

# Aquaculture Canada<sup>OM</sup> 2010 and Cold Harvest<sup>TM</sup> 2010

Proceedings of Contributed Papers

AAC Special Publication No. 17

CONFERENCE AND TRADESHOW

COLD HARVEST<sup>TM</sup> 2010



AQUACULTURE CANADA<sup>OM</sup> 2010

SUCCESSFUL PARTNERSHIPS FOR A SUSTAINABLE FUTURE

St. John's, Newfoundland and Labrador  
May 16-19, 2010

Tillmann Benfey and Gregor K Reid  
Editors



Aquaculture Canada<sup>OM</sup> 2010 and Cold Harvest<sup>TM</sup> 2010 – Proceedings of the Contributed Papers of the 27<sup>th</sup> Annual General Meeting of the Aquaculture Association of Canada, St. John's, Newfoundland and Labrador, May 16–19, 2010.

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Tillmann J. Benfey and Gregor K. Reid, editors

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**Aquaculture Canada<sup>OM</sup> and Cold Harvest<sup>TM</sup> 2010**

May 16-19, 2010

St. John's, Newfoundland and Labrador

Proceedings of Contributed Papers

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Gynogenesis and the genetic basis of sex determination of Atlantic cod (*Gadus morhua*)

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## President's Report

### **Aquaculture Canada<sup>OM</sup> 2010: A Retrospective**



Aquaculture Canada<sup>OM</sup> 2010 (AC10) was our 27<sup>th</sup> annual meeting, and the national forum on the science, technology and business of Canadian aquaculture. We were very fortunate to have partnered with the Newfoundland Aquaculture Industry

Association (NAIA) and the Government of Newfoundland and Labrador to co-host this event for the 4<sup>th</sup> time in the past three decades. The NAIA held its annual meeting jointly with AC10 and the synergies were tremendous.

By all accounts the attendance at the meetings was excellent, with producers, academics and government officials exceeding 350. More than 100 of the attendees were directly involved in aquaculture production. All social events sold out. The ever-enjoyable Dr. Joe Brown Aquaculture BBQ raised a record amount towards the Student Endowment Fund this year, and we truly appreciated the product donations from the aquaculture industry and the contributions of the Marine Institute in helping make this a success. Our major supporters for the joint conferences are listed in the next pages of these proceedings and we are extremely proud to acknowledge their contributions.

The theme of the meetings was “*Successful partnerships for a sustainable future*” and this reflects on the need to work in partnership among industry, academia, governments and other key stakeholders to provide for long term sustainable socioeconomic benefits for our coastal and rural areas of the country. The value of aquaculture in

Canada exceeds \$2 billion annually and we have barely tapped the potential. Aquatic protein is the most important source of a healthy daily diet for over 3 billion people on the planet, and the demand is growing. Canada has an increasingly important role to play in meeting this demand. Our conference agenda provided a good overview of how this challenge will be taken up by this important sector of the Canadian, and global economy. The successful program and logistics for the conferences would not have been possible without the leadership and support of our Program Chair, Tillmann Benfey, nor our Conference Coordinator, Joanne Burry, and for this I am personally indebted.

Lastly, the Aquaculture Association of Canada will continue to bring this national forum – Aquaculture Canada - to the Canadian aquaculture sector, to exchange ideas, and hopefully find solutions to development constraints in the future. I look forward to seeing many of you at these meetings in the future.

*Cyr Couturier*

*Conference Chair AC10 and NAIA CH10*

*President, Aquaculture Association of Canada  
2009-2010*

## **Aquaculture Canada<sup>OM</sup> 2010: Une rétrospective**

Aquaculture Canada 2010 a été notre 27<sup>e</sup> réunion annuelle, et le forum national sur la science, la technologie et les affaires en aquaculture canadienne. Nous avons été très chanceux d'avoir un partenariat avec le Newfoundland Aquaculture Industry Association (NAIA) et le gouvernement de Terre-Neuve-et-Labrador à la co-hôte de cet événement pour la 4<sup>ème</sup> fois au cours des trois dernières décennies. La NAIA a tenu sa réunion annuelle conjointement avec AC10 et les synergies ont été énormes.

Par tous les comptes la participation aux réunions a été excellente avec des producteurs, des universitaires et des responsables gouvernementaux de plus de 350. Plus de 100 des participants ont été directement impliqués dans la production aquacole. Le très agréable BBQ Dr. Joe Brown Aquaculture a recueilli un montant record pour le Fonds de dotation des étudiants cette année, et nous avons vraiment apprécié les dons de produits de l'industrie et les contributions du Marine Institute pour en faire un succès. Nos principaux sources de soutien pour les conférences conjointes sont répertoriés dans les pages suivantes de ce compte-rendu et nous sommes extrêmement fiers de reconnaître leurs contributions.

Le thème de ces rencontres était «Les partenariats réussis pour un avenir durable», ce qui reflète sur la nécessité de travailler en partenariat entre l'industrie, les universités, les gouvernements et autres intervenants clés pour assurer à long terme durable des retombées socioéconomiques pour les régions côtières et rurales de notre pays. La valeur de l'aquaculture au Canada dépasse 2 milliards de dollars par an et nous avons à peine exploité le potentiel. La protéine aquatique est la source la plus importante d'une saine alimentation

quotidienne depuis plus de 3 milliards de personnes sur la planète, et la demande est croissante. Le Canada a un rôle de plus en plus important à jouer pour répondre à cette demande. Notre programme de la conférence donne un bon aperçu de la façon dont ce défi sera relevé par ce secteur important du secteur canadien, et de l'économie mondiale. Le succès du programme et de la logistique pour les conférences n'aurait pas été possible sans le leadership et le soutien de notre président pour le programme, Tillmann Benfey, ni notre coordonnatrice de la conférence, Joanne Burry, et pour cela je suis personnellement redevable.

Enfin, l'AAC continuera d'apporter ce forum national - Aquaculture Canada - pour le secteur canadien de l'aquaculture, d'échanger des idées, et nous espérons trouver des solutions aux obstacles au développement dans l'avenir. J'ai hâte de vous retrouver nombreux lors de ces réunions à l'avenir.

*Cyr Couturier*

*Président de la Conférence AC10 et NAIA  
CH10*

*Président, Association aquacole du Canada  
2009-2010*



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- Department of Fisheries and Aquaculture, Province of Newfoundland and Labrador
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## Aquaculture Association of Canada – Lifetime Achievement Award



Al Castledine retired in the spring of 2010 from his position as Director of Aquaculture Policy, British Columbia Ministry of Agriculture and Lands. A degree in English and a desire to experience some of the world took him to Tanzania, East Africa with CUSO for several years in the early 1970's teaching English and Biology in a boys' secondary school. It was there that the idea came up to come back to Canada to pursue a passion for fish with the hope of some day returning to Tanzania to work on artisanal aquaculture. This was a simple, perhaps naïve, idea that has taken a number of twists over the years given the complexity of marriage, children and work. After Tanzania, a couple of years of undergraduate courses lead into a Master's Program in fish nutrition at the University of Guelph followed by a year's research on Asian carps in Malaysia compliments of a CIDA scholarship. Then off to the University of Victoria for a PhD in biochemistry focusing on fat metabolism in rainbow trout supported in part by NRC. A year in Ottawa (1981-1982) working with the Department of Fisheries and Oceans producing a report on the fish feed industry, amongst other duties, followed. Then off to Ontario from 1982 until 1987 as an aquaculture extension biologist with the Ontario Ministry of Natural Resources. In 1987 the opportunity to return to the west coast came up initially as aquaculture production specialist with the British Columbia government succeeded by various roles including both management and Director positions in seafood and aquaculture development in marine and fresh waters. In 2001, Al worked with DFO in Ottawa in an interchange agreement – it was an exciting time – the federal aquaculture policy framework was being developed, the Office of the Commissioner for Aquaculture Development was in full swing and many other new initiatives were being discussed and implemented.

