor failure was avoided by reducing the flowrate and solids content of the feed or by adding small amounts of hydrochloric acid to the feed and reactor. The first strategy was used around day 60 and again around day 230. The second strategy was used around day 100. The VFA produced during hydrolysis kept the pH in the feed tank around 5.5 and thereby provided a good environment for the leaching of phosphorus. The dissolved phosphorus concentration was high in the feed tank (200-800 mg/L) but decreased to about 30 mg/L in the digester.

**Conclusions**

The two-phase anaerobic digester was able to successfully treat fish waste solids with a TS content between 4 and 11 wt%. The reduction in the VS and TCOD fed to the digester was more than 60% and the overall methane yield was 0.14 m<sup>3</sup> CH<sub>4</sub>/kg TCOD removed. The reactor was stable despite the high pH and TAN concentrations in the digester but became unstable when the TAN concentration exceeded 3000 mg/L. The low pH conditions in the feed tank promoted the leaching of phosphorus from the solids whereas the high pH conditions in the digester forced the dissolved phosphorus to precipitate.



**Figure 2.**  
Digester performance.

## Acknowledgments

This work was funded by the Atlantic Innovation Fund and was performed in partnership with Cooke Aquaculture Inc. and Atlantech Engineering & Associates.

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# Phosphorus Leaching During the Hydrolysis of Fish Waste Solids

Jessica Conroy\* and Michel F Couturier

Recirculating Aquaculture Research Group, Department of Chemical Engineering, University of New Brunswick, PO Box 4400, Fredericton, NB, E3B 5A3, Canada, Tel.: (506) 453-4690, Fax: (506) 453-3591, E-mail: cout@unb.ca

In order to determine how phosphorus goes into solution, the hydrolysis of fish waste solids was investigated at room temperature. It was found that as the pH dropped due to the production of volatile fatty acids, the concentration of dissolved phosphorus increased. Within the range of solids concentrations tested (0.5 wt.% - 9.02 wt.%), solids content had little effect on dissolved phosphorus concentration. The effect of pH on dissolved phosphorus concentration is well described by an equilibrium model based on the solubility of  $\text{CaHPO}_4$ .



Jessica Conroy

## Introduction

Waste solids produced in recirculating aquaculture systems are typically collected in swirl separators and drum filters before being pumped to storage in off-line settling basins. If the swirl separators and basins are not frequently emptied, anaerobic conditions favorable to hydrolysis of the solids can quickly get established within the solids that settle in these units. Hydrolysis reduces water quality when it occurs within the recirculation loop of an aquaculture farm and increases water pollution when the overflow of off-line storage basins is discharged to surface water. The objective of this study was to determine the mechanism by which phosphorus is solubilized during the hydrolysis of fish waste.

## Materials and Methods

Several bench top experiments were performed to determine what factors cause phosphorus to leach from the waste solids into solution. These experiments consisted of placing a certain concentration of solids in a closed container and allowing them to stand for several days in order for hydrolysis to take place. Dissolved oxygen and pH were measured over time and water samples were taken at different times and analyzed for dissolved phosphorus, calcium, magnesium and potassium.

## Results and Discussion

Figure 1 shows the effect of pH on dissolved phosphorus concentration for different solids concentrations. As the pH dropped below 6.5 due to the generation of volatile fatty acids (VFA), the concentration of phosphorus increased rapidly. The solids concentration had little effect on the dissolved phosphorus concentration. As a result, the fraction of the solid phosphorus which was solubilized increased with decreasing solids concentration.

The dissolution of phosphorus was accompanied by the solubilization of several cations. As pH dropped from 7.8 to 5.5, the magnesium concentration increased from approximately 30 mg/L to 100 mg/L, the potassium concentration increased from approximately 15 mg/L to 30 mg/L, while the calcium concentration increased from about 100 mg/L to 800 mg/L. Since calcium saw the greatest increase in concentration, it is likely that the phosphorus in solution originated from calcium orthophosphate compounds. The dissolved concentration of phosphorus at equilibrium must thus be dictated by the dissociation reactions for phosphoric acid and the solubility of Whitlockite and calcium hydrogen phosphate.

According to the phase diagram in Figure 2(b), the only stable solid above pH 7 is Whitlockite,  $\text{Ca}_3(\text{PO}_4)_2$ . If the pH drops and crosses the boundary shown in the phase diagram, all of the  $\text{Ca}_3(\text{PO}_4)_2$  will be converted to  $\text{CaHPO}_4$  and the concentration of  $\text{Ca}^{2+}$  in solution will increase according to the following reaction:



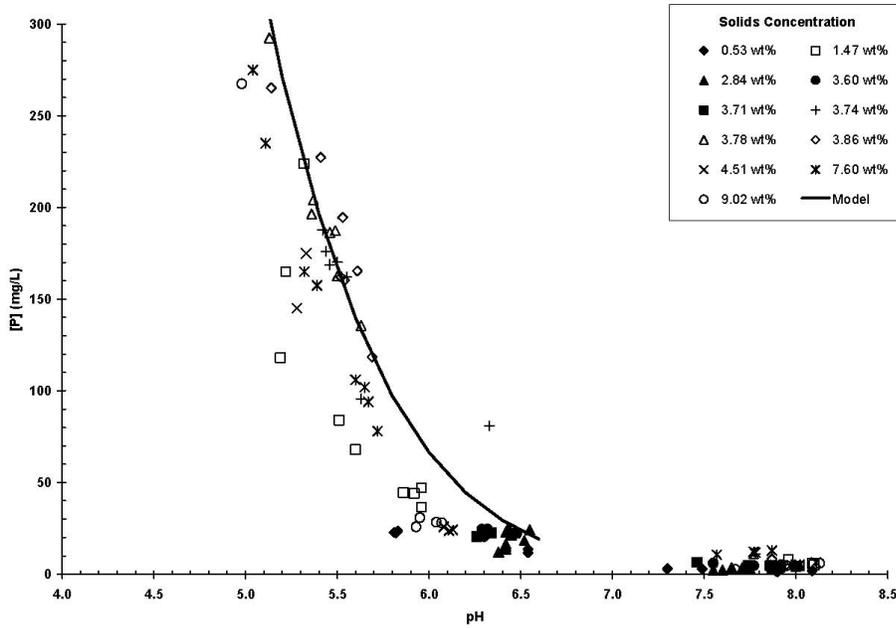
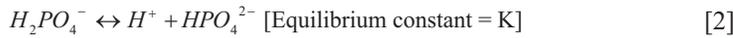


Figure 1. Effect of pH on phosphorus concentration.

Once  $\text{CaHPO}_4$  becomes the only stable solids compound, the effect of pH on the total molar concentration of phosphorus in solution,  $[P]$ , can be obtained from the following equilibrium equations:



Since the min phosphate species in solution over the pH range 5 - 6.5 is  $\text{H}_2\text{PO}_4^-$  (Fig. 2 (a)),  $[P] = [\text{H}_2\text{PO}_4^-]$ . If the initial phosphorus in solution is ignored, a mass balance on the dissolved  $\text{CaHPO}_4$  gives:

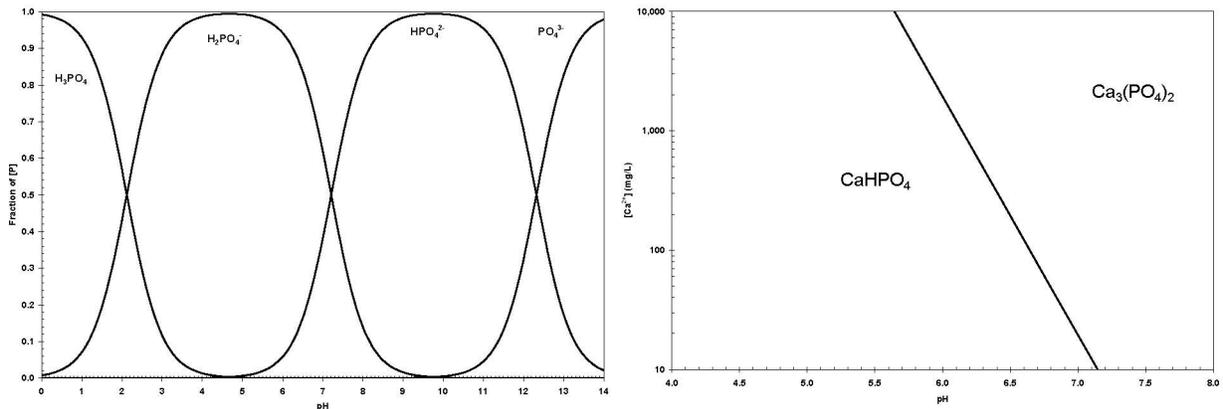
$$[\text{Ca}^{2+}] = [\text{Ca}^{2+}]_i + [P] \quad [4]$$

where  $[\text{Ca}^{2+}]_i$  = concentration of  $\text{Ca}^{2+}$  in solution after the conversion of  $\text{Ca}_3(\text{PO}_4)_2$  to  $\text{CaHPO}_4$ , mol/L.

Using equations 2, 3 and 4, the following equation can be derived for predicting the concentration of phosphorus in solution.

$$[\text{Ca}^{2+}]_i [P] + [P]^2 = \frac{K_{sp}}{K} \times \frac{10^{-pH}}{\gamma_{\text{Ca}^{2+}} \gamma_{\text{H}_2\text{PO}_4^-}} \quad [5]$$

Figure 2. Distribution diagrams for (below, left) phosphate species in solution and (below, right) calcium orthophosphate solids.



where  $\gamma_{Ca^{2+}}$  and  $\gamma_{H_2PO_4^-}$  are activity coefficients.

Using  $[Ca^{2+}]_i = 0.01$  mol/L,  $\gamma_{Ca^{2+}} = 0.32$  and  $\gamma_{H_2PO_4^-} = 0.75^{(2)}$  and published values for  $K$  and  $K_{sp}^{(1)}$ , equation 4 was plotted with the dissolved phosphorus data (Fig. 1). There is good agreement between the model and the data which confirms the hypothesis that calcium orthophosphate compounds were the main source of dissolved phosphorus.

## Conclusions

As the pH drops due to the generation of VFA during the hydrolysis of fish waste solids, the dissolved phosphorus, calcium and magnesium concentrations increase. The effect of pH on dissolved phosphorus concentration is well described by an equilibrium model based on the solubility of  $CaHPO_4$ .

## Acknowledgments

This work was done in partnership with Atlantech Engineering & Associates and Cooke Aquaculture Inc. We are grateful for the funding received from the Atlantic Canada Opportunities Agency under the Atlantic Innovation Fund program. We would also like to thank Dr. Dennis Connor for his help with the analysis of the water samples.

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# Preservation of Lipid Content in Microalgae Concentrates from Ultrafiltration Process

Diadié Diouf<sup>1</sup>, Réjean Tremblay<sup>1</sup>, Réal Fournier<sup>1</sup>, and Fabrice Pernet<sup>2</sup>

<sup>1</sup> Institut des Sciences de la Mer, Université du Québec à Rimouski, 310 allées des Ursulines Rimouski, QC, Canada, G5L 3A1 (Correspondence:

Tel: 418-724-1650, fax: 418-724-1842, email: diadiediouf@hotmail.com)

<sup>2</sup> Laboratoire Environnement Ressources en Languedoc-Roussillon, Pôle "Mer et Lagunes", Cedex Sète, IFREMER Sète, France 34203

Cultured microalgae from Eustigmatophyceae (*Nannochloropsis oculata*.), Prymnesiophyceae (*Pavlova lutherii* and *Isocrysis galbana*) and a cocktail of these three species was used to produce concentrates which were preserved and assessed by monitoring the lipid content during a eight week storage at 2°C. Experiment was conducted at the aquatic station of the marine science institute of the University of Quebec in Rimouski. Microalgae were cultured in 260 L photobioreactor using a continuous artificial light. After harvesting, they were concentrated by ultrafiltration and transferred in four polyethylene flasks (two preserved and two controls) and stored at 2°C. Sampling for lipid analysis was then conducted each week. Results showed that lipid class fatty acids (TAG, Sterol, polar lipids) did not vary significantly between the preserved concentrate and the control for all species. Concerning the fatty acids, the highly unsaturated fatty acids EPA, DHA and AA were well preserved for almost all species with the highest preservation rates attributed to the concentrate of mixed algae. Hence for the latter, preservation rate of EPA, DHA and AA were respectively 87.3%, 69% and 88.5%. For the sum of fatty acids, PUFA exhibited a good preservation rate of 85.2% while total saturated and MUFA increased respectively of 14.1% and 8.1%.

## Introduction

Due to their position at the base of the aquatic food chain, microalgae play a vital role in aquaculture where their main applications are related to nutrition. They are essential food source in the rearing of all stages of marine bivalve molluscs (oyster, clams, and scallops), the larval stage of some marine gastropods (abalone, conch), larvae of several marine finfish and penaeid shrimp and zooplankton.

The nutrient properties of the algae are critical for the growth and survival of larvae and adults<sup>(1)</sup> and lipids mainly polyunsaturated fatty acids PUFAs are reported to be essential for the growth and survival of larvae. According to Whyte et al.<sup>(2)</sup>, the fatty acids C16:0, C18:0, C18:1 (n-7), C20:5 (n-3) and C22:6 (n-3) are accumulated by larvae of the rock scallop *Crassadoma gigantea* indicating a possible requirement for these acids during larval phase.

The mass production of microalgae is a critical and often limiting step for hatchery operations. The need to dispose of quality and safe live microalgae at the right time is a real concern to aquafarmers who need to focus mainly on larval rearing tasks. In this regard, microalgae concentrates appear to be the solution in that they can be processed securely in centralised algal production facilities and supplied to hatcheries. However, lipid content is highly variable in time and the necessity to maintain nutritional value of algal feed has prompted to development and assessment of preservation methods in order to extend significantly the time storage of microalgae concentrates.



Diadié Diouf

## Material and methods

### Microalgal culture

Algae were cultured using semi-continuous system in 260L photobioreactors provided by NutrOcean Company at the university aquatic station of Pointe-Aux-Pères in Rimouski. Water is pumped from the Saint-Lawrence river and treated by filtration through gravel and filter bags (10µm, 1µm) followed by a sterilisation using ultrafiltration method. Guillard's medium F/2<sup>(3)</sup> was used for algal growth and photobioreactors were illuminated continuously with artificial fluorescent light. Culture vessels were equipped with a filtered air system and CO<sub>2</sub> was supplied when needed for pH control.

### Concentration

When cultures reached late exponential growth phase, they were harvested and transferred to a sterile stocking tank before concentration. Concentration was implemented by ultrafiltration, a

membrane sterilisation process which utilizes hydrostatic pressure to force seawater through a semi-permeable membrane. This process permits good water removal with a concentration factor of 200-300 allowing a final concentration of 1.5-5 billion cells/ml.

### Preservation

Once microalgae concentrates were made, they were dispatched in four plastic flasks of which two were preserved and two as control and stored at 2°C for lipid monitoring. Both of them were shaken and opened everyday to simulate daily utilization in hatcheries. Preservation was performed using a combination of two food acids (ascorbic and citric).

### Sampling and analysis

We performed a weekly sampling including cell counting using Z2 Counter Coulter, pH measure and lipid sampling for chemical analysis. Lipid classes were analysed by mean of Iatroskan MK6 TLC-FID and fatty acids by gas chromatography coupled with a mass spectrometry. Prior to analysis, methylesters were obtained using 2% H<sub>2</sub>SO<sub>4</sub> in MeOH and purified in silica columns to remove sterols. Results were analysed by non parametric Kolmogorov-Smirnov associated with a Poisson's distribution test.

### Results

Results are expressed as percentage of total µg/million cells and mean value of each treatment are presented. Lipid class did not show any significant difference between treatments ( $p > 0.05$ ) for all the species. Value of TAG comprises between 10% for *Isochrysis galbana* and 30% for the mix for preserved algae and between 9% and 30.2% for control. For Polar lipids, PL mean value of preserved concentrates range was 19.6% for *Pavlova lutherii* and 38.3% for mix. However, value of control concentrates of Pavlova was much higher than that of preserved algae. AMPL had almost the same tendency as TAG and value of the two treatments did not exhibit any significant difference. For example values for *Nannochloropsis oculata* were the highest and were 38.6% for preserved and 33.8% for control. Values of Sterol were very low for almost all species except *P. lutherii* of which Sterol represented 16.4% for preserved algae and 12.8% for control.

On the other hand results of fatty acids are presented as mean values of the two treatments as no significant difference were observed between them. Therefore mean values of each specie concentrate are presented as a function of storage duration.

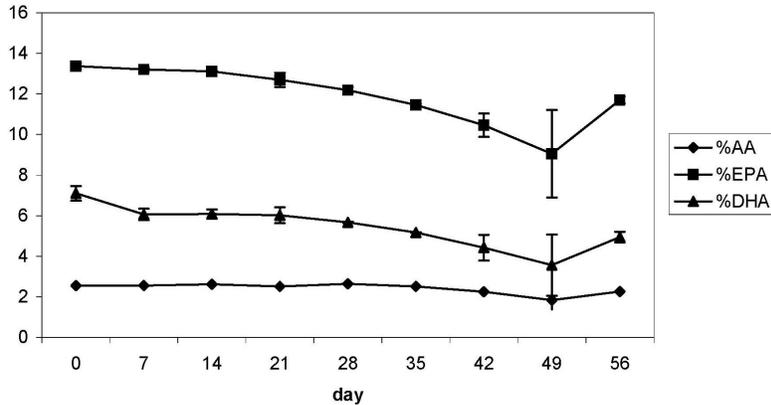
As for lipid class, fatty acids of both concentrates did not display any significant difference during the time length of the storage. Thus *I. galbana* concentrate showed a quite constant profile for arachidonic acid (AA) and eicosapentaenoic acid (EPA) and a docosahexaenoic acid (DHA) profile with slight variations between days 28-49.