Indeed, the ten years between 1999 and 2009 saw a lot of engagement by the Provinces with the federal government on aquaculture through the Canadian Council of Aquaculture Ministers Aquaculture Task Group (Al co-chaired the Task Group for a number of years). This Task Group provided an opportunity for the Provinces to shape and to support a number of key federal initiatives such as the National Aquatic Animal Health Program, the Aquaculture Collaborative Research and Development Program, and the significant resources currently deployed within the Department to support industry Market Access Program among others.

Several years ago, as Director of Aquaculture Policy in British Columbia, Al took the initial first steps toward aquaculture development focused on communities and area and ecosystem based approaches to management. Two conventional industry development positions were re-profiled to focus on social licence issues. These actions are recognition that social licence and not technology (at the moment) is the most important factor hindering further growth of aquaculture in British Columbia (and probably lots of other places).

Al was, for many years a member of the British Columbia Institute of Agrologists, taking the steps to qualify to become a member because of the logical connections between aquaculture and

agriculture (the irony of the recent British Columbia court decision declaring aquaculture to be a fishery, notwithstanding). He may have been the first Professional Agrologist in Canada to come from an aquaculture background and was recognized as Agrologist of the year for Victoria and the Islands Branch in 2000.

Al has been a member of AAC for many years and served on the Board in several capacities and as president in 1994-1995.

He would like to recognize the many wonderful and talented people he has met and worked with in what has been a very challenging and rewarding 35 years in aquaculture research, extension and management. As for Tanzania, Al and his wife Birgit, headed there in June 2010 to explore volunteer opportunities with high hopes that these will concern aquaculture.

### Association Aquacole du Canada – récompense de réussite de toute une vie

Al Castledine s'est retiré au printemps de 2010 de sa position comme directeur de la politique en aquaculture pour le ministère de l'agriculture et des terres de la Colombie-Britannique. Armé avec un baccalauréat en anglais et un désir de voyager, il s'est retrouvé en Tanzanie, Afrique de l'Est, avec CUSO pendant plusieurs années au début des années 70. Il enseigna l'anglais et la biologie dans une école secondaire pour garçons. C'était là que l'idée lui est venue de revenir au Canada pour poursuivre sa passion pour les poissons avec l'espoir d'un jour de retourner en Tanzanie pour travailler sur l'aquaculture artisanale. C'était une idée simple, peut-être un peu naïve, cette idée qui a pris un certain nombre de torsions au fil des ans avec la complexité du mariage, les enfants et le travail. Après la Tanzanie, quelques années au baccalauréat le porte vers une maîtrise en nutrition des poissons à l'université de Guelph, suivi par un projet de recherche sur la carpe asiatique en Malaisie par l'entremise d'une bourse de l'ACDI. Ensuite il retourna au Canada pour compléter son doctorat en biochimie sur le métabolisme de la truite arc-en-ciel, soutenu en partie par le CNR. Après les études il a passé un an à Ottawa (1981-82) avec le MPO pour faire un rapport sur l'industrie de l'alimentation des poissons avant de se trouver en Ontario pendant les prochaines cinq années (1982-87) comme biologiste pour le ministère des ressources naturelles. En 1987, l'occasion de se retrouver en Colombie-Britannique se présenta et il est retourné comme spécialiste en production aquacole pour le gouvernement de la Colombie-Britannique. Pendant les 15 prochaines années il fut gestionnaire et directeur pour le développement aquacole et des pêches au sein du Gouvernement de la C.-B. Il prit un poste d'échange à Ottawa en 2001 et c'était un temps très passionnant – le développement de la politique aquacole était en cours, le bureau du commissaire au développement de l'aquaculture était en pleine activité et les choses semblaient brasser.

En effet, les dix années entre 1999 et 2009 ont vu beaucoup d'enclenchement sur l'aquaculture par les provinces et territoires avec le gouvernement fédéral par le biais du groupe de travail du Conseil canadien des ministres des pêches et de l'aquaculture (Al fut co-président du GT pendant plusieurs années). Ce groupe de travail a pourvu un forum pour la participation des provinces dans l'élaboration d'un certain nombre d'initiatives fédérales, notamment le programme

aquatique national de santé animale, le programme de collaboration de recherche et développement en aquaculture, et le programme d'accès au marché d'industrie et qui sont tous en vigueur aujourd'hui.

Il y a plusieurs années, quand il était directeur de la politique en aquaculture pour la Colombie-Britannique, Al a pris les premières mesures envers le développement de l'aquaculture concentré sur les communautés et la gestion des ressources à base des écosystème. Deux positions d'agent de développement ont été reprofilées pour se concentrer sur les questions de comptabilité sociale. Ceci a démontré que le permis social, et non les développement en technologie, est le défis le plus important à l'heure actuelle qui empêche la croissance de l'aquaculture en Colombie-Britannique (et probablement un bon nombre d'autres endroits au pays).

Al était membre de l'institut d'agologie de la Colombie-Britannique pendant plusieurs années, en raison des raccordements logiques entre l'aquaculture et l'agriculture (malgré la décision récente du Tribunal de la Colombie-Britannique déclarant l'aquaculture comme étant de la pêche). Il a été le premier agronome professionnel au Canada venu de l'aquaculture et a été reconnu comme Agronome de l'année pour Victoria et les Îles en 2000.

Al a été un membre de l'AAC pendant beaucoup d'années et a servi sur le conseil d'aministration dans plusieurs capacités, incluant comme président en 1994-1995.

Il voudrait rendre hommage et reconnaître les nombreuses personnes merveilleuses et douées qu'il a rencontrées et a travaillées avec dans ce qui a été une carrière très enrichissante pendant les 35 dernières années en recherche et développement, en gestion et politique aquacole. Quant à la Tanzanie, Al et son épouse Birgit, se sont dirigés là en juin 2010 pour explorer les occasions volontaires avec des grandes espérances que ceux-ci concerneront l'aquaculture.

## Physical and biochemical properties of particles released from an onshore Atlantic cod *Gadus morhua* aquaculture facility in the context of integrated multi-trophic aquaculture

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### Abstract

To evaluate fish effluent as a food source for integrated multi-trophic aquaculture, particles released from an onshore aquaculture facility growing juvenile Atlantic cod (*Gadus morhua*) were collected. Particle distributions were determined with image analysis and a Coulter Multisizer, and samples were taken for dry weight, lipids and fatty acids. Effluent was filtered into three size fractions, <70  $\mu\text{m}$ , 70-500  $\mu\text{m}$  and >500  $\mu\text{m}$ , which comprised 36%, 31% and 33% of the dry mass, respectively. Particle diameter varied from 0.1  $\mu\text{m}$  to 2.4 mm, with the majority of particles in the 0.1 to 49  $\mu\text{m}$  range. Particles <70  $\mu\text{m}$  and >500  $\mu\text{m}$  had settling rates of 0.02 mm/sec and 0.04 cm/sec, respectively, so the 36% of effluent available for mussel consumption is that which has a greater potential to spread to surrounding areas. The top three lipid classes were free fatty acids (37-81%), phospholipids (4-21%) and triacylglycerols (6-15%). The essential fatty acids DHA and EPA, along with two zooplankton markers, were also present. Feeding experiments indicated mussels do ingest material in fish effluent.

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### Introduction

Aquaculture has several concerns associated with it such as water treatment<sup>1</sup> and unsustainable monoculture practices<sup>2</sup>. It has been suggested that aquaculture practices should focus on the use of extractive organisms as a form of bioremediation to create a more sustainable aquaculture<sup>3</sup>. The goals of this study are to describe the physical and biochemical properties of waste leaving an onshore Atlantic cod (*Gadus morhua*) aquaculture facility, and to determine its availability for ingestion by the blue mussel (*Mytilus edulis*). Mussel performance when reared on fish effluent as a diet was also examined in the context of integrated multi-trophic aquaculture.

### Methods

Effluent leaving an onshore juvenile Atlantic cod aquaculture facility was collected over a period of two weeks. Effluent was divided into three size fractions (<70  $\mu\text{m}$ , 70-500  $\mu\text{m}$  and >500  $\mu\text{m}$ ), and each was sampled for particle size, particle distribution, settling rate, dry weight, lipid and fatty

acid profile. Particle size distributions were determined using a Coulter Multisizer and image analysis. In order to avoid feces breaking into smaller particles, samples were gently swirled before analysis and the Multisizer's automated stirrer was not used. Lipid profile was determined using an Iatroscan Mark V TLC-FID and fatty acid profiles using a HP 6890 Series GC. A six-month feeding trial was undertaken to compare *M. edulis* growth on cod effluent compared to a commercial algal shellfish diet. Mussels were sampled on a monthly basis for shell length, dry weight, ash free dry weight, condition index, CHN content, lipid and fatty acid profile.

### Results and Discussion

A quarter of the mass fed to the fish left the tanks on a daily basis in the form of effluent, which is comparable to the 15-25% previously recorded<sup>4</sup>. The largest portion of effluent was found to be <70  $\mu\text{m}$  (36%), followed by >500  $\mu\text{m}$  (33%) and 70-500  $\mu\text{m}$  (31%). *M. edulis* selectively reject a significant proportion of particles >22.5  $\mu\text{m}$ <sup>5</sup> would therefore only a portion of the <70  $\mu\text{m}$  fraction be



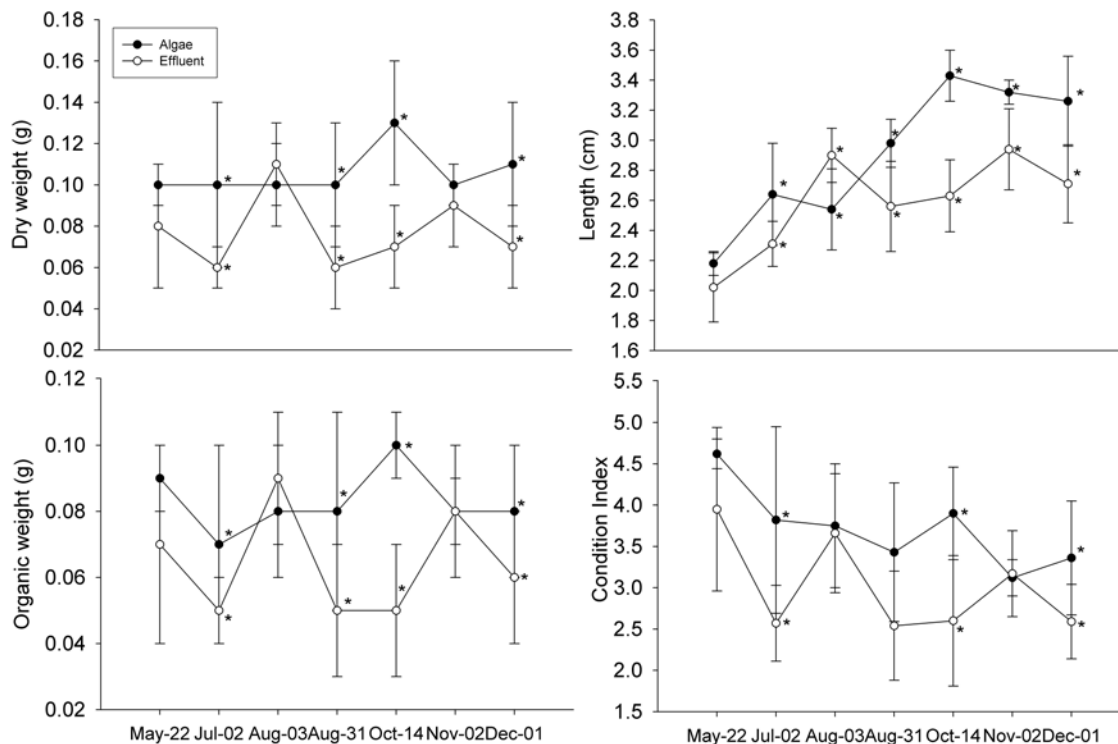


Figure 1. Dry weight, organic (ash-free) dry weight, shell length and condition index for mussels reared on algae and fish effluent during a six-month growth experiment. \* denotes a significant difference between diets at a given sampling date.

ingested. Particles 0.1 to 49  $\mu\text{m}$  were more numerous but comprised a smaller portion of the volume than larger particles. This is comparable to a previous study<sup>6</sup> in which the majority of particles in effluent from an Atlantic salmon hatchery were <20  $\mu\text{m}$  but the largest portion of the volume was occupied by larger particles. Particles >500  $\mu\text{m}$  settled at a rate of 2.6 to 0.04 cm/sec while particles <70  $\mu\text{m}$  settled at a rate of 0.02 mm/sec. This implies that the portion of effluent available for mussel ingestion has a greater potential to spread to surrounding areas due to its increased time in suspension. Particles <70  $\mu\text{m}$  had significantly lower lipid content ( $1.8 \pm 1.1\%$ ) than those 70-500  $\mu\text{m}$  ( $14 \pm 2.5\%$ ) and >500  $\mu\text{m}$  ( $7.4 \pm 3.6\%$ ). Lipid class composition was similar across size fractions; the bulk of lipids were free fatty acids (37-81%) followed by phospholipids (4-21%) and triacylglycerols (6-15%). Free fatty acids are breakdown indicators and have been reported to indicate the presence of cod feces in effluent<sup>7</sup>. Two essential fatty acids, DHA (22:6 $\omega$ 3) and EPA

(20:5 $\omega$ 3) were present in the effluent at 2.7 and 2.6%, respectively. Three zooplankton markers (20:1 $\omega$ 9, 20:1 $\omega$ 11 and 22:1 $\omega$ 11) were present at 2.1, 0.3 and 3.2%, respectively. These can be used to determine if mussels actually ingest the waste material by determining their presence/absence in mussels before and after being fed effluent.