For this species amount of AA preserved represented more than 80% and EPA 100%. Saturated and monounsaturated fatty acids increased respectively of 35.8% and 7.6% whereas polyunsaturated fatty acids (PUFA) preservation rate were 73%.

As for *P. lutherii*, DHA and EPA both displayed slight variations with time and preserved



**Figure 1.**  
Concentrated (left) vs. freshly harvested  
microalgae (right).



**Figure 2.**  
Evolution of essential fatty acids content of microalgal mixed concentrate during storage time.

amounts represented respectively 72.4% and 50.8%. Amounts of Saturated and MUFA increased of respectively of 13.6% and 19% and preserved PUFA was 63%.

For *N. occulata*, EPA presented a preservation rate of 77.4% and AA 74.5% whereas PUFA and saturated were preserved respectively to 79.3% and 84.9%. At the same time, MUFA increased of +58.6%.

The cocktail of microalgal concentrate (mix) exhibited the best lipid profile in that preservation rate of EPA, DHA and AA were respectively 87.3%, 69% and 88.5%. Moreover total saturated and MUFA increased respectively of 14.1% and 8.1% while PUFA demonstrated a good preservation rate of 85.2%.

## Discussion and conclusion

This study emphasizes the ability for live algae concentrates to maintain lipid content after long time storage. Microalgae offer excellent nutritional value for animals reared in aquaculture. As a whole, nutritional properties of the algae have been preserved at a very good rate for all the species. Effects of treatments have been found insignificant as well as that of time length.

According to Watson et al.<sup>(4)</sup> nutritional value of *Thalassiosira pseudonana* and *Chaetoceros calcitrans* treated with centrifugation process, declined at an unpredictable rate when stored at 4°C. Moreover Donaldson<sup>(5)</sup>, found that the nutritional value of centrifuged algal pastes fell rapidly on storage at 4°C with a shelf-life of only 10 days.

In this regard, results of this study are of great interest in the way that they can allow hatchery farmers to get rid of technical constraints and heavy costs of on-site algal production. Processed concentrates of high nutritional value would therefore be available at any time no matter distance and season.

## Acknowledgements

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# Challenges to Applying Eco-based Research – Analysis of a Two-Year Research Program in the Broughton Archipelago, British Columbia

Jon O’Riordan

*Research Director, BC Pacific Salmon Forum*

Public opinion for salmon aquaculture in British Columbia is highly polarized. The heart of the controversy is the conflicting science regarding the impact of fish farms on wild salmon in the Broughton Archipelago situated at the north end of Vancouver Island. Over the past five years there have been over 100 articles in various scientific journals and government reports examining the impacts of sea lice infestations on out migrating pink and chum salmon smolts in the Archipelago. This science is inconclusive, with some scientists reporting that if current trends continue unchanged, there could be extirpation of natural wild salmon populations in the area within the next eight years due to lice infestations. Other scientists report that populations of pink and chum have fluctuated widely over the past 40 years and that the most recent monitoring of fall escapements and spring out migration indicate a slight upward trend.

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The BC Pacific Salmon Forum was established in 2004 by the Premier of British Columbia to undertake a comprehensive research program for sustaining wild salmon in the Province. In view of the controversy in the Broughton, the Forum decided to mount a two year research program involving a range of science disciplines to provide a more substantive knowledge base for policy development in the Broughton in the future. The research was based on ecosystem science principles which have been supported by both the Federal Government<sup>(1)</sup> and the Provincial government. The key feature of the eco system based approach is the development of two models that are designed to analyze complex data on a whole systems basis.

Specifically, the objectives of the research program are designed to answer the following questions:

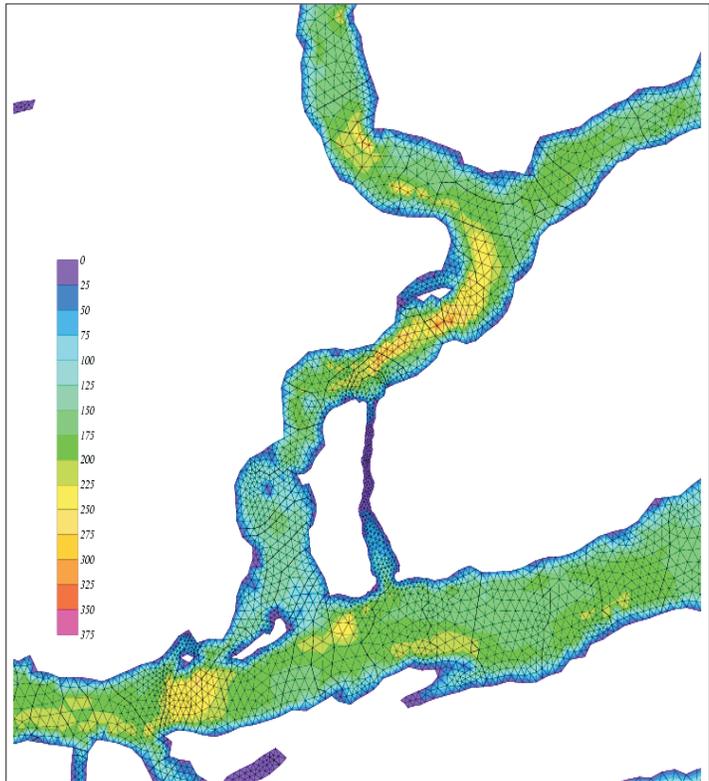
- Do lice populations on farmed infect wild salmon?
- Are lice loads on individual wild fish impacting their health and by extension the health of wild fish populations?
- What is the potential effect of adaptive management measures by farms to reduce risks of sea lice infection?
- Can an ecosystem-based model be used to monitor future farmed fish management regimes?

An oceanographic dynamic model has been developed by the Institute of Ocean Sciences, DFO and has become more sophisticated with the improved data inputs. With additional funding available from the Forum, the scientists have developed the model to account for wind forcing, improved modeling of water discharges into the Broughton, vertical and horizontal movement of lice, heat exchanges between the surface waters and the atmosphere and sub surface water current flows (Fig. 1). The model will be used to predict how lice move from point sources such as farms or natural sources and where they will travel during their development stages. The model can also be ‘back-tracked’ to evaluate where lice monitored on wild salmon may have originated. Scientists have measured lice populations on both farms and wild salmon in the Broughton in some detail over the past two years and the model will be run for 2007 and 2008 conditions later this year.

The second model has been developed by a team from the University of Alberta<sup>(2)</sup>. It is based on mathematical principle of infection dynamics and applies a ‘best fit’ set of mathematical calculations to determine if observed monitoring of lice on wild salmon is due to natural sources of lice dispersed in the area, or from point sources such as salmon farms or a combination of sources (Fig. 2). Based on observations undertaken prior to the Forum funded research in 2007 the hypothesis that lice originated from point sources fit the model best. However in the past two years the numbers of lice on both farmed and wild fish have dropped and so this hypothesis will be re-tested.

It is not yet clear why the lice populations have dropped. The farms have fallowed some of their locations on migratory routes in the spring and have applied the anti-parasitic chemotherapeutant SLICE on the farms during the winter months in advance of normal lice population increase on farmed salmon. However, the research has also indicated that resident sticklebacks in the Broughton have large populations of lice on them during the winter. The populations of

**Figure 1.**  
**Oceanographic dynamic model to account for wind forcing, improved modeling of water discharges into the Broughton, vertical and horizontal movement of lice, heat exchanges between the surface waters and the atmosphere and sub surface water current flows (Institute of Ocean Sciences, DFO).**



sticklebacks in 2007 were far greater than the population monitored in 2008.

Another key factor in the research is to assess the impacts of lice loads on wild smolts. This research involves analysis of their swimming abilities as well as the effects of lice on individual fish through a physiological model designed to calibrate fish health with survival and fitness to determine the threshold when fish become compromised by sea lice. Preliminary results indicate that if the intensity of lice is less than one per fish there is little or no effect on swimming ability and that the wild fish naturally shed the lice within 14 days. The prevalence of sea lice on wild fish in 2007 averaged about 20 percent though there were higher counts near to some farms, and the lice intensity ranged between 1.0 and 1.3 lice per fish or 0.24 to 0.81 per gram of fish<sup>(3)</sup>. Laboratory studies have shown that fish in controlled environments can sustain much higher intensities of sea lice without affecting their survival<sup>(4)</sup>. Previous research has indicated that effects on young salmon can occur with one fish per gram intensity<sup>(5)</sup>. The Forum funded research hopes to be able to clarify the differences in these research reports by comparing laboratory analysis with field research.

There are many challenges to undertaking eco-system based research in the Broughton. These include:

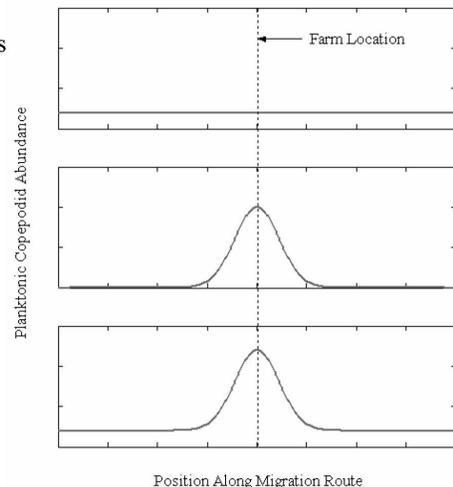
- Wide range of natural variability in wild salmon populations some of which appears to be linked to regional shifts in ocean productivity
- No scientifically substantiated linkage on source of lice on wild fish
- New information of lice loads on stickleback
- No confirmed threshold lice intensity level where wild fish are impacted at the population level
- Effects of changing operational practices by the farms to reduce lice populations on farmed fish

**Figure 2.**  
**Model based on the mathematical principle of infection dynamics to determine if observed monitoring of lice on wild salmon is due to natural sources of lice dispersed in the area or from point sources such as salmon farms or a combination of sources<sup>(2)</sup>.**

H1: all lice originate from salmon farms

H0: all lice originate from natural sources (farms have no effect)

H2: lice originate from natural and farm sources



Recently the two main operating farms in the Broughton – Marine Harvest Canada and Mainstream Canada – have developed a coordinated area management plan (CAMP) to follow each of the main migration routes for wild smolts in alternate years. The models developed for the Forum research project can be used to monitor the results of this new approach over the next several years under a variety of environmental conditions and should be able to assess the effectiveness of CAMP to reduce the risk of lice transmission from farms to wild fish. However the plan will require regulatory approvals for amendments to existing aquaculture licenses from the Provincial government as the companies will be required to move fish between sites to maintain net production levels across all farms in the Broughton. Under provincial policy such amendments require extensive consultation with First Nations in the Broughton to ensure that there is no infringement on traditional rights and this process can take time. Because of timing to implement CAMP in advance of spring 2009, such consultations and approvals need to be completed within a month to enable the plan to take effect in 2009.

In summary, the Forum research project has involved a team of interdisciplinary scientists who have undertaken an integrated approach to eco-system based research. The application of two models will enable the results of individual projects to be integrated to address the questions set out in the research project objectives as noted above. However the application of eco-system based research has its challenges—it is costly, requires a team of scientists to determine input data for running the model, which requires many days of computer time, and the results need to be communicated in clear language so that local interests can understand the results. This is a divergence from the normal communication of science in peer reviewed scientific journals on an individual project basis. Finally, government regulatory and consultation processes need to be more responsive to eco-system based research results such that adaptive management measures can be undertaken in a timely manner.

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# The Assessment of Impacts on the Benthic Environment from Suspended Oyster Aquaculture in Baynes Sound, British Columbia, Canada

WA Heath<sup>1\*</sup>, S Carroll<sup>2</sup>, R Devos<sup>2</sup>, and B Provan<sup>2</sup>

<sup>1</sup> BC Ministry of Agriculture and Lands, 2500 Cliffe Avenue, Courtenay, BC, V9N 5M6, Canada (Tel.: 250-897-7548, Fax: 250-334-1410, E-mail: Bill.Heath@gov.bc.ca)

<sup>2</sup> Environmental Technology Program, Camosun College, Lansdowne Campus, 3100 Foul Bay Rd., Victoria, BC, V8P 5J2, Canada.

A survey was conducted in Baynes Sound, BC, an important shellfish aquaculture area, to assess potential benthic impacts of suspended culture shellfish farms. Benthic grab samples and underwater video images were collected at oyster longline and raft farms and reference sites. Sediment samples were analysed for pH, porosity, sediment grain sizes, percent organic carbon and percent carbonates. Normalized data were analysed by multivariate methods of clustering and Principal Components Analysis. Some differences between conditions at shellfish farms and reference sites were noted, although benthic sediment conditions were in the normal, oxic classification. The main benthic impact observed at oyster longline and raft sites was an increase in fish habitat complexity, related to introduction of shell material to the benthic environment and increased presence of macroalgae and macrofauna, such as sea stars and crabs.

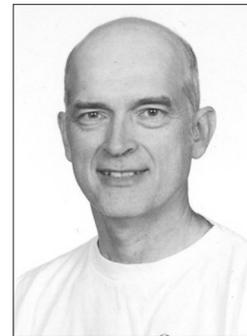
## Introduction

Baynes Sound, British Columbia (Fig. 1) is a channel comprising about 8500 ha between eastern Vancouver Island and Denman Island that has supported shellfish aquaculture since the 1920's. About 55% of the BC farmed shellfish production comes from Baynes Sound.<sup>(1)</sup> The major species cultured are Pacific oysters (*Crassostrea gigas*), Manila clams (*Venerupis philippinarum*), mussels (*Mytilus edulis* and *M. galloprovincialis*) and Pacific scallops (hybrid of *Mizuhopecten yessoensis* and *Patinopecten caurinus*). Environmental impacts of intertidal shellfish culture in Baynes Sound have been examined.<sup>(1,2)</sup> Although there are many reports that bivalves in suspended culture from rafts and long-lines play key roles in coastal systems because of their high filtration rates and biomass,<sup>(3,4)</sup> few studies have considered environmental interactions of suspended shellfish culture in British Columbia.<sup>(5)</sup>

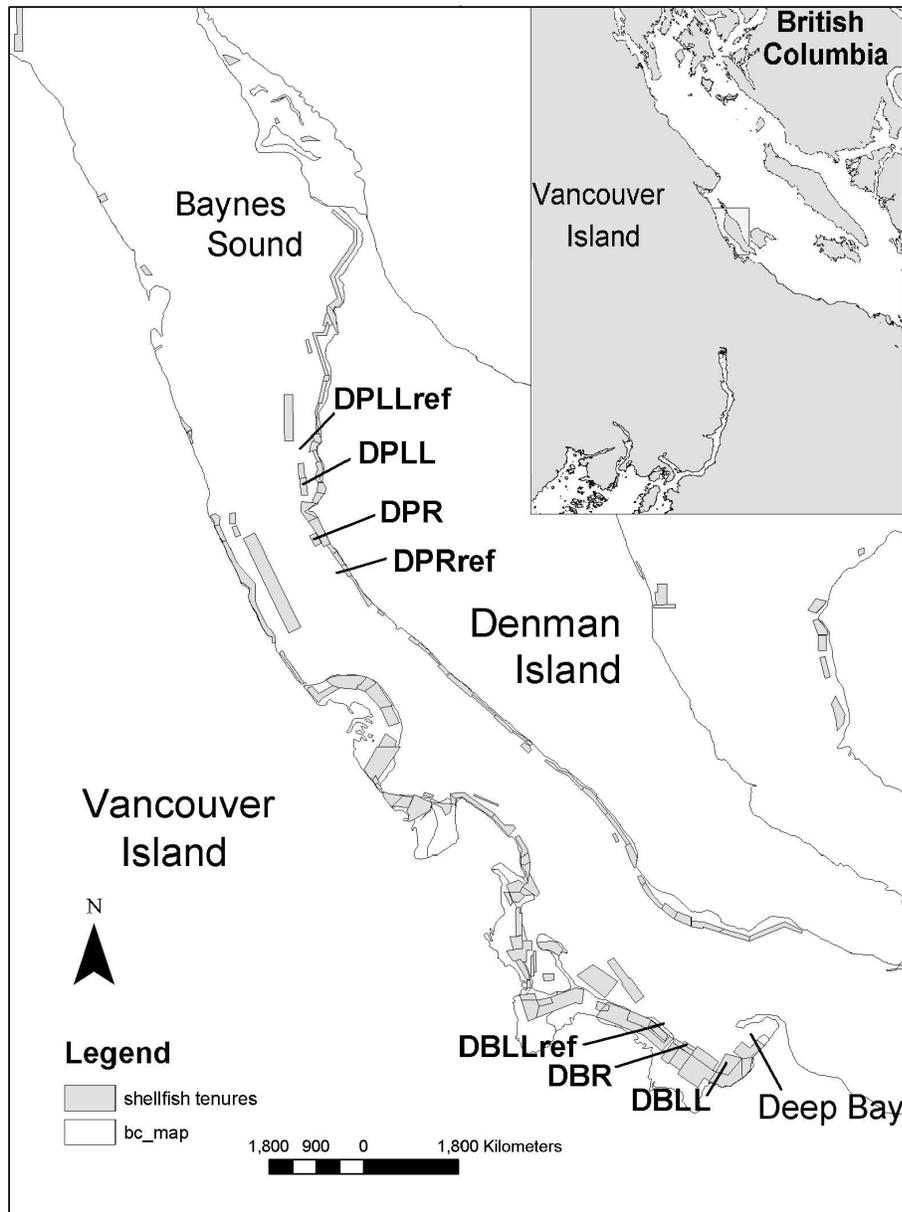
The objectives of this study were to compare current benthic environments potentially affected by suspended shellfish aquaculture with reference sites to determine if there were significant differences in benthic conditions as evident from visual observations and sediment geochemical properties.