Total and organic (ash free) dry weights of mussels fed both diets did not change significantly during the feeding experiment but shell length increased and condition index (dry weight/shell length  $\times 100$ ) decreased significantly on both diets (Figure 1). Carbon content decreased from  $444 \pm 8.5$  and  $450 \pm 11$  to  $425 \pm 7.5$  and  $426 \pm 8.8$  mg/g for mussels fed algae and effluent, respectively. Nitrogen content increased for mussels fed both diets but those fed effluent had significantly more nitrogen. Protein content, calculated from nitrogen via a conversion factor of 5.8<sup>8</sup>, was  $592 \pm 27$  and  $628 \pm 17$  for algal and effluent fed mussels respectively at the end of the experiment which is comparable to

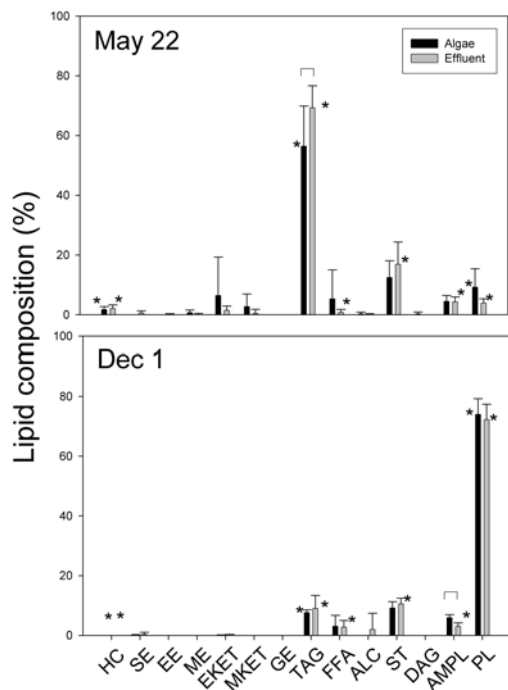


Figure 2. Lipid composition of mussels reared on algae and fish effluent at the start and end of a six-month growth experiment. Brackets denote a significant difference between diets a given sampling date and \* denotes a difference between dates for a given diet. (HC – hydrocarbon, SE – steryl ester, EE – ethyl ester, ME – methyl ester, EKET – ethyl ketone, MKET – methyl ketone, GE – glycerol ether, TAG – triacylglycerol, FFA – free fatty acids, ALC – alcohol, ST – sterol, DAG – diacylglycerol, AMPL – acetone mobile polar lipids and PL – phospholipids.)

the 44-57% dry weight recorded for *Mytilus trossulus*<sup>9</sup>. Lipid content decreased from  $2.6 \pm 0.77$  and  $2.4 \pm 1.1$  to  $1.5 \pm 0.79$  and  $1.7 \pm 1.3$  % wet weight for mussels fed algae and effluent, respectively. Lipid class composition changed significantly throughout the experimental, with a decrease in the proportion of triacylglycerols and an increase in proportion of phospholipids for both diets. Acetone-mobile polar lipids comprised a larger proportion of lipid in algal fed mussels than in those fed effluent (Figure 2). Fatty acid content decreased from  $16.5 \pm 4.4$  and  $19.5 \pm 10.4$  to  $9.8 \pm 5.4$  and  $10.9 \pm 7.6$  mg/g for algae and effluent fed mussels, respectively. Mussels fed effluent had a

significantly higher percent composition of the essential fatty acid DHA as well as two zooplankton markers (20:1 $\omega$ 9 and 20:1 $\omega$ 11) and the non-methylene interrupted diene (NMID) 20:2, while algae-fed mussels had a larger proportion of the essential fatty acid arachidonic acid (20:4  $\omega$ 6). The increased proportion of zooplankton markers in mussels offered effluent suggests that ingestion did occur, but the increased proportion of NMIDs suggests they may have been deficient in essential fatty acids as NMIDs have been speculated to be used as a substitute for essential fatty acids<sup>10</sup>.

## Conclusions

The 36% fraction of effluent from a cod aquaculture facility that is available for mussel ingestion is that with a greater capacity to spread to surrounding areas. Effluent does contain some essential FAs (DHA and EPA) for mussel growth and mussels do ingest effluent. Finally, zooplankton markers can potentially be used as an ingestion indicator.

## Acknowledgements

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## Aquaculture Collaborative Research and Development Program (ACRDP)



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### **Abstract**

The Aquaculture Collaborative Research and Development Program (ACRDP) is an industry driven research and development activity aimed at increasing the level of collaborative research between the aquaculture industry and Fisheries and Oceans Canada (DFO). This program has been running since 2001 and is jointly funded by DFO and industry partners. The ACRDP funding envelope is \$4.275 million annually (subdivided regionally), and must include a minimum industry contribution of 30% of the ACRDP amount requested (7.5% in-cash, 22.5% in-kind). There are three main research and development objectives to the program: (1) best performance in fish production, (2) optimal fish health, and (3) industry environmental performance. To date, over 290 projects have been approved and funded, totaling over \$66 million in aquaculture research. This includes minimum contributions of \$30 million in ACRDP funds, \$14.8 million from industry contributions, \$16.1 million in other DFO funding and \$5.2 million from other partners. The ACRDP also supports a number of projects on issues of national importance such as sea lice and Bacterial Kidney disease (BKD), national workshops and communications initiatives, including the development of project fact sheets, the bi-annual R&D Review and, most recently, an aquaculture science promotional video summarizing current DFO aquaculture research being undertaken in Canada. The program continues to strive for improvement through stakeholder engagement by seeking feedback on key priorities that address solutions for the sustainable development of the sector.

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### **Introduction**

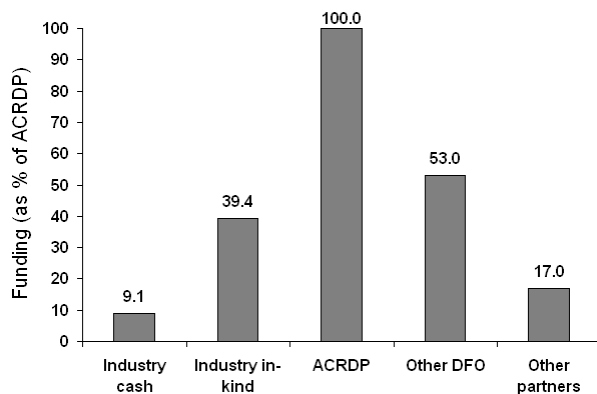
The Aquaculture Collaborative Research and Development Program (ACRDP) was launched in 2001 as part of the Program for Sustainable Aquaculture. The ACRDP is a Fisheries and Oceans (DFO) initiative to increase the level of collaborative research and development between the Canadian aquaculture industry and DFO. The program receives \$4.25 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



**Figure 1:** Funds leveraged by the ACRDP program through industry, DFO and other partner contributions, based on ACRDP expenditures from 2001-2008 and the allocated amounts from the 2009-2010 fiscal year. Values are up-to-date based on information in the ACRDP database as of June 15, 2010; any updates/changes after this date are not reflected.

responsible for the allocation of regional budgets, national priority setting and the allocation of the national fund. Membership is composed of DFO representation (two from science and one from aquaculture management), four provincial members, 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 and chosen to best represent the cross-section of the aquaculture industry in that region. These 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 to 2009, over 290 projects have been approved and funded. Of these, 57% fell under the best performance in fish production priority, 24% focused on environmental performance and 19% on optimal fish health. Research projects have focused on over 25 different species, including many marine and freshwater finfish species, several types of shellfish and some projects involving algae and invertebrates such as crayfish and sea urchins.

The ACRDP is administered by the Regional Management Committees, of which there are five. The Maritimes and Gulf regions are administered together by one of these committees. Each region is allocated a portion of the overall \$4.25 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). Recent examples of regional projects includes contributions to genetic improvement and development of marine fish broodstocks, development of strategies to improve shell growth performance in bivalves, environmental impact of freshwater cage culture, DNA-based family identification for Atlantic salmon, integrated multi-trophic aquaculture related initiatives, and development of fish diets to reduce total phosphorus output and improve feed conversions.

Since the beginning of the program, over \$30 million in ACRDP funds have been committed to research projects (Figure 1). These funds have been leveraged by \$14.8 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 \$16.1 million on top of the ACRDP funds. In total, over \$66 million in research has been funded through the ACRDP.

Of the \$4.25 million annual ACRDP envelope, approximately \$280,000 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; recent examples include contributions to the Productivity Improvement Fund to help alleviate the problems associated with aquatic invasive species in PEI, projects

looking at the withdrawal times for SLICE® which contributed to the final licensing of this product in Canada, support to the aquaculture industry for compliance monitoring associated with the use of bath therapeutants for sea lice and funding of the development of a white paper on Bacterial Kidney Disease (BKD). The National Fund also funds communications issues including the Canadian Aquaculture R&D Review (volumes I, II and III), and the design and writing of fact sheets for completed ACRDP projects and the newly developed aquaculture science video.

**Table 1:** Regional breakdown of ACRDP funding and total leveraged contributions. Based on ACRDP expenditures from 2001-2008 and allocated amounts from the 2009-2010 fiscal year. Values are up-to-date based on information in the ACRDP database as of June 15, 2010; any updates/changes after this date are not reflected.

Region	Number of projects funded	ACRDP funding	Total leveraged funding
Central and Arctic	25	\$4,158,386	\$4,345,683
Maritimes and Gulf	115	\$9,391,547	\$12,937,005
Newfoundland	18	\$4,870,800	\$4,249,316
Pacific	88	\$7,658,238	\$9,126,032
Quebec	50	\$4,339,863	\$5,384,132

### Moving into the Future

The year 2011 will mark the 10<sup>th</sup> Anniversary of the ACRDP. The program continues to be well received and is continuing to increase collaboration between DFO and the industry. Since 2008, the ACRDP is complemented by DFO's newer funding programs, The Program for Aquaculture Regulatory Research (PARR), a science program internal to DFO that supports research towards the environmental regulation of the industry, and Aquaculture Management Directorate's Aquaculture Innovation and Market Access Program (AIMAP). All three programs are working together towards economic prosperity and sustainability of aquaculture in Canada. The ACRDP continues to strive for improvement and stakeholder engagement by seeking feedback on key research priorities of the sector.

## Changes in the benthic macrofaunal community associated with sediment sulfide levels under salmon farms in southwestern New Brunswick, Bay of Fundy



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### **Abstract**

Sediment samples were collected under two salmon farms and a reference site in southwestern New Brunswick, Bay of Fundy. Sulfide data indicated that the reference site was unaffected, while a 150,000 fish farm had low impacts and a 500,000 fish farm had higher impacts. At the reference site, benthic macrofaunal diversity was high in all samples. At the farms, biodiversity was high in sediments with low sulfide levels and lower in sediments with higher sulfide levels, although there was considerable variability, especially at intermediate sulfide levels. Measures of community structure indicated that sediments under both farms were affected by organic enrichment, with greater impacts at the larger farm. The transition between normal and affected conditions (as reflected in the macrobenthic community) occurred at sulfide levels in the range 500–2000  $\mu\text{M}$ .

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### **Introduction**

The New Brunswick Department of Environment's Environmental Monitoring Program (EMP) evaluates the condition of sediments under finfish farms, using surficial sediment sulfide concentration (total  $\text{S}^{2-}$ ) as the indicator. Each operating farm must conduct regulatory monitoring between 1 August and 31 October each year. Farms are rated based on the average sulfide values from 6–21 samples: Oxidic A =  $<750 \mu\text{M}$ ; Oxidic B =  $750\text{--}1500 \mu\text{M}$ ; Hypoxic A =  $1500\text{--}3000 \mu\text{M}$ ; Hypoxic B =  $3000\text{--}4500 \mu\text{M}$ ; Hypoxic C =  $4500\text{--}6000 \mu\text{M}$ ; and Anoxic =  $>6000 \mu\text{M}$ . The use of sediment sulfide for monitoring programs has been promoted largely because of the lower cost and time required, compared to benthic macrofaunal community analysis<sup>1</sup>. This study collected data on the relationship between sediment sulfide levels and benthic macrofaunal communities at two salmon farms and a reference location in southwestern New Brunswick (SWNB), Bay of Fundy.

### **Materials and Methods**

Two salmon farms (sites A and B) and a reference site (C) in the Letang area of SWNB were studied.

Six locations were sampled at each farm site: one at each corner of the cage array plus two locations near the centre. Six similarly spaced locations were sampled at the reference site. Both farms were stocked with Atlantic salmon smolts in the fall of 2007. Site A had approximately 150,000 salmon and site B was an integrated multitrophic aquaculture (IMTA) farm with approximately 500,000 salmon as well as mussels and kelp. Benthic sediment samples were collected using a Hunter-Simpson grab which collected  $0.024 \text{ m}^2$  of sediment ( $16 \times 15 \text{ cm}$ ). Triplicate grab samples were taken at each location. Sites A and C were sampled on 27 October 2008 and site B on 28 October 2008. From each grab sample, three spatially scattered 5-ml syringe subsamples were collected from the top 2 cm of sediment. These subsamples were analyzed for total sulfides within 1 d. Sediment samples were sieved to retain material  $>330 \mu\text{m}$ . Identification of animals was performed to the lowest taxonomic unit possible (usually the species level) and the abundance data were adjusted to 1 kg sediment sample weight. Measures of biodiversity and community structure

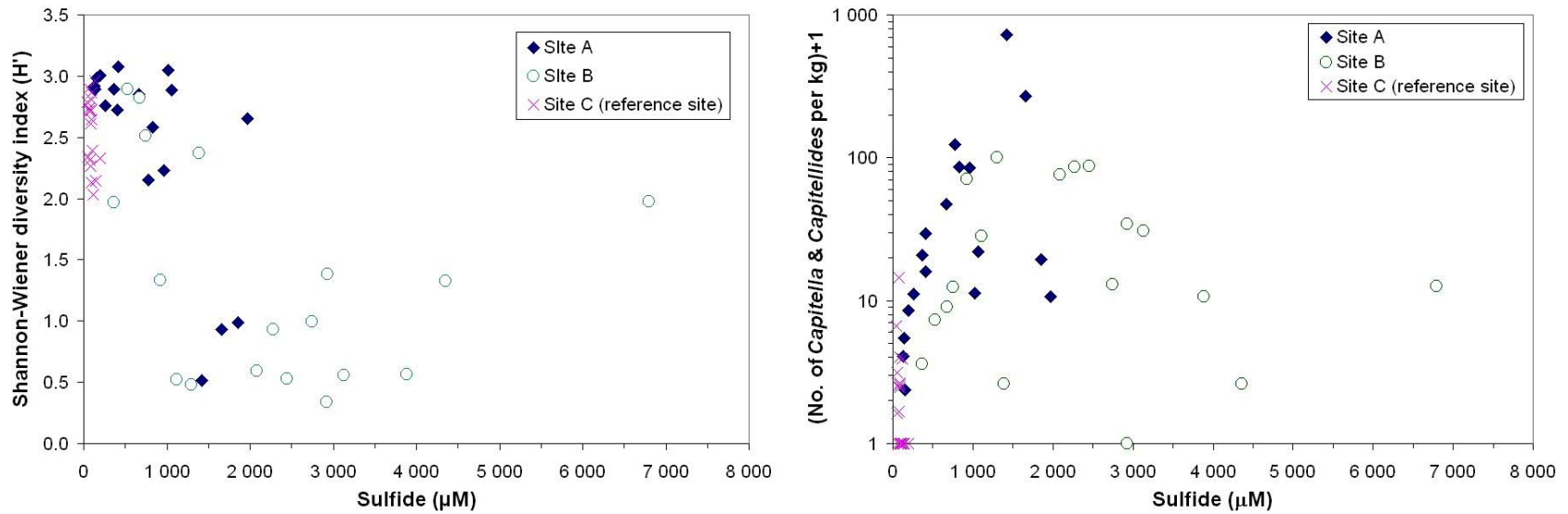


Figure 1. Relationships between the Shannon-Wiener diversity index ( $H'$ ) and sediment sulfide levels (left) and between the abundance of *Capitella* and *Capitellides* spp. and sediment sulfide levels (right) at two salmon farms (A and B) and a reference site (C).