## Materials and Methods

At two areas in Baynes Sound, oyster longline farms (DPLL and DBLL) and oyster raft sites (DPR and DBR) were selected for assessment of benthic conditions (Fig. 1). Two reference sites (DPLLref and DPRref) were selected at least 400m away from the Denman Point farm sites and at similar depths. Only one reference site was selected in Deep Bay (DBLLref) due to proximity of other suspension culture farms and industrial history in the inner Deep Bay area. Three replicate Van Veen grabs (0.05 m<sup>2</sup>) were taken at each of the seven sites and geo-referenced by handheld Garmin GPS. For each grab sample, a digital photograph was taken and the location coordinates, depth (m), pH, redox potential (Eh), colour, odour, texture and presence or absence of organisms and shells were recorded. Underwater video images were recorded from a Seamor remote operated vehicle (ROV). An acoustic seabed classification survey of Baynes Sound using Qester Tangent QTCView 4 with a 50 kHz sounder was conducted concurrently.<sup>(6)</sup> Laboratory analyses of sediment included porosity, % organics, % carbonates and sediment grain sizes. Porosity was determined by taking the wet weight and dry weight of each sample and calculating the % weight of water. For sediment grain sizing, dry samples were placed in a series of stainless steel sieves from 2mm to 63µm mesh size and shaken for ten minutes in a Tyler Ro-Tap<sup>®</sup> sieve shaker. The percent weight in each size class was then calculated by the Wentworth grade classification system.<sup>(7)</sup> Percent organics were determined by ashing of dry sediments for 6 h at 500°C in a muffle furnace. Percent carbonates were calculated after the ashed samples were burned for 2h at 950°C.



Bill Heath



**Figure 1.** Baynes Sound, British Columbia, Canada, shellfish tenures and sampling sites near Denman Point on Denman Island and Deep Bay, Vancouver Island.

Data analysis was by multivariate analysis using PC-ORD 4 software on normalized data.<sup>(8)</sup> Ordination by Principal Components Analysis (PCA) was used to assess dissimilarities between the samples and to determine which environmental parameters accounted for most of the variability in the data.

## Results and Discussion

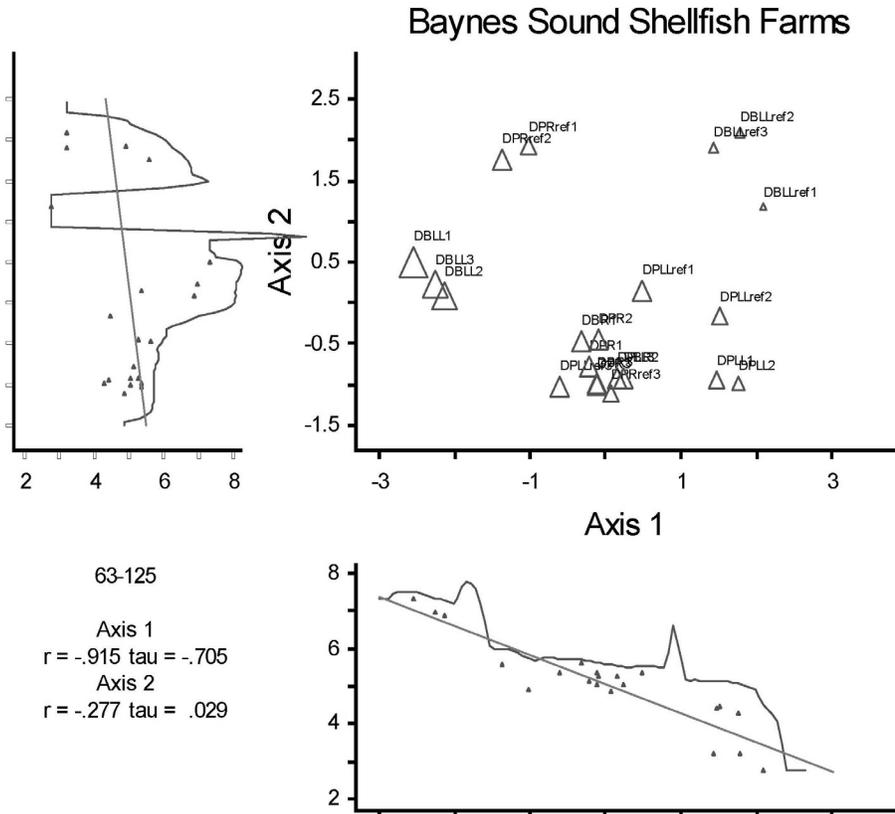
Of all the ordination results, some highlights are presented here. Generally, there were notable dissimilarities between the farm site samples and the reference site samples. However, the major differences appeared to be attributable to variation in natural sediment properties rather than to changes from aquaculture activities. For example, variability between samples for the very fine sand component (63-152  $\mu\text{m}$ ) of grain size distribution is shown in Figure 2. Axis 1 accounts for 83.7% and Axis 2 for 7.7% of the variability; this variable, thus accounts for most of the variation in the data. The largest dissimilarity is between the Deep Bay longline farm (which has higher levels) and the Deep Bay reference site, 1300 m away, with more coarser

sand.

The parameters, % organics and redox potential, Eh, are often used as indicators in Environmental Quality Objectives (NS EMP). Sediment organic content is a measure of organic loading, while Eh is a measure of oxidation-reduction potential in sediment and is an indirect index of aerobic versus anaerobic conditions. In this study, for the % organics variable, Axis 1 explained only 28.2 % and Axis 2 just 0.1% of the variability. Samples with the highest organics were at the Denman Point Raft reference site (6.7 – 15.7 %), compared to 4.2 – 5.7% at the Denman Point Raft site. Organics were lower (2.8 – 3.2%) at the Deep Bay Long-Line farm, at the Deep Bay Raft farm (2.3 – 3.0%) and at the Deep Bay Reference site (1.2 – 2%). The results for % organics (above) and redox potential (range 87 -363 mV) indicate that all of the samples were in the normal-oxic or Type A classification for marine sediments.<sup>(9,10)</sup>

The underwater video images were very useful for comparing benthic conditions beneath the shellfish farm and reference sites. Longline farms at Denman Point and Deep Bay tended to have more shells (mainly oyster), shell fragments, macroalgae, and epifauna, such as crabs and sea stars. Oyster raft farms generally had less shell debris, except underneath one product handling raft where tray oysters were graded in a rotary tumbler. This location had a large amount of shell on the seabed, presumably from discarded oyster mortalities accumulating over time. In contrast, the reference

**Figure 2.**  
**The contribution of sediments between 63-152µm (very fine sand) to the ordination of sample sites. DPR=Denman Point Raft Farm Site, DPRref=Denman Point Raft Reference Site, DPLL=Denman Point Longline Farm Site, DPLLref=Denman Point Longline Reference Site, DBR=Deep Bay Raft Farm Site, DBLL= Deep Bay Longline Farm Site, DBLLref=Reference Site for both Deep Bay Longline and Deep Bay Raft Farm Sites.**



sites had the least number of shells and shell fragments and epifauna.

The addition of large oyster shells to otherwise soft-sediment systems may significantly alter the physical structure of the benthic environment by changing fairly homogeneous two-dimensional environments into complex three-dimensional ones.<sup>(11)</sup> This encompasses the shell surfaces, the cavities within and around them, the sediments that accumulate in the matrix and the habitats formed by the associated species.<sup>(12)</sup> The resulting increase in the number and type of habitats available leads to increases in the abundance and number of species,<sup>(11)</sup> as was observed for macrofauna at the longline and raft farm sites in this study. This effect, which has been observed under mussel farms<sup>(13)</sup> has been termed the development of a “benthic hard bottom community” on soft bottom, which functions similarly to natural beds of bivalves in increasing the abundance of associated species, including fishes and macro-invertebrates.<sup>(11)</sup>

In summary, the suspended shellfish farm sites in this study showed limited impact on the benthic environment, including the addition of fish habitat complexity to soft-bottom sites in the case of oyster longline sites and one oyster raft site.

### Acknowledgements

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# How Does the Spotted Wolffish (*Anarhichas minor*) Adjust to Constant and Fluctuating Oxygen Concentrations?

F Larouche\*<sup>1</sup>, NR Le François<sup>1,2</sup>, SG Lamarre<sup>3</sup>, and D Chabot<sup>4</sup>

<sup>1</sup> Département de biologie, chimie et géographie, Université du Québec à Rimouski, Rimouski, QC, G5L 3A1 Contact:

<sup>2</sup> Nathalie\_Le-Francois@uqar.ca

<sup>3</sup> Centre Aquacole Marin de Grande-Rivière, Ministère de l'Agriculture, des Pêcheries et de l'Alimentation, Grande-Rivière, QC, G0C 1V0

<sup>4</sup> Memorial University of Newfoundland, St-John's, NL

<sup>4</sup> Institut Maurice Lamontagne, Pêches et Océans Canada, Mont-Joli, QC, G5H 3Z4 Contact : denis.chabot@dfo-mpo.gc.ca

In Quebec, efforts have been applied for the establishment of a profitable spotted wolffish (*Anarhichas minor*) production. However, more production data and verifications are necessary before initiating the commercial phase. Land-based facilities will likely be the preferred rearing technology on Quebec's territory. Production in large raceway units and at fairly high rearing densities (> 200 kg/m<sup>3</sup>) is currently the most efficient rearing technology for this species, but fish reared in such systems will likely be exposed to high variations in dissolved oxygen (DO) that might translate in growth reduction. The impact of fluctuations in DO on fish performance, the compensation mechanisms and the physiological adjustments of spotted wolffish should be more fully characterized. Not much information can be found in the literature on the effects of fluctuating DO levels on spotted wolffish. Our research will concentrate on the effects of DO on growth, stress level and oxidative metabolism of juvenile spotted wolffish exposed to normoxic, hypoxic and hyperoxic conditions, the latter two being either constant or fluctuating.

## Introduction

The formation of winter ice coverage is a pronounced characteristic of Québec's coastal environment during the winter and this situation seriously limits the use of sea cages for commercial aquaculture operation. Land-based structures are thus the next best alternative to diversify the aquaculture industry of Québec to include cold-water marine fish species. The spotted wolffish (*Anarhichas minor*) is a calm fish that can easily be reared in shallow raceway at very high density<sup>(1-2)</sup>. This system has the advantage to increase terrestrial space available to the producer. A disadvantage of their utilisation is the rapidity with which water quality can deteriorate, leaving only little time to the operators to react in case of emergency<sup>(3)</sup>. In low depth rearing tanks, dissolved oxygen (DO) is one of the most affected parameters. High densities and low water volumes make it more difficult to maintain stable oxygen concentrations<sup>(4)</sup>.

Hypoxia tolerance varies widely among fish species<sup>(4-7)</sup> and in an intensive aquaculture production context, it is essential to know the impacts of suboptimal DO levels and/or DO fluctuations on growth performances and survival of a given species. The spotted wolffish is a very tolerant species when exposed to hypoxia. Indeed, Foss et al.<sup>(4)</sup> observed in juveniles ( $\pm 68.5$  g) exposed to four DO levels over six weeks a significant growth reduction compared to the normoxic group. However, the hypoxia conditions (40% and 60% sat.) were adequate to sustain juveniles growth with a recorded daily specific growth rate of 0.46% and 0.71%·day<sup>-1</sup> whereas for the normoxic group it was 0.90%·day<sup>-1</sup>. A similar experiment was undertaken by Le François et al.<sup>(8)</sup> on Atlantic wolffish (*A. lupus*), a close relative of the spotted wolffish. After a 72-hour exposure without pre-acclimation period, there was no survival at 16% DO, but interestingly there was no mortality at 22% DO.

When fish are confronted to a lack of oxygen, they must initially maintain their oxygen supply through various acclimation mechanisms. In addition to increased gill ventilation rate, the number of erythrocytes and its affinity with oxygen can also be increased allowing a better oxygen acquisition and organ delivery<sup>(9-10)</sup>. If oxygen concentration remains insufficient, fish will have to switch to anaerobic glycolysis<sup>(11-12)</sup> and downregulate ATP consuming processes until oxygen supply resumes. Since anaerobic pathway has a very poor yield in ATP, the energy pool of the fish will be negatively affected. The DO level at which anaerobic glycolysis become the most prevalent contribution in ATP can be measured by the catalytic capacity of enzymes implied in the anaerobic and aerobic pathways (acid citric cycle and electron transport system)<sup>(12-14-15)</sup>. There is a threshold level of DO at which anaerobic metabolism will prevail. Fish acclimated at DO below this threshold are generally acclimating by adjusting the activity levels of key metabolic enzymes. Therefore, this threshold can be determined by comparing the activities of these enzymes in fish acclimated at different DO and identifying the DO at which activities of critical enzymes are compensated. Hypoxia and hyperoxia and especially the transition between these two had been associated to an increased



François Larouche

production of reactive oxygen species (ROS)<sup>(15-16)</sup>. To prevent or at least alleviate the negative effects of ROS, fish can rely on different antioxidant enzymes and low molecular weight molecule. The extents to which these antioxidant systems are acclimating to different environmental conditions appear to vary according to fish species<sup>(16-17)</sup>.

Literature is scarce on tolerance of spotted wolffish exposed to DO fluctuations, and not much is known about the metabolic and physiological adjustments to hypoxia and hyperoxia environments in this species. The aim of our project is to explore the responses of the spotted wolffish exposed to constant and fluctuant DO levels in terms of growth performances, stress and metabolic adjustments.

## Materials and Methods

The experiments will be performed at the Maurice Lamontagne Institute (Qc, Canada). Experiment I will occur in summer 2009. Juveniles (200-800g) will be randomly distributed among 16 circular tanks and following a pre-acclimation period (100% sat.) of one month, each tank will be assigned one of eight different DO levels (30, 45, 60, 75, and 100% sat.) for a period of two months. In Experiment II, specimens of experiment I will be redistributed among eight tanks ( $50 \times 2 = 100$  fish) at four fluctuant DO levels (20-120, 40-120, 20-150 and 40-150% sat.) for three months. DO levels will be adjusted by adding nitrogen or oxygen monitored by computer. For both experiments, 15 fish per replicate will be weighed (g) and measured (cm) and two fish will be sampled monthly (T0, T30, T60, T90). Gills, liver, muscle, heart and blood sample will be collected from each of these fish for physiological measurements. Enzymatic activity of the glycolytic enzyme pyruvate kinase (PK), the mitochondrial enzyme citrate synthase (CS), the antioxidant enzymes catalase (CAT) and glutathione peroxidase (GP) will be measured. Lipid peroxidation represented by thiobarbituric acid reactive substances (TBARS) and the sensitive enzyme to oxidative stress aconitase will also be measured. Finally, we will quantify haematocrit and plasma glucose levels to determine fish oxygen extraction capacity and stress level.

## Expected results

To compensate the lack of oxygen in a hypoxic environment and thus optimise oxygen extraction capacity, fish generally increase the number of erythrocytes and gill ventilation rate<sup>(10-13)</sup>. These modifications are energetically costly and as a consequence we should observe a diminution of their levels and activity as the organism oxygen needs will increase. In prolonged hypoxia, fish must reduce their energy expenditure to the minimum<sup>(11-12)</sup>. In these experiments, we propose the evaluation of adjustments of the aerobic and anaerobic energetic pathways in presence of DO variations. Increases in the level activity of PK and a reduction of CS are likely to occur. Following the potential excessive production of ROS in the organism in hypoxic or hyperoxic environments, we believe that the activity of antioxidant enzymes like GP and CAT or oxidative damage (TBARS and aconitase loss activity) can be enhanced.

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# Canadian Trout Industry: Competitive Advantage and Strategic Options

**Rashed Nabi**

*Fisheries and Oceans Canada, 200 Kent St., Ottawa, Ontario, K1A 0E6, Canada (Tel: 613-990-0244, Fax: 613-993-8607, E-mail: rashed.nabi@dfo-mpo.gc.ca)*

The Canadian trout industry has potential to grow but lacks a benchmark to demonstrate competitiveness and attract investors. Assessing the competitive advantage, this paper shows that despite certain advantages, the industry is trapped between low investment, low domestic price and slow growth of US imports. The paper concludes that the industry can enhance its competitiveness and re-position itself by focusing both on domestic and US markets, creating new market segments, initiating market promotion, and creating horizontal clustering.



**Rashed Nabi**

## Introduction

Despite its small size, the Canadian trout industry does have growth potential. Although freshwater trout farms flourish in almost all provinces, the average trout production growth rate fell from 8.0% to 1.7% between 1987-96 and 1997-2006 when the world aquaculture growth rate increased exponentially. In recent years, production has been stagnant in Ontario and Quebec, where suitable freshwater supply is abundant. Production growth in the Prairie Provinces relies mainly on Saskatchewan. These two regions accounted for over 90% of trout production of 7100 tonnes in 2006.