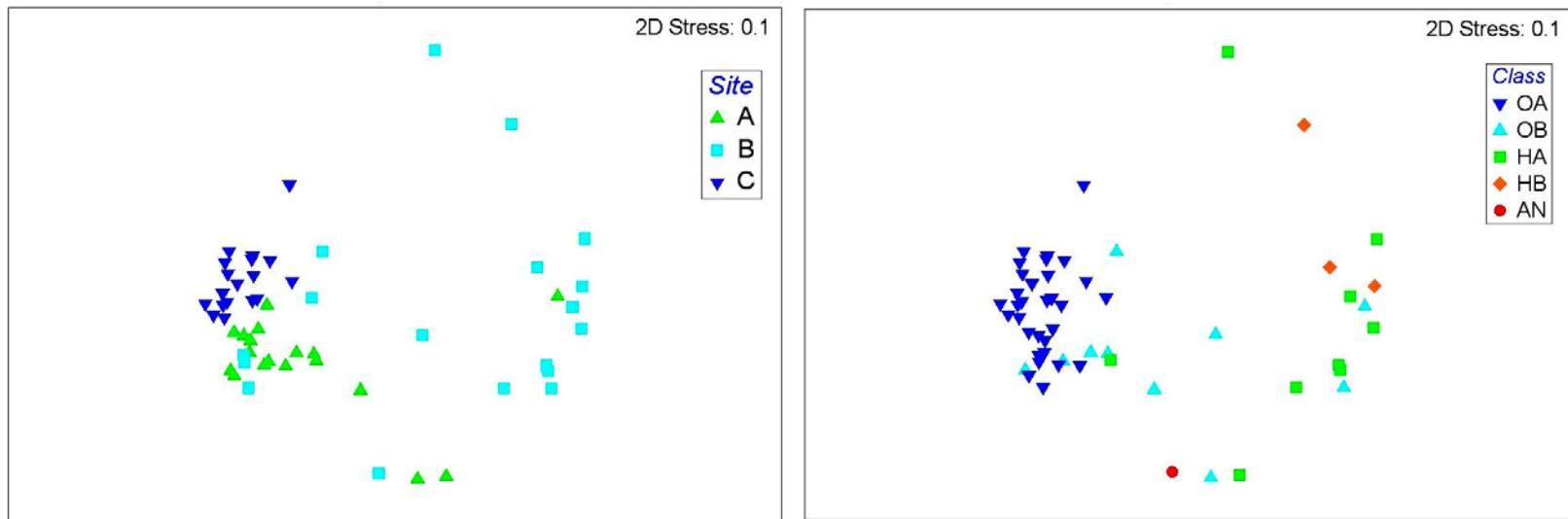


Figure 2. Multi-dimensional scaling plots of benthic macrofaunal species abundance data (after square root transformation) collected at two salmon farms (A and B) and a reference site (C). Left: symbols represent the sites A-C (18 samples per site). Right: symbols represent sediment sulfide classification: OA = Oxidic A (31 samples); OB = Oxidic B (10); HA = Hypoxic A (9); HB = Hypoxic B (3); AN = Anoxic (1).



were calculated using PRIMER v6 software, index ( $H'$ ) and non-metric multi-dimensional scaling (MDS) plots (after square root transformation of data).

## Results

Sulfide levels in all 18 grab samples at reference site C were in the Oxidic A category ( $\leq 205 \mu\text{M}$ ). At site A, nine samples had Oxidic A sulfide levels, six were Oxidic B and three were Hypoxic A. At site B, four were Oxidic A, four were Oxidic B, six were Hypoxic A, three were Hypoxic B and one was Anoxic. At site C, the Shannon-Wiener index ( $H'$ ) ranged from 2.0–3.0 (Fig. 1). At site A,  $H'$  was  $>2.0$  when sulfide levels were  $<1100 \mu\text{M}$ ; at higher sulfide levels  $H'$  was quite variable but mostly  $<1.0$ . At site B,  $H'$  was  $\geq 2.0$  when sulfide levels were  $<750 \mu\text{M}$ , and decreased at higher sulfide levels except for a relatively high value (2.0) in the sample with the highest (anoxic) sulfide level. The abundance of *Capitella* and *Capitellides* spp. was low ( $<15$  per kg of sediment) in all samples at site C (Fig. 1). At sites A and B, numbers were low in samples with sulfide levels  $<500 \mu\text{M}$ , increased with sulfide levels up to about  $1400 \mu\text{M}$ , peaking at 720 individuals/kg of sediment at site A, and decreased at higher sulfide levels.

The MDS plot of data from the three sites showed all of the site C samples to be clustered together and not overlapping with samples from the two farm sites (Fig. 2). Site A samples were spread across much of the horizontal axis but confined to the lower half of the plot. Site B samples were spread out both horizontally and vertically, overlapping with samples from site A. The MDS plot of the same data, but using sediment classification (based on sulfide levels), showed all of the Oxidic A samples to be clustered together at the left (Fig. 2). Oxidic B and Hypoxic A samples were spread quite widely across the plot, while the three Hypoxic B samples were all at the far right and the one Anoxic sample was near the bottom.

## Discussion and Conclusions

The results agree with the classification system proposed by Hargrave et al.<sup>2</sup>, in which they associated Oxidic A conditions with high biodiversity of benthic macrofauna (normal

including the Shannon-Wiener diversity conditions), Oxidic B with moderate biodiversity (transitory), Hypoxic with reduced biodiversity (polluted), and Anoxic with very low biodiversity (grossly polluted). We found impacts on the benthic macrofaunal community associated with organic enrichment (as measured by sediment sulfide levels) at both farms, with higher impacts at site B which also had higher sediment sulfide levels and more fish. The Shannon-Wiener diversity index values indicated that the transition between high and reduced biodiversity occurred at sulfide levels of about  $750\text{--}2000 \mu\text{M}$ , while trends in abundance of *Capitella* and *Capitellides* spp. indicated that the transition between normal and affected conditions may occur at sulfide levels of about  $500\text{--}1000 \mu\text{M}$ . Overall, the data indicate that sediment sulfide can serve as an indicator of impacts on the benthic macrofaunal community, but there can be considerable variability in the relationship, especially at intermediate levels of organic enrichment, and the transition from background or affected to affected can occur between  $500\text{--}2000 \mu\text{M}$  sediment sulfide.

## Acknowledgements

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## Quantitative trait loci (QTL) for body weight in Fraser strain Arctic charr (*Salvelinus alpinus*) reared in fresh and brackish water



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### **Abstract**

The identification of chromosomal regions related to commercially valuable traits such as growth are important because such information, when coupled with marker assisted selection (MAS), can be used to increase productivity.

The goal of this study was to identify quantitative trait loci (QTL) for variation in body weight among multiple families of Arctic charr in fresh and brackish water. Thirty full-sib families were produced at the Coastal Zones Research Institute and transferred to a commercial facility where they were grown communally in both fresh (FW) and brackish water (BW) from May 2008 to September 2009. Wet weight and fork length were measured on four sampling dates. To test for QTL for variation in body weight, 11 microsatellite markers were selected from salmonid QTL studies. Overall survival and mean body weight of charr in FW tanks had the tendency to be greater than in BW tanks. QTL for variation in body weight varied among families and between environments, indicating that some families had a genetic predisposition to perform better in FW and others in BW. Knowledge of QTL positions and relative family phenotypic performance is essential for the application of MAS on a commercial scale.