The growth of the industry is constrained by a complex set of planning and environmental regulations. The onerous approval process causes delays in access to new production sites, increases costs of access, and diminishes investor confidence. This constraint is encountered in many countries that strive to keep the competitive edge in the global market. The feed quota regulation in Denmark, the new environmental regulation in Peru, and the legal battle over water use in Idaho, US, are but a few such examples. Besides regulatory constraints, the Canadian industry lacks a benchmark to demonstrate market competitiveness and thus fails to attract new investors.

This paper provides a basic assessment of the competitive advantage of the industry with a few recommendations to enhance competitiveness in domestic and American markets—the only existing markets for the industry. The assessment excludes European and Asian markets because the industry has yet to show any readiness to enter these markets.

## Competitive advantage and the trout industry

This paper draws on published sources and key informant inputs and uses the competitive advantage framework of Porter<sup>(1)</sup> to provide a diagnostic assessment of the industry. Sectoral competitiveness assessments generally focus on two aspects: sustained profitability and increased market share. Porter offers the framework to understand these aspects by examining four mutually reinforcing attributes of a sector: factor conditions, demand conditions, related and supporting industries, and firm structure and strategy. Government policies and regulations as well as chance factors have influence over these attributes. Government's role can both enhance and diminish competitiveness. For the trout industry, the existing regulatory requirements complex set of regulations is a diminishing factor. Chance factor is often beyond the control of the industry or government. For example, a sudden contraction of meat supply can increase demand for seafood and spur the growth of the seafood industry.

### Factor conditions

Factors are essential inputs — the important factors “are not inherited but are created.” For example, the inheritance of abundant freshwater resources can become a comparative advantage for the industry when it has the desirable access to it. The regulatory constraint reduces access to this factor in every province but more so in Ontario, where the scope of cage culture expansion in the lake water is greater. Specialized workforce, which is in moderate supply in Canada, is another factor condition. However, trout producers face competition in the labour market from salmon and other industries that offer higher wages. Labour costs on trout farms in Canada are higher than those in the US (Table 1). Nonetheless, Canadian producers managed to keep the average production cost down

	Labour cost (\$/kg)	Feed cost (\$/kg)	% feed cost of total cost	Production cost (\$/kg)
Canada <sup>(2)</sup>	0.90	1.3	57%	2.3
US <sup>(3)</sup>	0.50	1.0	70%	2.5

Table 1.  
Comparison of average  
costs.

to \$2.3/kg in 2006.

### **Demand conditions**

The quality of demand matters as much as the quantity. Over 85% of Canada's trout is sold in the domestic market. The farm gate price has been stagnant at below \$4.0/kg whereas the import market appears to be growing. Trout imports in Canada rose from 1200 tonnes in 2002 to 2,300 tonnes in 2007. Changing demographic and ethnic composition places increasing demand on freshwater fish with the likelihood of raising demand for trout.

By comparison, Canada's trout export to the US has been flat. In 2007, Canada exported 1,038 tonnes, less than 20% of total 5300 tonnes of US trout imports. The rest 80% of US imports came from South American countries including Argentina, Peru and Chile<sup>(4)</sup>. Imports from these countries have gone up owing to the low prices of their frozen products while US imports also have grown. Canada's frozen trout has been too expensive (US\$8.0/kg) to compete with these countries. Nearly 80% of US trout imports in 2007 comprised frozen products.

Canada fares better in the US with fresh rather than frozen trout. The export price of fresh products increased from US\$4.0/kg in 2002 to US\$6.0/kg in 2007, still lower than the prices offered by others. Fresh trout products meet US consumers' preferences<sup>(5)</sup> and Canada should be able to capitalize on it, although high fluctuation of fresh US trout imports causes concern. Canada also has an advantage in its pigmented trout products over white flesh products. In 2007, pigmented products brought more than US\$2/kg higher price<sup>(3)</sup>. However, pigmented products are vulnerable to competition with salmon products. Trout is rated as sustainable seafood by environmental groups. Sea Choice, Monterey Bay Aquarium, Environmental Defense Fund and Seafood Choices Alliance have variously identified it as the best seafood choice.

### **Related and supporting industries**

The cage culture operations are clustered in Northern Ontario and they benefit from the clustering of suppliers<sup>(5)</sup>. This clustering is absent elsewhere where producers depend on the US supplies of eggs. Further, small and isolated producers in the Prairies and Southern Ontario do not have good access to processing facilities. Market linkage is a general problem for them as they are not supported by market research and promotion. There are five associations but they rarely engage in market promotion.

### **Firm structure and strategy**

Trout operations are small and family-run with an average production of 10 tonnes. This also characterizes trout industries elsewhere. The cage culture operations are larger although many of them are family managed. There are three large cage operations with an average production of 1000 tonnes — two in Ontario and one in Saskatchewan. Many cage operations vertically integrate their production and sale. For the land-based operations, smallness is a deterrent to integration and new product development. Being dispersed and unable to benefit from clustering, they are forced to operate in local markets.

### **Discussion and Conclusions**

A synthesis of the attributes shows that the Canadian trout industry has several advantages: the average production cost is not inordinately high; the home market is growing; the export product types (fresh and pigmented) meet consumer preferences; export prices of the products have increased. But the industry has many disadvantages as well: new investors are difficult to attract; the farm gate price is stagnant; US import growth for fresh products is slow; and product development and clustering are challenged by smallness and dispersion. In other words, the industry is trapped between low investments, stagnant domestic price; and slow growth of US imports of fresh trout.

Against this backdrop, how can the industry sustain profitability and increase market competitiveness? The regulatory reform through the "Sustainable Aquaculture Programme" programming

is expected to spur the expansion of the industry. As this reform opens up new growth opportunities, the industry can enhance competitiveness and re-position itself by pursuing a combination of strategies stated below. First, there is a need to establish a strategic goal for the industry for the next five years. Second, to support further growth, it needs a simultaneous focus on increased profitability in the domestic market and increased share of fresh products in the US market. Third, production cost is a concern but not a hurdle; therefore, the industry should direct its energy to creating new products or new market segments. Fourth, trout needs to get into the mind of consumers as a distinct item; this is where the industry needs to initiate its own market research and promotion, or collaborate with the more advanced segment of the seafood industry, such as salmon. The smallness of the industry size and positive ratings from environmental organizations offer the industry an opportunity to take a lead in emerging market requirements such as certification. Finally, since geographical clustering is difficult in the Prairies and other provinces, the industry can initiate a horizontal clustering, which is tantamount to networking with a characteristic of the producer cooperative. A more innovative clustering, especially in the Prairies, could also include a strategy of integrating small trout farms with hog farming. The horizontal clustering will not only help to address the problem of economies of scale but also to diversify the industry.

### **Acknowledgments**

The author wishes to thank Éric Gilbert, Director, Aquaculture Management Directorate, Fisheries and Oceans Canada, for his support and useful comments.

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# Effects of Poultry Oil as a Replacement to Fish Oil in Atlantic Salmon (*Salmo salar*) Diets

David Deslauriers\* and Keith Rideout

School of Fisheries, Marine Institute of Memorial University of Newfoundland, P.O. Box 4920, St. John's, NL A1C 5R3

The aim of this study was to determine the impacts of replacing up to 40% of total marine oil with poultry oil in Atlantic salmon (*Salmo salar*) diets. Growth, feed conversion ratios and digestibility as well as flesh and faeces proximate analysis were evaluated. The experimental design consisted of four treatments with triplicate groups of 20 fish each. The diets were fed twice daily until apparent satiation to 32.1 g fish during 54 days at 9°C. Final weight, specific growth rate and condition factor did not differ among the dietary treatments. Hepato-somatic and viscero-somatic indexes as well as fillet yield of fish from the four treatments did not show any significant differences. Flesh proximate analysis showed a significant difference ( $P < 0.05$ ) in moisture for the 20% inclusion of poultry oil diet. Digestibility of protein and lipid decreased in the 10% diet compared to the control. The results suggest that marine oil can be replaced up to 40% with poultry oil without compromising growth performance.

## Introduction

The major concern for fish growers is to get the most out of the feed they are giving to the fish at a minimum cost. They want performance which can be translated by growth, feed conversion and health. Lipids in salmonid diets constitute an important component of their daily energy intake. Early juvenile stages require significant levels of polyunsaturated fatty acids (PUFA) such as eicosapentanoic acid (EPA) and docosahexanoic acid (DHA)<sup>(1)</sup>. These PUFAs are mainly found in marine oils. By replacing these marine oils with vegetable or terrestrial oils, the usual fatty acid profile could become modified<sup>(2)</sup>. Terrestrial animal oils in fish food may also cause problems such as gastric dilation and lead to regurgitation of part of these oils<sup>(3)</sup>. The other concern might be associated with the increases in marine oil prices due to the declining fish stocks worldwide. Fish aquaculture is a growing industry that consumed 81% of the world's fish oil in 2002 and is expected to use up to 97% of the total supply by 2010<sup>(4)</sup>. Terrestrial animal fats are by contrast inexpensive because of their abundance. The problems are therefore economically and health-related.

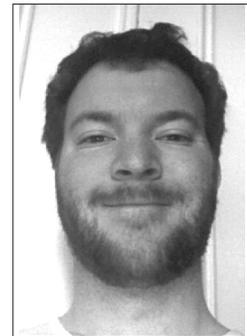
Fish growers want to attain maximum production with minimum input. By using terrestrial animal fats as replacement fats in high concentrations, the growers potentially induce lipid regurgitation. This lipid regurgitation can be observed on the surface of the water at certain marine cage sites of either Atlantic salmon (*Salmo salar*) or Rainbow trout (*Oncorhynchus mykiss*). The accumulation of lipids on the surface of the water means that these quantities have not been assimilated by the fish and therefore are a waste of food and money. The grower should find out what is the optimal ingredient formulation so that fish are able to assimilate the maximum quantities of food.

This study will test the effects of a new diet that is based on different concentrations of poultry oil as replacement to marine oils. The effects of this diet on growth, food conversion, digestibility and energy loss will be quantified and the conclusion should be able to discern whether poultry oils should be used in salmonid diets and if so, at which concentration.

The objective, which can be divided into sub-sections, will be to test the effects of poultry oils as a replacement to marine oils. More specifically, the study will attempt to demonstrate if poultry oil causes regurgitation and if it does, at what concentration and what kind of energy loss is associated with it. Also, the study will give a good idea of which one of the different treatment offers optimal growth. From there it will be possible to find a new range in which to test for even more optimal feed formulations.

## Materials and Methods

Triplicate tanks of Atlantic salmon (*Salmo salar*), obtained from Daniel's Harbour, NL, were fed one of four experimental diets in an experiment lasting 54 days at the aquaculture facility of the Marine Institute, Memorial University of Newfoundland, Canada. The fish had an average weight of 32.1 g and were randomly distributed in 12 x 190 Liter Swedish tanks. Twenty fish were assigned to each tank with an initial stocking density of 5.14 kg/m<sup>3</sup>. The tanks were supplied with freshwater at constant temperature of 9°C. The tanks were serviced with a flow-trough system and a flow rate of 250 L/hour. Dissolved oxygen was kept above 9.0 mg/L. The fish were exposed to a light regime of



David Deslauriers

		Diet			
		Control	10%	20%	40%
Initial weight	g	30.63 ± 7.58	33.03 ± 2.28	34.3 ± 1.05	30.43 ± 6.56
Final weight	g	51.43 ± 9.92	54.07 ± 6.31	56.63 ± 3.33	51.77 ± 8.91
SGR	%/day	0.98 ± 0.14	0.91 ± 0.09	0.93 ± 0.05	1.00 ± 0.12
CF	%	1.19 ± 0.02	1.19 ± 0.02	1.19 ± 0.01	1.22 ± 0.11
FCR		1.79 ± 0.21	1.74 ± 0.44	1.60 ± 0.27	1.68 ± 0.34

Mean ± standard deviation. Values in the same row with different superscript are significantly different at  $P < 0.05$ ;  $n = 20 \times 3$

**Table 1.**  
Initial and final weights as well as specific growth rate (SGR), condition factor (CF) and feed conversion ratio (FCR) according to the different diets.

12 hour light and 12 hour darkness. The fish were fed manually twice daily to apparent satiation.

The diets were produced using pelletization technique. A mash provided by Corey Aquafeeds was used as the base of every diet. To this mash, the different sources of fat were added. The control diet contained 100% marine fish oil. The other diets contained 90%, 80% and 60% marine oil and 10%, 20% and 40% poultry oil respectively. The poultry oil was provided by Country Ribbon Inc.

The fish were all individually weighed and measured at the beginning, mid-point and the end of the experiment. Faeces were collected once daily during week 3, 4, 7 and 8 and kept frozen until analysis. Faeces were kept separately for every tank and collections from week 3, 4 and 7, 8 were combined.

The different diets as well as the faeces and fillets were all analysed using proximate analysis techniques. Moisture and ash content, crude protein (Kjeldahl method<sup>(5)</sup>) and crude fat (Soxhlet method<sup>(5)</sup>) were determined. The gross energy content was assessed using a bomb calorimeter. Digestibility was determined using acid insoluble ash methodology.

All data were subjected to one-way analysis of variance (ANOVA) using Tukey's test at  $P < 0.05$  to detect significant differences among the means. The comparison was made between the mean of every measured parameter in respect to the associated diet.

## Results

No significant differences were found in final weights or lengths. Specific growth rate (SGR), condition factor (CF) and feed conversion ratio (FCR) did not show any significant differences amongst the means. (see Table 1).

Hepato- and viscero-somatic indexes and fillet yield were compared between the fish submitted to the different diets and did not show any significant differences. No statistical test was done on the digestibility data as negative values were obtained for the 20% and 40% diets. As for the control and 10% diets, there seems to be a reduction of digestibility for protein and lipid in the 10% diet compared to the control.

## Discussion

Specific growth rate calculation was used to determine and compare growth between the various treatments. The results showed no significant differences and the same can be said for the condition factor values. This means that the inclusion of a maximum of 40% poultry as replacement to marine oil does not negatively affect growth for the specific life stage, time frame, and temperature that were used for this study.

The fact that growth was independent of the diet gives a good indication that biometric calculations should also be similar. That is exactly what was observed for hepato- and viscero-somatic indexes as well as for fillet yield where the mean values were similar between the treatments and thus independent of the diet.

Protein and lipid digestibility were calculated using % ash and % acid insoluble ash of the diets and the faeces as well as % of protein or lipid found in the diet and the faeces. Negative values were obtained for the fish fed the 20% and 40% diets. The negative results may be explained by the fact that very small quantities of ash and AIA for the faeces were used to determine the coefficients and the error associated with the scale might be enough to throw off the results completely. It is still interesting to look at the positive values and compare both the control and 10% diets even though no statistical analysis was performed. A decrease is observable for both protein and lipid digestibility as the level of poultry oil increases. This might suggest that lipid and protein are digested less effi-

ciently as marine oil % of total lipid decreases or as poultry oil % of total lipid increases.

Feed conversion ratios (FCR) were calculated and were in the order of 1.60 to 1.79, which is higher than what is predicted in the literature. This has nothing to do with the fish but with the way the FCRs were calculated. Indeed, the fish were fed to more than apparent satiation and the excess food accumulated at the bottom of every tank. This excess food was not quantified and subtracted from the initial weight of the food that was given to every tank. This included the uneaten food in the equation, explaining the high values that were obtained.

Oil accumulation on the surface of the water was supposed to be collected if present, but there was none. This suggests that regurgitation might occur when there is inclusion of terrestrial fats in the diet combined with variations in temperature and/or salinity.

## Conclusion

The present study showed that inclusion of up to 40% poultry oil as a replacement to marine oil did not negatively affect the growth performances of the fish. There seems to be a negative correlation between digestibility and poultry oil inclusion. Finally, inclusion of up to 40% poultry oil does not provoke regurgitation at this particular life stage, time frame and temperature.

## Acknowledgments

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# Modelling the Transport and Deposition of Particulate Effluent from Fish Farms in Southwestern New Brunswick, Bay of Fundy

FH Page\*, RJ Losier, BD Chang, and EP McCurdy

Fisheries and Oceans Canada, Biological Station, 531 Brandy Cove Road, St. Andrews, NB, E5B 2L9 Canada (E-mail: [pagef@mar.dfo-mpo.gc.ca](mailto:pagef@mar.dfo-mpo.gc.ca))

This report examines approaches for predicting the spatial distribution of effluent particles originating from salmon farms in the southwestern New Brunswick portion of the Bay of Fundy. A simple model of particle transport was developed and tested using current meter data collected at an operating salmon farm and the results were compared with those generated using the commercially available DEPOMOD model. The results from both models were compared with actual sediment sulfide data. The results from the models and the sediment surveys showed some differences in smaller scale patchiness, but both models predicted benthic spatial domains of particle distributions that were similar in extent to the observed zones of elevated sediment sulfide.



Fred Page

## Introduction

In the context of risk assessment and management, the potential impacts of the release of a substance can be broken down into components: release, exposure, consequence, and acceptability. *Release* is the introduction of a substance into the environment from a source such as a fish farm. Transport, dispersal, and deposition of the released substance result in a temporal zone of *exposure* (or influence) to the released substance. The zone of exposure overlaps with ecosystem components, and the interaction results in ecosystem *consequences*: some degree of change in the ecosystem state, structure, and/or function. A judgement must then be made by decision-makers, stakeholders and the public concerning the *acceptability* of these consequences. Prediction and monitoring are used to keep track of the exposure zone and the consequences, thus allowing the determination and implementation of adaptive actions to manage and mitigate any impacts. Modelling is one tool that can be used to predict potential consequences of substance releases, but the models must be tested to determine their suitability for a given substance type and geographic area.

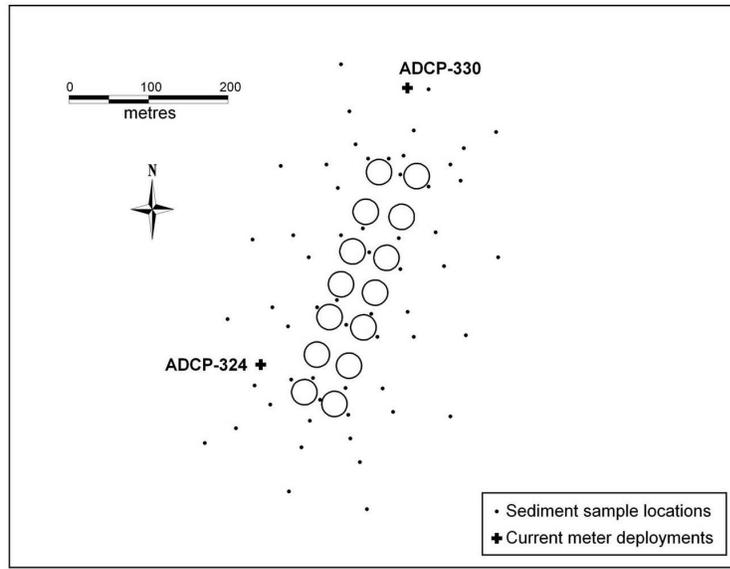
In this report, we look at some modelling challenges and approaches for predicting the bottom distribution of feed related particulates released from salmon farms in the southwestern New Brunswick (SWNB) area of the Bay of Fundy and compare these predictions to observed surficial sediment sulfide, an observed index of the organic enrichment of the seafloor. Some preliminary results from this project were previously reported in this series<sup>(1)</sup>.

## Materials and Methods

We conducted our study primarily at one salmon farm in SWNB. The farm (Site A) consisted of fourteen 32-m diameter (100-m circumference) polar circle cages. The average water depth at the farm site was approximately 20 m (relative to lowest normal tide). The daily tidal range in the area varies from 4-8 m. Acoustic Doppler Current Profiler (ADCP) meters were deployed a few metres off the seafloor at two locations near the farm (Fig. 1). These meters recorded data on current speed and direction at 1-m depth intervals throughout the water column at the deployment location and at time intervals of 15 min. Deployment ADCP 324 was from 12 January to 19 April 2005 (97 d) and deployment ADCP 330 was from 13 September to 24 October 2005 (41 d).

We collected sediment samples at several locations at Site A on 22 September 2005 (Fig. 1). The sediment samples were analyzed for surficial sulfide levels, which are an indicator of organic enrichment. Sulfide data were log transformed and normalized by subtracting the mean log transformed value and dividing by the standard deviation of all log transformed samples from the farm. Contour plots were produced from the normalized data using MapInfo Vertical Mapper (version 3.0) software (interpolation by triangulation with smoothing). Details on the sediment sampling are published in a separate report in this series<sup>(2)</sup>.

We tested two models for predicting the zone of exposure to organic particles released from the farm. The simple model is described below. The more complex model was the commercially available DEPOMOD model<sup>(3,4)</sup>. For DEPOMOD, we used the following input values: the feed had 10% water content; 3% of the feed was wasted (uneaten); 10% of the consumed feed was egested as fe-



**Figure 1.**  
Locations of Acoustic Doppler Current Profiler (ADCP) deployments and sediment sampling at study site A. Circles represent approximate locations of fish cages.

ces; the feed input per cage was the average daily amount of feed delivered to each cage during September 2005 (the month when sediment sulfide data was collected; feed data were provided by the farm operator); water current data were obtained from the ADCP deployments. DEPOMOD predicts organic benthic accumulation rates; the results are presented as contour maps showing relative rates of organic deposition. We compared the predictions from the two models with the contour plots of the sediment monitoring data.

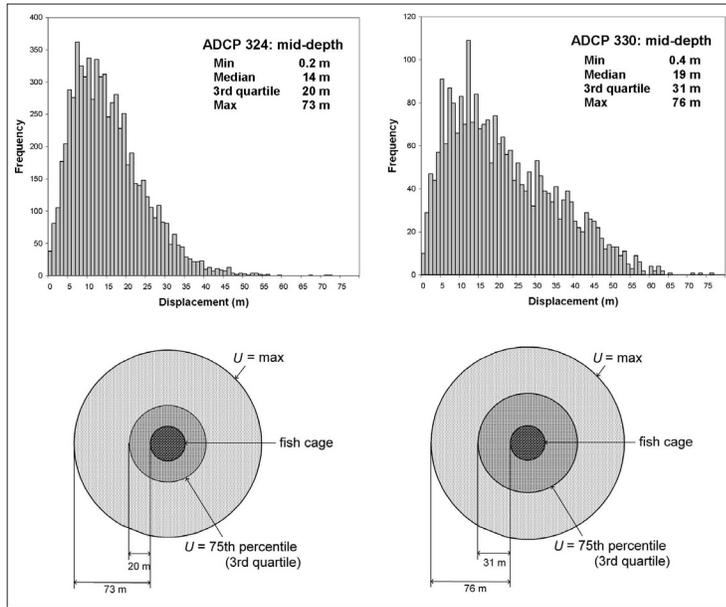
**A simple model of organic enrichment at a fish farm**

In our conceptual model, feed and feces are introduced into floating salmon cages at the water surface. The feed and feces begin to sink, until they reach the seafloor, creating a zone of influence (or exposure) on the seafloor. If there are no horizontal water currents ( $U=0$ ), the particles will land directly under the cages. Water currents will result in horizontal displacement of the particles, thus affecting where they will hit the seafloor. The horizontal distance ( $x$ ) travelled by a particle is calculated as the horizontal water velocity ( $U$ ) multiplied by the time required to sink to the bottom ( $t_s$ ). The time to sink to the bottom ( $t_s$ ) is calculated as the water depth ( $h$ ) divided by the particle sinking rate ( $w_p$ ). Table 1 gives some calculations of horizontal displacement for different sinking rates and horizontal water currents, if it is assumed that the current flow is in one direction; the sinking rates we used for feed and feces were in the ranges reported by Cromey et al. (3).

We know that the currents in southwestern New Brunswick are not unidirectional, hence particle displacement will not be in only one direction. In the simplest case of incorporating varying current direction, we assume that the current flows equally in all directions. This results in a circular zone of exposure for each cage, with the area of the zone dependent on the horizontal current velocity, the particle sinking rate, and the water depth. Using actual data on the frequency of current velocities, we can estimate statistics describing the particle displacements such as the maximum distance travelled by particles, and the distance travelled by particles transported at the slowest 75% of velocities. For these calculations, we used a water depth of 20 m and a particle sinking rate of  $10\text{ cm s}^{-1}$ , resulting in a sinking time of 3 min (Fig. 2). A schematic map of the zone of influence for an entire farm can be produced by combining the zones of influence of all cages.

Particle type	Sinking rate ( $\text{cm s}^{-1}$ )	Sinking time at depth = 20 m (min)	Horizontal displacement (m)	
			$U = 10\text{ cm s}^{-1}$	$U = 50\text{ cm s}^{-1}$
Feed	15.0	2.2	13	65
	10.0	3.3	20	100
Feces	5.0	6.7	40	200
	1.0	33.3	200	1 000
Fines	0.1	333.3	2 000	10 000
	0.01	3 333.3	20 000	100 000

**Table 1.**  
Calculations of horizontal displacement of different particle types under different horizontal current velocities ( $U$ ), with no directionality.



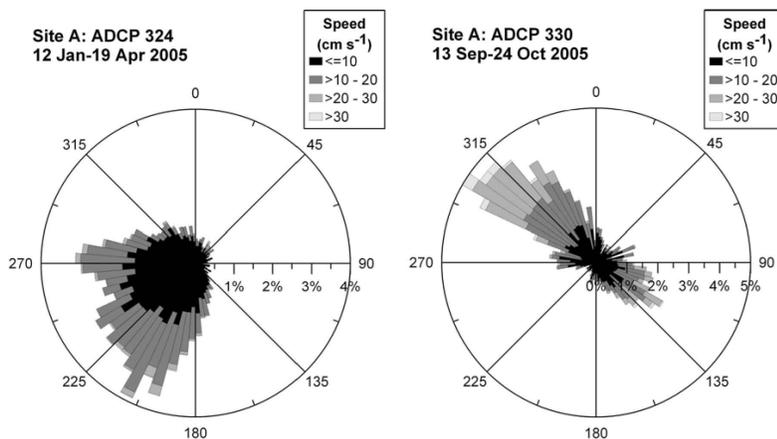
**Figure 2.** Predicted horizontal displacement of particles released from a fish cage. Top figures show non-directional particle displacement estimates, using a water depth of 20 m, a particle sinking rate of  $10 \text{ cm s}^{-1}$ , and horizontal current velocity ( $U$ ) data from Acoustic Doppler Current Profiler deployments ADCP 324 (left) and 330 (right). The lower figures are schematic representations of the corresponding benthic zones of exposure associated with particles released from a single fish cage, assuming that the current direction is equal in all directions, using current velocities from the ADCP deployments; the outer circle represents the distance travelled by particles transported at the maximum horizontal velocity; the next inner circle encloses 75% of the particles.

**Sensitivity of models to variations in water current data**

We used water current data from the ADCP deployments to estimate the horizontal particle displacement with our simple model and with DEPOMOD. Maximum current velocities were similar in the two current meter deployments, but the median velocities and the slowest 75% of velocities were considerably lower in deployment ADCP 324 (Fig. 2). The current directions were different in the two deployments: in ADCP 324, currents were mainly to the west and southwest, and in ADCP 330, they were mainly to the northwest and somewhat to the southeast (Fig. 3). Because the deployments were not simultaneous, the differences may reflect both spatial and temporal factors.

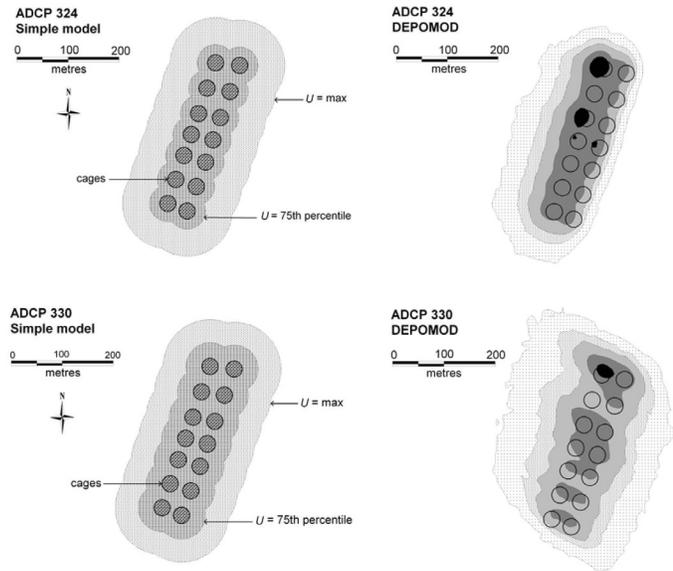
Using the simple model, the predicted areas of maximum particle displacement were similar using data from the two current meter deployments, since the maximum velocities were similar (Fig. 4). The deposition of particles within the cage area would be expected to be greater when the horizontal particle displacement is smaller, which would occur when current velocities are lower (Table 1). Using data from deployment ADCP 324, where the median and 75<sup>th</sup> percentile current velocities were lower, the simple model predicted that the area of higher particle deposition would be smaller, compared to that predicted using data from ADCP 330 (Fig. 4). This would suggest that the particle density within the higher deposition area would be higher using data from ADCP 324, since the particles would be spread over a smaller area than in the scenario using data from ADCP 330.

DEPOMOD, using data from ADCP 324, predicted that the overall area of particle displacement would extend to the west and southwest of the cages, while with data from ADCP 330, the overall area of particle displacement would extend to the northwest and southeast, reflecting the current di-



**Figure 3.** Mid-depth current velocities from Acoustic Doppler Current Profiler deployments ADCP 324 and 330 near study site A (see Fig. 1). The shading indicates the speed ( $\text{cm s}^{-1}$ ). The length of each radial bar indicates the relative frequency (percent of the current records within the full deployment time series) and the bars within each direction bin are stacked so the total length of each bar represents the cumulative frequency of currents in that direction bin. Direction bins are in 5 degree increments. Zero degrees indicated flow toward true North; 90 degrees indicated flow toward the East.

**Figure 4.**  
**Predicted particle displacements using mid-depth current data from two Acoustic Doppler Current Profiler deployments, ADCP 324 (top) and 330 (bottom). The left figures show the predicted particle displacements for the simple model, using current velocity data ( $U$ ), assuming that current flows equally in all directions. The right figures show the DEPOMOD predicted particle deposition rates, using current speed and direction data (shaded areas represent relative rates of particle deposition, with darker areas indicating higher rates).**



rection data (Fig. 4). DEPOMOD predicted that the overall area of particle displacement would be slightly smaller when using ADCP 324 data, but there would be a large area with high particle deposition rates under most of the cages, although shifted slightly to the west and southwest (Fig. 4); the highest particle densities were under the cages which received the greatest amount of feed. Using data from ADCP 330, the overall area of particle deposition predicted by DEPOMOD was slightly larger, but the area of high particle deposition was much smaller, mainly under the two cages which received the greatest amount of feed. These predictions reflect the higher current velocities in the ADCP 330 data, which would result in greater particle displacements, but lower particle densities in the cage area.

**Comparisons of model predictions with field observations**

Our simple model predicted relatively uniform conditions under the farm, with the highest input under the cages. DEPOMOD indicated some patchiness, related to the amount of feed delivered to each cage, but the zone of influence remained mostly under the cages (Fig. 4). When we compare these predictions with actual sediment sulfide data (Fig. 5) we see that the field data indicated more patchiness within the domain, and a westward shift of the higher sulfide areas. The scale of the elevated sulfide domain was similar to the zones of influence predicted by both models.

The finer scale patches indicated by the sediment sulfide data (Fig. 5) did not always occur under the cages which received the most feed; at this farm, there was considerable variability in the amount of feed delivered to each cage. While the field data showed high sulfide patches in the northwestern area of the farm, in the vicinity of the two cages which received the most feed, there was also a high sulfide patch at the southern end of the site, where cages received relatively less feed. DEPOMOD predicted high deposition patches under the two cages in the northwest area, but did not predict a high deposition patch at the southern end.

We obtained sediment sulfide samples (see Chang et al.<sup>(2)</sup>) and feeding data from two other farms in SWNB. At both of these farms, the sediment sulfide showed a patchy distribution, despite the relatively equal amounts of feed among the cages (Fig. 6).

**Figure 5.**  
**Contour plots based on sediment sulfide data at study site A. Sulfide data have been log transformed and normalized to the mean and standard deviation of all values; <-1.0 represents background levels. Circles represent cage locations, with the circle sizes in proportion to the amount of feed delivered to each cage up to the date of sediment sampling. Dots represent sediment sampling locations.**

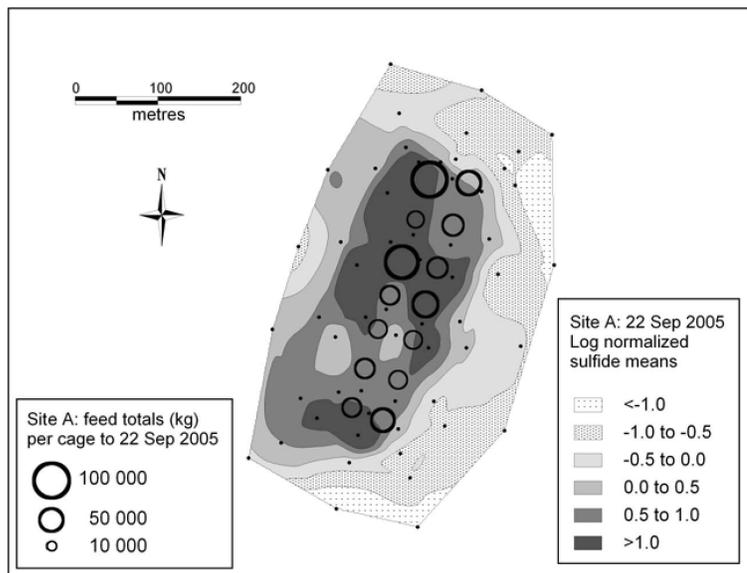
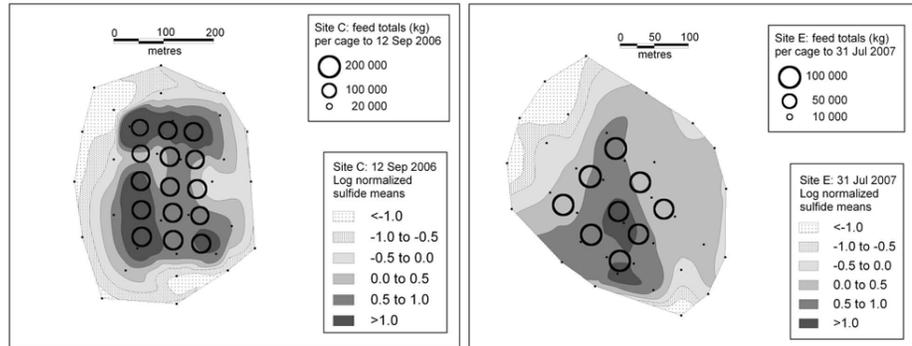


Figure 6.