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### **Introduction**

In terms of world aquaculture production, Canada ranks 23<sup>rd</sup> and only accounts for 0.3% of the total production<sup>1</sup>. Expansion of the industry requires development of new technology to enhance the efficiency of breeding programs and development of alternative aquaculture species. Arctic charr (*Salvelinus alpinus*) is an example of one such alternative species. Traits like growth and maturation are highly variable in charr; however, this species exhibits many characteristics which make it appealing for culture. For example, charr are tolerant of low temperature and perform well under high density growing conditions<sup>2</sup>.

The goal of this study was to use microsatellite markers to identify quantitative trait loci (QTL) related to variation in body weight among multiple families of Arctic charr. Specific research objectives were to evaluate the relative phenotypic

performance of the Coastal Zones Research Institute's (CZRI; Shippagan, NB) broodstock families in fresh and brackish water and to determine if published QTL identified from genome scans have detectable effects across the CZRI broodstock for variation in body weight. This is the first study to investigate the effect of QTL across multiple families in a commercial production line of charr and there is potential to implement marker assisted selection in the CZRI broodstock at the conclusion of the study.

### **Materials and Methods**

The Arctic charr used in this experiment originated from the breeding program based at the CZRI. Thirty full-sib families were reared under standard husbandry conditions at CZRI until the experiment began. At a mean weight of 230g (February 2008), 120 PIT-tagged individuals from each family were transferred to a commercial facility (CanAqua

Seafoods Ltd., Advocate, NS) where 30 fish from each family were housed in each of four 16 m<sup>3</sup> tanks, for a total of 900 fish per tank. In May of 2008, the salinity in two of the four tanks was gradually increased to ~20ppt. All fish were measured (weight and length) twice annually from May 2008 to September 2009. Tissue samples were collected from all parents and progeny. Eleven microsatellite markers previously identified as QTL for body weight were selected for the analysis<sup>3,4,5</sup> and all individuals were genotyped at these loci using standard methods for PCR, electrophoresis and scanning. Allele peaks were scored visually and the QT-test was used for QTL analysis.

### Results

Mean body weight of freshwater families was greater than that in brackish water at every sampling date. Preliminary statistical analysis, using a mixed model with family as a fixed effect and separate residuals variances for each treatment, shows that rearing environment had a significant effect on family body weight ( $p \leq 0.05$ ). QTL on linkage groups AC-8 and AC-20 contributed the most to juvenile growth. Growth as adults was influenced by many more QTL regions which varied depending on the environment. To investigate the possibility of a genotype-by-environment interaction, an animal model was used. This analysis showed that the phenotypic correlation ( $r_p$ ) between body weights in brackish and fresh water was negligible but genetic correlations ( $r_G \pm SE$ ) between body weights in these environments were positive and ranged from moderately strong to strong. At the October 2008 sample date, body weight of fish in brackish water was strongly correlated with body weight in fresh water ( $0.671 \pm 0.148$ ). Heritability ( $h^2$ ) for body weight was moderate for each environment, ranging from  $0.302 \pm 0.080$  in October 2008 to  $0.278 \pm 0.006$  in September 2009. However,  $h^2$  in brackish water was approximately half that in fresh water at each sampling date.

### Discussion and Conclusions

The identification of chromosomal regions related to commercially valuable traits such as growth is

important for the expanding aquaculture industry. Current selective fish breeding programs generally rely on phenotypic selection rather than marker assisted selection. CZRI broodstock families perform better in fresh than in brackish water. However, the standard deviation of body weight in brackish water was much greater than in fresh water. Two of the 30 families had a statistically greater mean body weight in brackish water, suggesting that some genetic backgrounds will perform well in this environment. Additionally, because the genetic correlation of body weight in fresh and brackish water was strong and positive, the current selection for body weight that occurs in freshwater culture should also improve populations for future culture in brackish water. Genetic parameters showed that body weights in fresh and brackish water were affected by genotype-by-environment interactions.

QTL identified from genome scans have detectable effects across the CZRI broodstock for variation in body weight. The results of this study are similar to those of Moghadam et al.<sup>5</sup>, who found significant QTL for body weight on AC-8, -13 and -25 and a suggestive QTL on AC-20 during the juvenile growth phase of Arctic charr. Few QTL studies have followed the experimental population beyond one year. In the present study, growth as adults was influenced by many more QTL regions which varied depending on the environment. This suggests that a large number of loci are responsible for total variation in body weight, each contributing small effects.

In conclusion, it appears that selection based on a single marker for increased body weight is not a realistic option for the Arctic charr aquaculture industry. Marker assisted selection must take into account the phenotype and select for multiple loci associated with this trait of interest.

### Acknowledgements

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## From culture to conservation: a workshop to develop advanced reproductive technologies for sturgeon

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### **Abstract**

A workshop supported by NSERC and DFO (ACRDP) was held in March 2010 at the Biodôme de Montréal to identify major gaps related to reproductive technology in sturgeon and to target R&D efforts and groups to address them. The workshop featured industry participation and had as its objectives to review the current available methods in regard to all aspects of sex differentiation in sturgeon, in order to target preferred methods that could be useful for the aquaculture industry as well as for wild stock managers, and to identify the R&D necessary to put into practice the target methods and their commercialization in aquaculture as well for the conservation of wild stocks. Participants included representatives from Germany, California, British Columbia, New Brunswick and Québec.

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### **Introduction**

Sturgeon is ranked second in Canada among most preferred species for targeted development in the freshwater aquaculture sector<sup>1,2</sup>. The main product, caviar, is one of the most valuable fish products in the world and, despite recent developments in aquaculture production, the demand over the next 20 years cannot be met by current availability<sup>3</sup>. Since 1997, the production of shortnose and white sturgeon in New Brunswick and British Columbia, respectively, has revealed many positive commercial aspects of these species (e.g., extreme disease resistance and hence no use of antibiotics, good adaptation to culture techniques, amenability to land-based recirculation systems and unlimited site potential). Likewise, lake sturgeon has great opportunity to develop the freshwater aquaculture sector in Québec. The expansion of this industry is, however, difficult because sexual maturation requires significant time, including significant risk and essentially lack of financial backing to support the 5-6 year startup investment<sup>1</sup>. Moreover, the lack of knowledge about sexual dimorphism requires additional infrastructure to grow both

males and females until the more valuable females can be identified. Therefore, R&D for improvement of sturgeon culture must develop effective and noninvasive methods for early sex identification.

In order to address these existing problems, we wanted to identify the major gaps related to reproductive technology in sturgeon and target R&D efforts and groups that meet the actual demand of the industry. Our specific objectives were:

1. To review the advantages and disadvantages of currently available methods regarding all aspects (morphology, physiology, reproduction) of sex differentiation in sturgeon
2. To target preferred methods that could be useful for the aquaculture industry as well as for wild stock managers.
3. To identify the R&D needs to put into practice the target methods and their commercialization in industry as well as uses for wild stock management.