Contour plots of sediment sulfide levels at two other farms in southwestern New Brunswick. Sulfide data have been log transformed and normalized to the mean and standard deviation of all values within each site. Circles represent cage locations, with the circle sizes in proportion to the amount of feed delivered to each cage up to the date of sediment sampling. Dots represent sediment sampling locations.



## Discussion

The patchiness observed in the field data may be caused by several factors. The transport and deposition of the particles can be affected by processes and variables including: spatial and temporal distribution of feeding; the proportion of feed to feces; variations in sinking rate(s) of feed and feces; variations in water depth; and spatial and temporal variation in water currents. Once particles have settled on the bottom, their distribution can be affected by additional processes including bio-geochemical degradation processes and re-suspension.

The simple model and DEPOMOD both predicted the coarse scales of the domains of elevated sulfide. However, the models did not accurately predict the fine scale patchiness that was observed in field monitoring data. Although DEPOMOD includes spatial variation in feed input among cages, and this did influence the model predictions, the results did not always match the field data. At site A, which had uneven feed distribution among the cages, DEPOMOD predicted patchiness, but some patches that were observed were not predicted.

DEPOMOD incorporates current speed and direction data, but uses data from just one current meter deployment. Our current meter data show that currents vary considerably within the spatial domain and scale of the fish farm. Despite this variation when we compared model results using different current data, we found that although the different water current scenarios did influence the results, the predicted spatial domains of exposure were similar. This was due in part to the relatively shallow depth at the farm, which meant that particles did not remain in the water column for long, and hence the differences in current velocities did not have time to be manifested at the scales examined here, before being deposited on the seafloor.

Differences between the model results and observations may be due to inherent spatial and temporal variation in the sediment sulfides; a lack of spatial variability in the model water velocities; and/or processes not included in the models. Whether or not the details matter depends upon the purpose for which the models are to be used. For example, do regulators need the small scale spatial details for site application evaluations? Or are the details needed for evaluating potential mitigation strategies or empirical monitoring strategies?

## Acknowledgements

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# Building a Collaborative Research Program

**Pamela Parker**

*BC Pacific Salmon Forum, L-427 Fitzwilliam Street, Nanaimo, BC V9R 3A9*

**T**his presentation will introduce the BC Pacific Salmon Forum and its mandate and discuss the 'process' followed in building a collaborative research program in British Columbia in the area of wild/farmed salmon interactions.

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The BC Pacific Salmon Forum began operation on April 1, 2005. Appointed by Premier Campbell to provide policy recommendations on ways to protect and enhance wild salmon, to enhance the sustainability of aquaculture and to increase public confidence in fisheries and aquaculture management, we were given a three-year mandate and funding to support applied scientific research. In spring 2008 we received a one year extension to enable us to complete and report out on our research initiatives.

Honourable John Fraser, a former federal fisheries minister and Chair of the Pacific Fisheries Resource Conservation Council was appointed as Chair along with six other individuals with a variety of backgrounds and expertise; all are well informed about fisheries issues.

When the Forum began operation the first step was to develop a research framework with three goals: healthy ecosystems, sustainable livelihoods and social equity and two principles: sound science and good governance. The Forum felt that if there could be progress in using science to understand and manage the interaction of farmed and wild salmon, there could be progress in gaining public confidence in aquaculture as a legitimate user of the marine resource.

It was the judgment of the Forum that public concerns about the role of sea lice in reducing wild salmon stocks had become the single greatest obstacle to public confidence in salmon aquaculture in BC. Resolution of this issue could, therefore, have a significant impact in gaining support for salmon aquaculture growth in the province – thereby meeting the mandate to enhance the economic, social and environmental sustainability of aquaculture for all coastal communities.

In 2005 there were strongly held differences of opinion among scientists and very little collaboration among them. There were no widely shared research protocols for conducting sea lice research. There was still very little known about the complex ecosystem in which both salmon and sea lice are born, live and reproduce.

As a first step Dr. Tony Farrell was contracted to lead in the development of a Reference Manual for Research in this area. The manual was developed by an extensive network of specialists in the field of sea lice research from Norway, the UK, USA and Canada. There were 17 authors and 9 contributors active in writing the various chapters; the chapters were reviewed by 34 international researchers and the result was a manual of protocols and guidelines intended to address data quality and/or provide a reference for research.

The Forum then commissioned Dr. Bill Pennell and Dr. Paige Ackerman to conduct a gap analysis for us. *A Review of Research Priorities on Sea Lice, Wild Salmon and Farmed Salmon Interactions* was developed with input from 43 active sea lice researchers. The document provided the basis for a workshop that included researchers and representatives from government, First Nations, industry and conservation sectors. The workshop resulted in the identification of three areas of critical importance for future research: sources of sea lice, factors that influence distribution and survival and impacts to hosts. The workshop participants also agreed that collaborative research teams should be used to conduct the work. A call for proposals was issued based on the workshop results, and research was contracted for the 2006 field season.

To provide support to the Forum and for the research program a multi-disciplinary scientific advisory committee (SAC) was created. This was also done through a consultative process in the fall of 2005. Members of the SAC were chosen for their scientific credentials, and to ensure a broad range of interests and scientific expertise to inspire public confidence in the committee's ability to be both competent and fair. The committee held their first meeting in January 2006.

In the fall of 2007, researchers funded by the Forum met to share their interim research findings and discuss continuing research gaps. There was general consensus that greater value would be gained if a directed research program was designed that took a broader ecosystem approach. This would ensure that the program included both the biological and environmental sciences necessary to address this subject.

Because the Broughton Archipelago area of BC is where a large number of the province's salmon farms are located and the region had been the epicenter of the sea lice debate this area was chosen as

the centre for the research program.

A two-day workshop of over twenty research professionals was hosted to identify the areas of focus for the research in 2007 and 2008 and to begin the development of the collaborative research teams that would be charged with completing the research. Four areas of focus were identified:

- The out-migration period: quantification of fish and lice dynamics
- The impacts of lice on individual pink and chum salmon smolts
- Pink and chum population dynamics
- Community Engagement

Within each topic area there are a series of individual research projects which results in approximately twenty individual research projects.

Community engagement was recognized as a critical component to gaining support for research development and results. However, despite our best efforts, significant progress in gaining stakeholder involvement in our research has been slow in coming. In both years of the program we've managed to engage some sectors but not others. Keeping the various parties engaged throughout the process is also difficult.

Community engagement is a social science and is an area that certainly requires further attention.

The Broughton Research Program itself does include a wide variety of stakeholders and research perspectives. Over the past two years we have engaged researchers from government, industry, academia and also independent researchers in our research projects. In addition to providing scientific expertise these partners have also contributed over \$2 million dollars in cash and in-kind, which, coupled with the \$1.8 million contributed by the Forum makes for a very robust research program.

Research funded by the Forum is generally aimed at meeting the needs of resource managers dealing with a high profile resource issue – this requires high quality science-based information, including research and analysis done to the highest standard and based on the best available information.

There have been difficulties in the past with access to data from researchers and industry in both directions. Researchers need farm data to complete their analysis. Industry is interested in data to enable them to better understand the marine ecosystem dynamics and apply this information to their production planning.

Companies and researchers both seek assurance that data they provide is used in accordance with certain guidelines and in a transparent manner and that confidentiality remains in place until such time as research is published. Trust is a difficult thing to achieve.

In an attempt to meet the needs of both sides, a data sharing protocol has been drafted by the Forum, and is being used for research that we are funding. This protocol will allow researchers to formally communicate their data requirements and both parties will be held accountable for information and how it is used and disseminated.

While the Forum expects that research funded will be published in peer-reviewed journals or technical papers; to meet our obligations we must report on interim findings in advance of these publications. Therefore, the Forum requires that all researchers we fund report interim findings in the fall of each year. This is done through an agreed-upon communications protocol. This early reporting has enabled us to make adjustments to our research direction and provide current information on this topic to government officials and the general public.

Most recently a *Summary of 2007 Interim Research Findings* was issued which contains interim reports from each of the research projects funded by the Forum in 2007. The report contains a summary of key findings, based on preliminary reports from each project, that was examined and approved by the Science Advisory Committee.

Two additional initiatives by the Forum that build upon the knowledge base emerging from the Broughton Research Program include a sea lice research review entitled *Science and Sea Lice: What Do We Know?* by Dr. Brian Harvey. This is an annotated bibliography of almost 100 peer reviewed sea lice research papers published between 2004 and 2008. While this is largely a dispassionate review of the various research papers, the author has included his own analysis and commentary on the topic.

The second is the *Broughton Archipelago: A State of Knowledge* by Dr. Isobel Pearsall.

This is a historical data report that contains a chronology of environmental factors that have impacted the Broughton Archipelago since the early 1950s. The report provides a historical context and overview of the trends in wild salmon and the various factors that influence those trends. The potential exists to plot this information in a GIS based format. The report can also serve to underpin an ecosystem management approach in the area.

While achieving 100% consensus on research results isn't likely; the Forum is of the opinion that having a broad range of collaborators engaged in the research will contribute to a broader base of support for the research results and contribute to broader communication of the results.

The mandate of the Forum is to provide policy recommendations to the Provincial government. In June 2007 the Forum issued an interim report that tabled a number of findings and recommendations.

- The main threats to wild salmon are changes in ocean productivity and in the capacity of watersheds to support wild salmon
- Coordinated decision making in watersheds was critical to maintain proper function
- Establishment of a single research body to pool expertise and funding to advance research based on ecosystem based principles
- That closed containment is an unproven approach to salmon farming that requires a scientific, technical and economic analysis be pursued without delay, followed by extensive piloting, before it can be considered as a public policy requirement for the industry
- That the salmon farming industry should share information with the public consistent with its use of a public resource
- That internationally recognized, independent, third-party certification of wild and farmed salmon should be supported to help build public trust
- That decisions regarding future development of salmon farming should be based on scientific consensus within each ecological area

The Forum is now beginning the development of the final report it will present to government in late, 2008. The report will contain recommendations to achieve the Forum's vision for sustainable wild salmon, sustainable aquaculture and building public trust. The intent for the Forum in the area of aquaculture is to provide recommendations that could provide the basis to enhance the economic, social and environmental sustainability of aquaculture for all coastal communities – recommendations to also increase public confidence will also be a factor.

It is our hope that we will be able to continue to build on the relationships and collaborations we are developing with the salmon farming industry to achieve our goals.

Building collaborations in a highly charged and emotional climate has not been easy; however, we've tried to keep our focus on our mandates and our efforts to develop a new paradigm in British Columbia that will, in the end benefit both the wild and the farmed salmon sectors.

# Virulence of Infectious Salmon Anaemia Virus (ISAV) Isolates in Atlantic Salmon (*Salmo salar* L.) in the Bay of Fundy: A Review

RJ Ritchie<sup>1\*</sup> and N Gagné<sup>2</sup>

<sup>1</sup>Research & Productivity Council (RPC), 921 College Hill Rd., Fredericton, NB E3B 6Z9 Canada (\*Corresponding author:

Rachael.ritchie@rpc.ca)

<sup>2</sup>Department of Fisheries and Oceans, Aquaculture and Environmental Sciences, Moncton NB E1C 9B6 Canada

ISAV surveillance in the Bay of Fundy has identified the existence of a large number of genetically distinct ISAV isolates which appear to be of variable virulence, but it has been difficult to elucidate a clear association between these molecular variations and variations in virulence. This has stymied the establishment of proactive management decisions regarding infected fish, and ISAV infections, regardless of type, must be treated as one. Field data of ISAV infections is difficult to collect and to compare between infections owing to a wide range of confounding factors including time of year, fish stock, cage site location, mitigating factors and stressors. Quarantine studies can control for many of these factors, and although they can't mimic pathogen transmission in the field, they are often good surrogates for epidemiological field studies. In recent years, several studies investigating the *in vitro* virulence of common and recent ISAV isolates have been performed and the link between differences in mortality and different ISAV molecular isolates is becoming clear. These studies are reviewed here, and the implications of these studies to the management of ISAV infections is discussed.



Rachael Ritchie

## Introduction

Infectious Salmon Anaemia (ISA), a serious disease of Atlantic salmon, was first identified in Norway in 1984<sup>(1)</sup> and has had significant economic impact on salmon farming regions around the world. The genome encodes for two major surface proteins, the haemagglutinin-esterase (HE) encoded by segment 6 and fusion protein (F) encoded by segment 5, which mediate cell entry and lysis<sup>(2-5)</sup>. Genetic variation has been found in both genes, although the hyper polymorphic region (HPR) of the HA gene is best characterized.

In New Brunswick, more than twenty different HPR variants and three segment 5 variants have been identified<sup>(4-6)</sup>. Variants such as HPR2 and HPR4 are commonly found year after year in the Bay of Fundy, and there is growing anecdotal evidence that the difference in HPR types is associated with differences in pathogenicity<sup>(7-9)</sup>. Elucidation of such a link would significantly improve management of ISAV infections in the field allowing implementation of risk-appropriate depopulation guidelines.

Preliminary field studies and significant anecdotal field observations suggest that some ISAV types (i.e., HPR4) are more pathogenic than others<sup>(8)</sup>. However such field work is often hampered by incomplete or small datasets and the need to identify and control for a range of confounding factors such as the presence of other diseases (BKD and co-infection by ISAV), seal attacks, husbandry, feed and fish. In order to get a rough indication of pathogenicity, researchers have used quarantine challenges<sup>(7, 9-14)</sup> looking at different infectious routes and species, to understand the pathogenicity of ISAV isolates and progression of associated disease.

These studies are typically performed using intraperitoneal (IP) injection or by cohabitation challenge models and for many diseases have been shown to be a good surrogate for field studies. In the early days of ISAV research these studies were used to study dynamics of ISAV infection and to develop effective challenge models for vaccine studies. However in recent years these studies have evolved to study the effect of different viral isolates or strains. In 2005, Mjaaland et al.<sup>(13)</sup> published the results of a large comparative challenge of European ISAV isolates, and in 2006, Kibenge et al.<sup>(7)</sup> compared the survival of multiple North American and European ISAV isolates. In 2008, we published the results of quarantine study of high and low virulence ISAV isolates in the Bay of Fundy<sup>(9)</sup>.

In this paper we review the results of quarantine studies to date and discuss the implications for this work on management of ISAV.

## Results and discussion

Three large quarantine studies have investigated the relationship between ISAV molecular type and virulence<sup>(7,9,13)</sup>. In all, a total of 28 isolates (representing 13 molecular types) of European or North American origin spanning a 10-year period have been studied. These studies clearly show differences in mortality in quarantine challenge and look set to provide a basis for improved management decisions of ISAV infection in the field. For the Bay of Fundy region, the studies of most relevance to fish health management are those performed using Saint John River stock fish by Ritchie et al.<sup>(9)</sup> and Kibenge et al.<sup>(7)</sup>. Although each group used difference challenge models, they included one strain in common and together they provide some interesting and useful data on the relative virulence of different isolates.

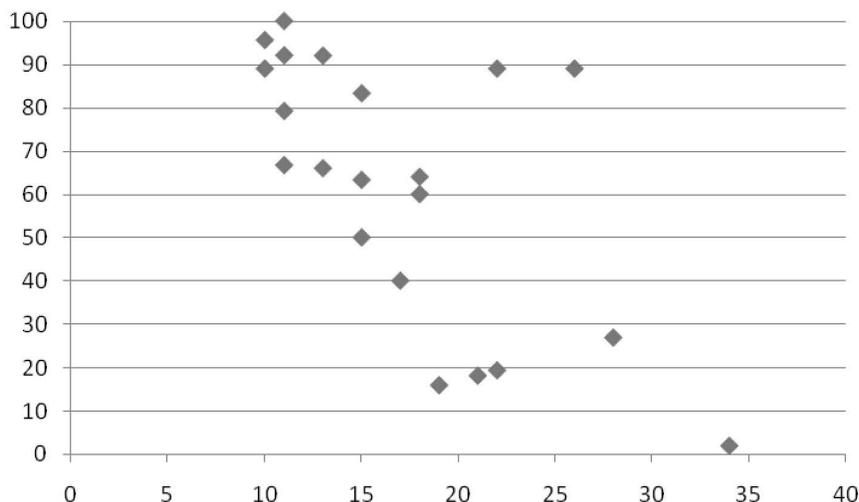
Summary data for the 16 ISAV isolates comprising 9 different HPR types included in the Canadian studies is shown in Table 1. A review of the data for these isolates reveals considerable variation in mortality among the different isolates. The majority of the data has been gathered using IP challenge models, however Ritchie et al.<sup>(9)</sup> performed a combined cohabitation-IP challenge and it is worth comparing the results from these two methods first. As we might expect fish infected by IP succumbed to disease more quickly (10-22 days post infection) than their cohabitant counterparts (22-34 days post infection) and typically experience greater mortality. However for strains with high mortality (e.g. isolates 970-1 and 61-1) this difference in mortality was not significant<sup>(9)</sup>. The delay in time to first mortality likely reflects a delay in exposure of the cohabitants to significant doses of pathogen. The difference in mortality between fish exposed to specific Hpr types by different methods is interesting and suggests that the Hpr 4 isolates are extremely efficient at entry and colonization of the fish no matter the method of exposure. This is in contrast to Hpr 2 and Hpr 5 isolates in which exposure to higher titres through IP infection effected higher overall mortalities.

Turning now to data from fish challenged by IP we can see wide variation in mortality. We can describe mortality as high (>75%+), medium (40-75%) and low (<40%) and we can see an inverse correlation between mortality level and time to first mortality (Fig. 1). This mirrors the disease progression seen in similar studies<sup>(13,15)</sup>.

As viral doses were standardized in the two studies, variation in mortality and variation in time to first mortality is not likely due to viral doses, but rather variation in viral type or differences in host. Although family-based variation in response to ISAV has been shown<sup>(13,16)</sup> the two studies here used pooled stocks of similar origin to minimize the influence of host variation on mortality. Thus, a significant portion of variation in virulence seen in Table 1 is due to differences in viral type.

One of the first things we observe is that isolates of both European and North American origin are capable of causing moderate to high mortality. This is consistent with field data showing high mortality associated with North American isolates. Few field outbreaks of European isolates have been identified and followed and it is difficult to assess how well this reflects field data. However, it suggests that identification of European isolates in the field, with the notable exception of the supposedly avirulent Hpr0, should be considered as significant causes of mortality and be managed accordingly until proven otherwise.

The data suggests that some Hpr types (Hpr 2, Hpr5 and Hpr7) are associated with low levels of mortality. This includes the Hpr 5 isolate (85-1) used in both studies, and supports anecdotal and published field studies<sup>(9)</sup>. However care must be taken when extrapolating results from these types



**Figure 1.** Comparison of mortality level vs. time to first mortality for fish exposed by IP. Graph showing cumulative mortality for ISAV isolates (in percentage on Y-axis) compared against day post infection on which mortality began (days, X-axis). Data compiled from isolates in Kibenge et al.<sup>(7)</sup> and Ritchie et al.<sup>(9)</sup>.

Isolate name	HPR type	Isolate origin	Geographic location	Stock	Infection method	Viral titre (TCID <sub>50</sub> )	Original study reference	Date of 1st mortality	Cum. mortality
300-2	2	NA	NB	SJR	Cohab	n/a	(9)	28	27.0
300-2	2	NA	NB	SJR	IP	4	(9)	17	40.0
u5575-1	3	Euro	NS	SJR	IP	5.8	(7)	18	64.0
61-1	4	NA	NB	SJR	Cohab	n/a	(9)	22	89.0
970-1	4	NA	NB	SJR	Cohab	n/a	(9)	26	89.0
61-1	4	NA	NB	SJR	IP	4	(9)	10	89.0
970-1	4	NA	NB	SJR	IP	4	(9)	13	92.0
85-1	5	Euro	NB	SJR	Cohab	n/a	(9)	34	2.0
04-085-1	5	Euro	NB	SJR	IP	5.5	(7)	21	18.2
85-1	5	Euro	NB	SJR	IP	4	(9)	19	16.0
390/98	7	Euro	Scotland	SJR	IP	6.13	(7)	11	79.2
485/9/97	14	Euro	Norway	SJR	IP	5.8	(7)	18	60.0
810/9/99	15	Euro	Norway	SJR	IP	6.13	(7)	11	92.1
NB98-0280-2	20	NA	NB	SJR	IP	5.8	(7)	11	66.7
ultNB01-0973-3	21*	NA	NB	SJR	IP	5.8	(7)	22	19.5
NB01-0593-1	21*	NA	NB	SJR	IP	5.8	(7)	15	50.0
NB02-0775-14	21*	NA	NB	SJR	IP	5.8	(7)	15	63.3
7833-1	21*	NA	Chile	SJR	IP	5.8	(7)	13	66.0
NB02-1179-4	21*	NA	NB	SJR	IP	6.3	(7)	15	83.3
NBISA01	21*	NA	NB	SJR	IP	5.8	(7)	10	95.6
NB98-049-1	21*	NA	NB	SJR	IP	5.8	(7)	11	100.0

**Table 1.**  
**Response of Atlantic Salmon to ISAV isolates with sequence variation in the HPR. (\*HPR21 is the designation used by Europeans to describe a deletion variant known as HPR 3 in North America).**

of studies. Although both hpr4 isolates studied here produced high mortality in the quarantine study, one of these isolates was associated with low mortality in the field<sup>(9)</sup> and isolates containing the Hpr21 motif show considerable variation in mortality (19.5-100%) in the quarantine study<sup>(7)</sup>.

The quarantine studies performed to date provide some useful information on mortality levels associated with different ISAV isolates and molecular types. However it is also clear that additional quarantine studies controlling for host and viral factors, and analysis of field data are required to extend these findings and pave the way for improved management of ISAV infections in the field.

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# Protein Hydrolysates and Trypsin Inhibitor Enhanced Digestive Capacities, Growth and Survival of Newly Hatched Spotted Wolffish

A Savoie<sup>1\*</sup>, NR Le François<sup>1</sup>, SG Lamarre<sup>2</sup>, PU Blier<sup>3</sup>, L Beaulieu<sup>4</sup>, and C Cahu<sup>5</sup>

<sup>1</sup> Université du Québec à Rimouski / Centre Aquacole Marin, Rimouski, QC G5L 3A1, CANADA (Tel: 418-385-2251 Fax: 418-385-3343 Email:

<sup>2</sup> Nathalie\_Le-Francois@uqar.ca; Arianne\_Savoie@uqar.ca)

<sup>3</sup> Memorial University of Newfoundland, St. John's, NL A1C 5S7

<sup>4</sup> Laboratoire de Biologie Évolutive, Université du Québec à Rimouski, QC G5L 3A1

<sup>5</sup> Centre de Technologie des Produits Aquatiques (CTPA), Gaspé, QC G4X 2V6

<sup>\*</sup> Unité Mixte INRA IFREMER de Nutrition des Poissons, B.P. 70, 29280 Plouzané, FRANCE

Spotted wolffish is a fish species particularly well suited for cultivation under cold northern climates. However, despite larval robustness, this species displays highly variable survival at first-feeding (40-80%). We propose to investigate the use of protein hydrolysates (pre-digested proteins (PH)) to improve survival and growth at first-feeding. To determine if protein digestion (as expressed by trypsin activity) is a growth limiting process, we also added a trypsin inhibitor (Soy Bean Trypsin Inhibitor, SBTI). Four different diets were evaluated: control standard feed (C); standard feed + protein hydrolysate 20% (H); standard feed + protein hydrolysate 20% + inhibitor (HI) and standard feed with inhibitor (I). As expected, diet I yielded the lowest survival rate ( $44.7 \pm 10.9\%$ ) compared to the control diet ( $67.3 \pm 3.5$ ). Inclusion of protein hydrolysates with or without the inhibitor had a positive impact on survival rate ( $82.7 \pm 5.7$  and  $84.7 \pm 5.9\%$  respectively). Enhanced palatability of the diet and therefore a stimulation to initiate first-feeding could be responsible. Trypsin inhibitor was detrimental only when PH was not present in the diet, indicating that PH favoured protein assimilation and accretion in the presence of trypsin inactivation. Surprisingly, the only diet that enhanced significantly growth compared to control diet is HI. This might be linked to an easier assimilation of peptides or to a concurrent overcompensation of trypsin secretion due to the presence of the protease inhibitor.



Arianne Savoie

## Introduction

Spotted wolffish is a marine fish species particularly well suited for culture in cold northern climates<sup>(1,2)</sup>. In the east of Québec (Canada), a project aimed at creating an experimental farm with a production of 10–20 metric tons is actually under evaluation. The advantages of this species include 1) their high growth rate at cold water temperature, 2) the low complexity of the larval-juvenile period and 3) their farming-friendly behaviour, all of which should facilitate technological transfer to an aquaculture industry that is currently solely based on salmonid culture. However, despite larval robustness, high variability of survival at first-feeding is still frequently reported.

Protein hydrolysates (PH) are pre-digested proteins that could enhance the larval performance of fish species based on the assumption that an “immature” digestive system limits nutrient absorption<sup>(3)</sup>. In several species, the inclusion of PH has been reported to have a positive effect on larval performance<sup>(4-7)</sup>.

Amount and activity of proteolytic enzymes as well as amino acid absorption are known to affect growth performance<sup>(8)</sup>. Furthermore, complete functionality of the digestive organs (i.e., the availability of digestive enzymes and of key metabolic enzymes) could set substantial physiological limitations on the growth and (or) survival of juveniles<sup>(9-11)</sup>.

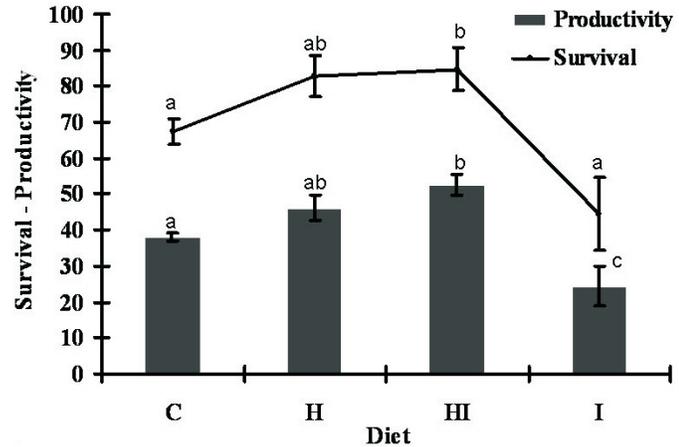
In this study, we have used a nutritional approach to enhance larval performances by the replacement of 20% protein fraction by PH. Also, in order to evaluate the implication of trypsin in larval digestion, we assessed the impact of the addition of soy bean trypsin inhibitor to the feed.

## Materials and Methods

### Experimental animals and rearing conditions

The study was carried out at the facilities of the Centre Aquacole Marin (Grande-Rivière, QC, Canada). Spotted wolffish eggs were incubated as described in Savoie et al.<sup>(12)</sup>. Fifty newly hatched fish (mean weight  $0.103 \pm 0.01$ g and mean length  $24.5 \pm 1.4$  mm) were randomly placed in each of the twelve low-level rearing units. Once a day, mortalities were recorded, dead fish removed and rearing units carefully cleaned. The different treatments (diets) were randomly assigned to the 12

**Figure 1.**  
Survival (%) and productivity (g/tank) (mean weight X survival) of spotted wolfish at the end of the experiment (60 DPH).



rearing units (4 diets in triplicate).

**Diets**

Fish were fed by hand each hour from 8am to 5pm for the entire experimental period. The four experimental diets contained exactly the same ingredients except for hydrolysates and soy bean trypsin inhibitor (SBTI) content. In the diets “C” and “I”, the protein fraction was fish meal. In the diets “H” and “HI”, 20% fish meal was replaced by an equal amount of protein hydrolysates (PH). The diets “I” and “HI” contained 750 mg/kg SBTI. The trypsin inhibitor was purchased from Sigma (T-9128) and PH were made from shrimp by-products (HPC90) (Ocean NutraSciences, Matane, Qc, Canada, www.oceannns.ca). The diets were formulated to be isonitrogenous and processed at Ifremer, Centre de Brest (France) as described in Savoie et al.<sup>(12)</sup>.

**Sampling and analysis**

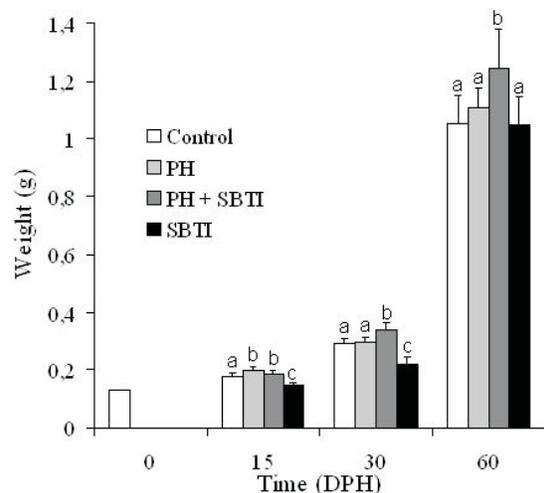
Twenty-five fish were sampled at day 0 from the initial fish stock. Thereafter, fifteen fasted fish per tank (18 hours before sampling) were weighed and measured at day 15, 30 and 60. Four of the fifteen fish were sampled and quickly frozen at -80°C until analysis (total: 205 fish). Whole individuals were thawed on ice and homogenised in 9 volumes of Tris-HCl buffer. Aspartate aminotransferase (AAT), trypsin (TRY) and chymotrypsin (CHY) were measured and total protein content was determined. Productivity was calculated as “mean weight x survival” in a particular tank.

**Results**

At the end of the trial, there was a significant effect of diet on survival and productivity (Fig. 1). Diet H enhanced non-significantly productivity and survival but diet HI enhanced both productivity and survival compared to the control group (p=0.02 and 0.085 respectively). Productivity and survival were significantly lower for group I compared to HI (p=0.001 and 0.006 respectively) and lower for group I compared to control for productivity only (p=0.029). At day 15, fish weight was higher for both groups receiving diets containing hydrolysates (H and HI, p<0.000 and p=0.002 respectively) and lower for the group I (p=0.021) compared to control (Fig. 2). At day 30 and 60, group H had the same mean weight as control but group HI was heavier (p=0.001 and 0.008 for day 30 and 60 respectively).

**Discussion**

Protein hydrolysates seem to promote the initiation of exogenous feeding behaviour in both H and HI experimental groups especially in the first 15 days. As a result, newly-hatched spotted wolfish that were offered PH showed improved survival and growth trajectories. Growth was improved in the first 15 days post-hatch in group H and throughout the experiment in group HI probably via an optimal supply of amino acids. Protein hydrolysates are also known to present a greater bioavailability<sup>(13)</sup> and to improve growth through stimulation of the digestive functions<sup>(14)</sup>.



**Figure 2.**  
Mean weight of spotted wolfish according to diet (C, H, HI, I) and days post-hatching (DPH).

The deleterious effect of the added SBTI was unequivocal in group I: weight was lower at day 15 and 30 in this group that also displayed the lowest survival rate (45%). These fish were probably lacking sufficient amount of amino acids due to the lack of trypsin proteolytic activity. Their digestive capacities were hampered by the presence of SBTI and contrarily to group HI, did not have access to pre-digested proteins to counterbalance. Hydrolysates incorporated in feed of group HI were providing the fish with all the essential and non essential amino acids needed for their normal development, thereby removing the negative effect of SBTI.

There was a clear beneficial effect of adding PH in combination with SBTI in the diet for newly-hatched spotted wolffish. It is quite surprising that only the group HI, containing the trypsin inhibitor, improved its growth after 60 days. Sveier et al.<sup>(15)</sup> realized a similar experiment on Atlantic salmon (185 g) and obtained best growth rates when both protease inhibitor and protein hydrolysate were added to the diet. Analysis of the enzymatic activities should help us determine the physiological adjustments involved; we suspect some kind of overcompensation in the presence of SBTI and PH.

## Acknowledgements

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# Effect of Rearing Density on Growth and Plasma Ion Levels of Juvenile Spotted Wolffish (*Anarhichas minor*): Preliminary Results

S Tremblay-Bourgeois<sup>1\*</sup>, NR Le François<sup>1</sup>, RL Roy<sup>2</sup>, TJ Benfey<sup>3</sup>, and AK Imsland<sup>4</sup>

<sup>1</sup>Université du Québec à Rimouski, Rimouski, QC, Canada G5L 3A1. Nathalie\_Le-Francois@uqar.ca

<sup>2</sup>Institut Maurice-Lamontagne, Pêches et Océans Canada, Mont-Joli, QC, Canada G5H 3Z4

<sup>3</sup>University of New-Brunswick, Fredericton, NB, Canada E3B 5A3

<sup>4</sup>University of Bergen, Postbox 7803, 5020 Bergen, Norway

**D**etermining the optimal rearing density of fish is important to maximise the productivity of aquaculture operations. We describe some initial findings from a density trial with juvenile (50-100 g) spotted wolffish (*Anarhichas minor*), a promising candidate for cold-water aquaculture. Initial rearing densities of 10, 20 and 40 kg/m<sup>2</sup> were used to compare growth and stress level. We allowed density to increase as the fish grew. Fish size and plasma ion (Na<sup>+</sup> and K<sup>+</sup>) concentration were measured at the start of the experiment (time 0) and at days 15 and 30 (all parameters), and 59 and 120 (size only). Preliminary results indicate that growth is better at 10 and 20 kg/m<sup>2</sup> than at 40 kg/m<sup>2</sup> (p<0.001) and that optimal rearing density of spotted wolffish of this size is around 30 kg/m<sup>2</sup> (~ 215 kg/m<sup>3</sup>).

## Introduction

Rearing density is a crucial parameter in aquaculture since space utilisation is directly related to costs and benefits<sup>(1)</sup>. Higher productivity can be obtained through a compromise between high densities and fast growth<sup>(2)</sup>. The optimal density is achieved when food conversion efficiency and growth rate are maximised<sup>(3)</sup>.

Fish requirements for space also represent important health and welfare concerns<sup>(3)</sup>. Consequences of keeping fish at too high densities include higher level of aggression (due to increased hierarchy and competition for food or territory), lower water quality and decreased feeding<sup>(3,4)</sup>. This may lead to chronic stress, resulting in energy transfer from growth to maintain of homeostasis<sup>(5,6)</sup>. A reduction in growth can also be seen at very low densities<sup>(7)</sup>, possibly because fish have more space available to develop hierarchies and express aggression. Optimal rearing density is highly species-specific<sup>(7-9)</sup> and may also vary according to age and life stage<sup>(10,11)</sup>. It is therefore essential to evaluate this parameter for every species used in aquaculture.

Spotted wolffish (*Anarhichas minor*) is a promising species for cold-water aquaculture<sup>(12)</sup> for which only a few studies have been conducted on optimal densities at a given size<sup>(13,14)</sup>. No studies could be found in relation to the stress response of this species. Rearing density is especially important for this species because its production will mainly be land-based. Wolffish are demersal and solitary, but they tolerate crowding well<sup>(15)</sup>. As for other demersal fish, rearing density in spotted wolffish is measured in terms of biomass per surface area rather than volume.

According to Imsland et al.<sup>(14)</sup>, adult spotted wolffish grow well at densities as high as 90 kg/m<sup>2</sup> and could possibly be kept at even higher densities. Juveniles, however, seem to be less tolerant. Jonassen<sup>(13)</sup> reared smaller spotted wolffish (0.5-1 kg) at initial densities of 25 and 40 kg/m<sup>2</sup> and found that growth was 10 % better at 25 kg/m<sup>2</sup>. However, no studies were conducted on smaller wolffish.

The aim of this study was therefore to determine the optimal rearing density for 50-100 g spotted wolffish.

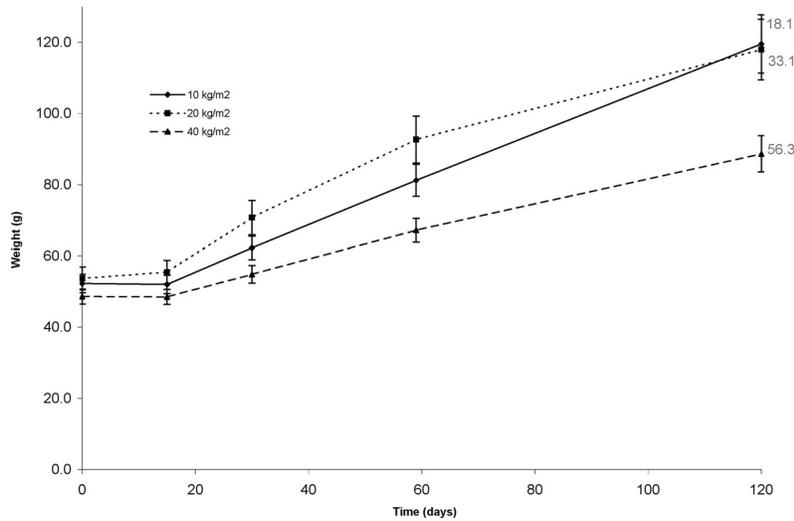
## Materials and Methods

This experiment was conducted at the Centre Aquacole Marin (Grande-Rivière, QC). A total of 432 tagged fish were held in tanks supplied with flow-through water (2 L/min) for 120 days during the summer of 2007. All fish were initially measured (weight and length) and blood samples were taken from 18 individuals for determination of initial plasma ion (Na<sup>+</sup> and K<sup>+</sup>) concentration (day 0). The fish were then randomly distributed among six tanks to give duplicate tanks at initial densities of 10, 20 and 40 kg/m<sup>2</sup> (~ 71, 142 and 148 kg/m<sup>3</sup>).

Fish were fed to satiation three times a week and kept under natural photoperiod. Temperature was 8.3 ± 0.8 °C, dissolved oxygen 84 ± 6 % saturation and salinity 28.7 ± 1.1 ppt. Nitrite and union-



Sarah Tremblay-Bourgeois



**Figure 1.** Weight gain over time. Number represent real densities in kg/m<sup>2</sup> at final sampling time.

ized ammonia concentrations remained very low ( $0.022 \pm 0.016$  mg/L and  $0.14 \pm 0.06$  mg/L, respectively) throughout the experiment. All the fish were measured after 15, 30, 59 and 120 days. Blood samples were collected from 3 individuals per tank on days 15 and 30 for plasma ion measurement, using an automatic ion chromatograph (model ICS-3000, Dionex, Oakville, ON, Canada).

Mean values of weight and length were compared by a nested two-way analyse of variance (ANOVA) using Systat 11 software. Data from replicate tanks were pooled after first demonstrating that there was no tank effect. A three-way nested ANOVA was per-

formed to compare Na<sup>+</sup> and K<sup>+</sup> concentration values. Tukey post-hoc tests were used when significant differences were found. Treatments were considered to be significantly different when  $p < 0.05$ . Results are shown as mean  $\pm$  standard error.

### Results and Discussion

There was a significant effect of density on weight gain over time ( $p < 0.001$ , Fig. 1). Fish initially stocked at 10 and 20 kg/m<sup>2</sup> showed better growth than those starting at 40 kg/m<sup>2</sup> ( $p < 0.001$ ). Fish at 20 kg/m<sup>2</sup> initially grew slightly better than those at 10 kg/m<sup>2</sup> ( $p = 0.040$ ), but after 59 days their growth seemed to decline. Density of the 20 kg/m<sup>2</sup> group reached approximately 30 kg/m<sup>2</sup> at this time, which suggests that over this latter density growth is depressed and that optimal rearing density is therefore around 30 kg/m<sup>2</sup>. Growth in length showed comparable trends (Table 1).

The condition factor increased from day 0 to 120 for all groups. Slightly lower final condition factor for the two higher densities might indicate suboptimal food ingestion and feeding methods.

No differences were detected among densities for ion values ( $p > 0.7$ ). Mean values ranged from  $170 \pm 8$  to  $192 \pm 5$  mmol/L for Na<sup>+</sup> and from  $3.49 \pm 0.06$  to  $4.89 \pm 0.76$  mmol/L for K<sup>+</sup> between 0 and 30 days. Imsland et al.<sup>(14)</sup> obtained similar values for spotted wolffish. Gomes et al.<sup>(2)</sup> also found no effect of rearing density on Na<sup>+</sup> and K<sup>+</sup> concentrations in tambaqui (*Colossoma macropomum*). Studies that have found changes in ion levels seem to be related more with acute stress than with long-term stress, such as high rearing density, and a fast return to baseline values is often reported<sup>(16,17)</sup>.

### Conclusion

Our results suggest that the optimal rearing density is around 30 kg/m<sup>2</sup> for juvenile spotted wolffish (50 g-100 g). To confirm this, a second growth trial is currently taking place with three fixed densities running in triplicate, at: 20, 30 and 40 kg/m<sup>2</sup>. Particular attention is being given to feeding methods.

**Table 1.** Weight, length and condition factor at the beginning and the end of the experiment.

Growth parameters	Sampling time (days)	Density (kg/m <sup>2</sup> )		
		10	20	40
Weight (g)	initial (0)	52.3 $\pm$ 2.6	53.8 $\pm$ 3.2	48.7 $\pm$ 2.1
	final (120)	119.6 $\pm$ 8.2	118.0 $\pm$ 8.5	88.7 $\pm$ 5.1
Length (cm)	initial (0)	18.6 $\pm$ 0.3	18.8 $\pm$ 0.4	18.4 $\pm$ 0.3
	final (120)	23.1 $\pm$ 0.4	23.4 $\pm$ 0.5	21.7 $\pm$ 0.3
Condition factor	initial (0)	0.78 $\pm$ 0.01	0.75 $\pm$ 0.01	0.75 $\pm$ 0.01
	final (120)	0.91 $\pm$ 0.02	0.84 $\pm$ 0.01	0.83 $\pm$ 0.01

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# Aquaculture Collaborative Research and Development Program (ACRDP): History and Future Initiatives

Christie L Whelan\* and G Jay Parsons

*Fisheries and Oceans Canada, Aquaculture Science Branch, 200 Kent Street, Ottawa, Ontario, K1A 0E6, Canada*

The Aquaculture Collaborative Research and Development Program (ACRDP) aims to increase the level of collaborative research and development activity between the Canadian aquaculture industry and Fisheries and Oceans Canada (DFO). The industry-driven program has been in operation since 2001 and is jointly funded by DFO and industry partners. The ACRDP funding envelope is \$4.5 million per year (subdivided regionally), and must be matched by a minimum industry contribution of 30% of the ACRDP amount requested (7.5% in-cash, 22.5% in-kind). Since the beginning of the program (2001-2008), over 230 projects have been approved and funded. In total, over \$56.8 million in research has been conducted through the ACRDP. This includes \$25.9 million in ACRDP funds, \$12.7 million from industry contributions, \$13.0 million in other DFO funding and \$5.2 million in contributions from other project partners. Program history, research priorities, and future plans for the program discussed at a recent national ACRDP stakeholders meeting in Ottawa will be discussed.



Christie Whelan

## Introduction

The Aquaculture Collaborative Research and Development Program (ACRDP) was launched in 2001 as part of the Program for Sustainable Aquaculture. The ACRDP is a Fisheries and Oceans (DFO) initiative to increase the level of collaborative research and development between the Canadian aquaculture industry and DFO. The program receives \$4.5 million per year in research funds. These funds must then be matched by a minimum 30% contribution from the industry partner (7.5 % in cash, 22.5 % in kind).

The key goals of the ACRDP are to improve the competitiveness of the Canadian aquaculture industry, to increase collaboration between the department and industry on scientific research and development that will enhance aquaculture in Canada, to facilitate and accelerate the process of technology transfer and research commercialization through closer collaboration with the Canadian aquaculture industry, and to increase scientific capacity for essential aquaculture research and development in the aquaculture sector. The program has three broad research and development objectives; best performance in fish production, optimal fish health and industry environmental performance.

## Structure of the Program

The ACRDP is managed by a two-tiered system. The National Steering Committee is responsible for the allocation of regional budgets, national priority setting and the allocation of the national fund. The National Steering Committee membership is composed of DFO representation (two from science and one from aquaculture management), four provincial members from the Canadian Council of Fisheries and Aquaculture Ministers – Aquaculture Task Group, five members representing the aquaculture industry (one from the Canadian Aquaculture Industry Alliance, one from the Aquaculture Association of Canada and three members representing regional industry associations), and one member representing an environmental NGO. Each region has a Regional Management Committee with membership structure similar to that of the National Steering Committee. The Regional Management Committee membership is chosen to best represent the cross-section of the aquaculture industry in that region. The Regional Management Committees are responsible for setting the regional priorities, for the evaluation of research proposals and for the allocation of the regional ACRDP funds. The ACRDP secretariat (part of the Aquaculture Science Branch), coordinates and manages the program from the DFO office in Ottawa.

## Program Overview

Since the beginning of the program in 2001, over 230 projects have been approved and funded. Of these, 55% of the projects fell under the best performance in fish production priority, 24% of the projects focused on environmental performance and 21% on optimal fish health. Research projects

**Table 1.**  
Regional breakdown of number of projects, ACRDP funding and total leveraged contributions.\*

Region	Number of projects funded	ACRDP funding	Total leveraged funding
Pacific	72	\$6 800 000	\$15 300 000
Central and Arctic	18	\$3 400 000	\$7 200 000
Quebec	43	\$3 800 000	\$8 300 000
Maritimes and Gulf	90	\$8 000 000	\$19 100 000
Newfoundland	12	\$3 900 000	\$6 900 000

have focused on over 25 different species included many marine and freshwater finfish species, several types of shellfish and some projects involving algae and invertebrates such as crayfish and urchins.

The ACRDP is administered by five regional management committees. The Maritimes and the Gulf regions are administered together by one Regional Management Committee. Each region is allocated a portion of the overall \$4.5 million per year to fund research projects. Given that each region is challenged by a different set of priority issues, the number of projects and amount of leveraged funds varies regionally (Table 1).

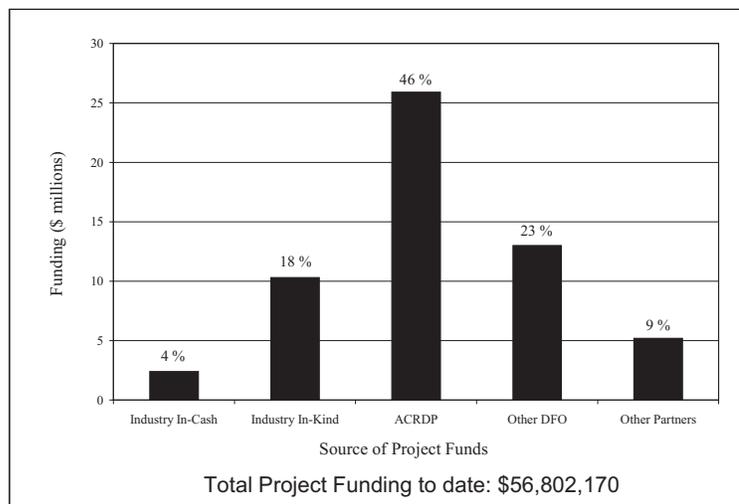
Since the beginning of the program, \$25.9 million in ACRDP funds has been committed to research projects (Fig. 1). The ACRDP funds have been leveraged by \$12.7 million in industry contributions (cash and in-kind) and \$5.2 million from other project partners. Over the course of the program, DFO has contributed an additional \$13 million on top of the \$4.5 million ACRDP yearly allocation. In total, over \$56.8 million in research has been funded through the ACRDP.

Of the \$4.5 million annual ACRDP envelope, \$300 000 remains in a National Fund. Any funds not allocated in the regions through calls for proposals are also rolled into the National Fund later in the year. The National Fund is distributed by the National Steering Committee and serves several purposes. Research for high priority or emergency issues can be funded through the National Fund. Two recent examples of the national money being used in this situation include money that was allocated towards dealing with aquatic invasive species in PEI and a project in Maritimes and Gulf looking at the withdrawal times for SLICE®. Part of the National Fund has often been used towards funding communications initiatives such as the Canadian Aquaculture R&D Review (volumes I and II), the design and writing of fact sheets for completed projects and the development of AquaPort.

### Moving into the Future

A large meeting was held in Ottawa at the beginning of March 2008 that included all Regional Management and National Steering Committee Members, the Canadian Aquaculture Industry Alliance board members and several other significant stakeholders. The meeting focused on the achievements of the program and discussion for a path forward ensuring the success of future collaborative research and benefits to the Canadian aquaculture industry. Discussion during the meeting indicated that overall the ACRDP is delivered well and the stakeholders are pleased with the management of the program. Greater collaboration between scientists, regions and academia, and taking advantage of leveraging opportunities were noted as areas for improvement. The need for increased and better communications on aquaculture issues and project results resonated as the most important issue that the ACRDP needs to focus and improve upon.

**Figure 1.**  
Funds leverage by the ACRDP program through industry, DFO and other partner contributions. Please note that all funding values presented are calculated based on the ACRDP expenditures from 2001-2006 and the allocated amounts from the 2007/08 and 2008/09 fiscal years. All values presented are up-to-date based on information in the ACRDP database as of April 30, 2008, any updates/changes after this date are not reflected.



## **AQUA-L – the AAC Aquaculture Discussion Group**

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# Aquaculture Canada<sup>OM</sup> 2008 Committees

## Conference Organizing Committee

### **Alistair Struthers - AAC President and Conference Chair**

Caroline Graham – AquaFair President  
Cyr Couturier - Program Chair  
Joanne Burry - Conference Coordinator  
Catriona Wong - Aquaculture Association of Canada  
Susan Waddy - Aquaculture Association of Canada  
Tim Jackson – National Research Council – IRAP

## Program Committee

### **Cyr Couturier - Chair**

Alistair Struthers – AAC President  
Caroline Graham – AquaFair President  
Andrew Vickerson - Contributed papers  
Tim Jackson – Chair Local Organizing Committee  
Ruth Salmon – Canadian Aquaculture Industry Alliance  
Debbie Martin-Robichaud – AAC President-elect  
Bettie Arsenault – Professional Shellfish Growers Association of New Brunswick  
Jules Gaudet - Professional Shellfish Growers Association of New Brunswick  
Jason Mullen - Awards and Student Affairs  
Linda Duncan – Prince Edward Island Aquaculture Alliance  
Rod Penney – Canadian Food Inspection Agency  
Brian Muise – Aquaculture Association of Nova Scotia  
Joanne Burry – Conference Coordinator

## Local Organizing Committee

### **Tim Jackson – Chair**

Caroline Graham – AquaFair President  
Sybil Smith - New Brunswick Salmon Growers Association  
Bettie Arsenault – Professional Shellfish Growers Association of New Brunswick  
James A. Smith - New Brunswick Salmon Growers Association  
Charlane Hatt - New Brunswick Department of Agriculture and Aquaculture  
Karen Coombs - New Brunswick Department of Agriculture and Aquaculture  
Scott Walker – Atlantic Canada Opportunities Agency  
Amber Garber – Huntsman Marine Science Centre  
Thierry Chopin – UNB Saint John  
Eddy Kennedy – Fisheries and Oceans Canada  
Nell Halse – Cooke Aquaculture Inc.  
Jason Mullen – Student Affairs Committee Chair  
Joanne Burry - Conference Coordinator