## Materials and Methods

The workshop was an initiative of the Biodôme de Montréal and the Université Laval, in partnership with the Canadian sturgeon industry, and was held on March 8-9, 2010, at the Biodôme. Workshop participants included two international researchers (from the University of California at Davis and the Leibniz Institute of Freshwater Ecology and Inland Fisheries), eight Canadian researchers (from Université du Québec à Rimouski, Université Laval, University of New Brunswick, Freshwater Fisheries Society of British Columbia and Aquarium du Québec), six representatives from the Canadian industry (Interprovincial Partnership for Sustainable Freshwater Aquaculture Inc. (IPSFAD), Target Marine Products Ltd., Supreme Sturgeon & Caviar Ltd., Huntsman Marine Sciences Centre, Québec Caviar and La Société de Recherche et de Développement en Aquaculture Continentale (SORDAC)) and five government representatives (Fisheries and Oceans Canada (DFO), Natural Sciences and Engineering Research Council of Canada (NSERC), Le Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ) and Biodôme de Montréal).

Day 1 was devoted to the presentation and discussion of several topics including farming industry issues, producer perspectives, sturgeon management, reproduction and sex differentiation in sturgeon, target species, and techniques and practices (including their advantages and disadvantages) of current methods for sex differentiation in sturgeon. On day 2, a survey of funding programs was realized and a discussion was also undertaken to identify a number of major problems faced by the industry. This was followed by a ranking exercise using a paired comparison analysis (matrix provided by Daniel Stechey, Canadian Aquaculture Systems Inc.) that provides a systematic, organized way to evaluate and prioritize the various alternatives presented for review.

## Results

Several needs were identified during the workshop, but only 9 major problems faced by the industry

were chosen for the paired comparison analyses. Results are given in Table 1.

Table 1. Comparative analysis of RDC issues for sturgeon culture

Issue	Rank*
Induce onset of puberty	2
Early sex determination	1
Impact of recirculation on reproduction	3
Policies related to access to seed	6
Variation in caviar quality/yield	3
Stress effects on vitellogen	7
Evaluation of lake sturgeon	9
Impact of feed on reproduction /caviar quality	5
Sperm cryopreservation	7

\*2 sets of scores were considered tied

## Discussion and Conclusions

Although impractical at this time, the production of all-female populations for caviar will be a critical success factor for going forward (i.e., maximize profit per production unit)<sup>3</sup>. It is not thus surprising that early sex determination was found to be the most important problem to resolve in the next future. Sex can be determined using a variety of invasive techniques (direct inspection of the gonads, biopsy samples or endoscopy) as well as non-invasive sonography<sup>4</sup>. However, these methods only work for large individuals, are time-consuming, can cause damage or stress to the fish and/or are not efficient for differentiating between immature males and females. Novel techniques based on plasma hormones<sup>5</sup>, gene expression<sup>6</sup>, biometry<sup>7</sup> and comparative proteomics<sup>8</sup> are still far from being applied in the industry. New methods that can be used on small individuals and not cause damage or stress must thus be developed. The development of non-invasive techniques for early sex identification in sturgeon has promise for great economic value for current commercial aquaculture and its future expansion and can potentially be extremely useful in the protection and restoration of the remaining wild stocks<sup>3</sup>. Accurate determination of the sex ratio within a population is

particularly important for understanding population dynamics and successful management<sup>4</sup>.

Invariably, understanding the impacts of numerous factors on the onset of puberty and on caviar quality/yield (feed, recirculation, stress, etc.) will be essential to improve product quality. Moreover, sperm cryopreservation could allow for use of limited rearing space for the growth of females only, but techniques have not been consistent to date. Species-specific market access issues, mostly related to endangered species status, are also affecting North American producers. The development of hybrids (e.g., shortnose x Atlantic as developed by Supreme Sturgeon and Caviar) could allow for the management of these issues.

In the near future, the main outstanding R&D requirements identified will be revisited and evaluated by a broader audience and action items will be developed for looking ahead over the next five years of furthered industry development. Lake sturgeon will be the subject of special attention because of its weak documentation and expressed interest by Québec private partners and institutions as a native species that has no major legal constraints for sale in the United States and has strong similarities with shortnose sturgeon such as egg size. We are now working on the formation of a new research group in partnership with industry, conservation and academic researchers and to increase its visibility via a congress and review publication. Research proposals will also be developed. For instance, we plan to evaluate if geometric morphometrics, unlike traditional methods, can be used to track size and shape variation during ontogeny until sex can be determined (in collaboration with the research group of Dr. Richard Cloutier, UQAR).

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## Production dynamics, self-thinning and profitability of blue mussel populations reared in suspension culture in Cascaféria Bay



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### **Abstract**

Part-time mussel farming is a potential way for fishers to diversify their activities. This can be achieved by growing mussels on spat collectors, bypassing spat sleeving. However, this implies that population density is no longer a control variable for profit-minded producers, and intraspecific competition is exacerbated. Therefore, we explored the profitability and production dynamics of mussel culture on collector ropes. We randomly collected 30.5-cm lengths from triplicate collectors sampled at two depths from October 2003 through July 2007. In addition to biomass and population density, we monitored size structure, length-mass relationships of individual mussels, multilayering of mussels, proportions of bare substrate, depth of the mussel lines and water temperature. Production costs were estimated on the basis of information from the mussel growers. Using modifications of Faustmann rotation methodology, we found positive profits at the standard terminal period in Québec of 36 months, with 2.2 kg/30.5 cm rope. Production dynamics were governed by self-thinning, but this constraint was not as binding on profitability as we thought it would be.

### **Introduction**

In order to diversify their activities, fishers in Carleton, Québec, are considering growing mussels on a part-time basis. Usual mussel culture techniques, however, involve a series of activities such as spat declumping, grading and sleeving. In addition, attention must be paid to longline buoyancy, maintenance and management. These activities are time-consuming and therefore mussel culture based on sleeving is not well suited for part-time activities. Therefore, an alternative method, called self-regulated collectors (SRC) or autocollectors, has been proposed.

The SRC technique consists of raising mussels directly on the structures used for spat collection. With the standard mussel technique, longlines are held at mid-water depth, with collectors, sleeves

and flotation buoys attached directly to the longline. In contrast, a self-regulated longline consists of pairs of 3-m long collectors that are drawn upwards from the main line by a float<sup>1</sup>. Compensation weights attached to the main line with 1-m long ropes fall from the main line. Spat are left to grow on the collectors. The longlines eventually sink in the water column due to the increasing weight increase from settlement and growth of mussels and fouling organisms. However, the compensation weights touch the bottom before the main line does and, as they touch the bottom, they are no longer supported by the long line. Consequently, the main line benefits from a buoyancy reserve and is prevented from touching the bottom, and mussel growth continues. Use of the SRC technique allows operators to save costs associated with stripping the collectors and placing the spat in sleeves. One possible drawback



of the method is that there is a greater variability of sizes at harvest and a slower growth rate because spat size and mussel density during grow-out cannot be adjusted over time. This study examined the performance and production dynamics of the SRC technique according to criteria such as profitability, size of mussels and limits on the production.

**Methods**

We studied two variants of the method in two closely located farms in Cascapédia Bay, an open body of water. The design of the longlines was the same in both cases, except for the size of the buoys. Technique A used 16 L buoys (farms 1 and 2) and Technique B used 40 L buoys (farm 2 only). Temperature and depth recorders (2 at the end of each line) were installed on the longlines of farm 1.

We randomly sampled 30.5-cm lengths from triplicate collectors sampled at two depths from October 2003 through July 2007. In addition to biomass and population density, we monitored size structure, length-mass relationships of individual mussels, multilayering of mussels, proportions of bare substrate, depth of the mussel lines and water temperature. Profitability of both techniques was studied using modifications of the Faustmann rotation methodology, which was originally developed to optimise forest harvesting schedules based on profit-maximization goals. Here we modified the method to account for the fact that mussel price varies with individual size. Production costs were estimated on the basis of information from the mussel growers.

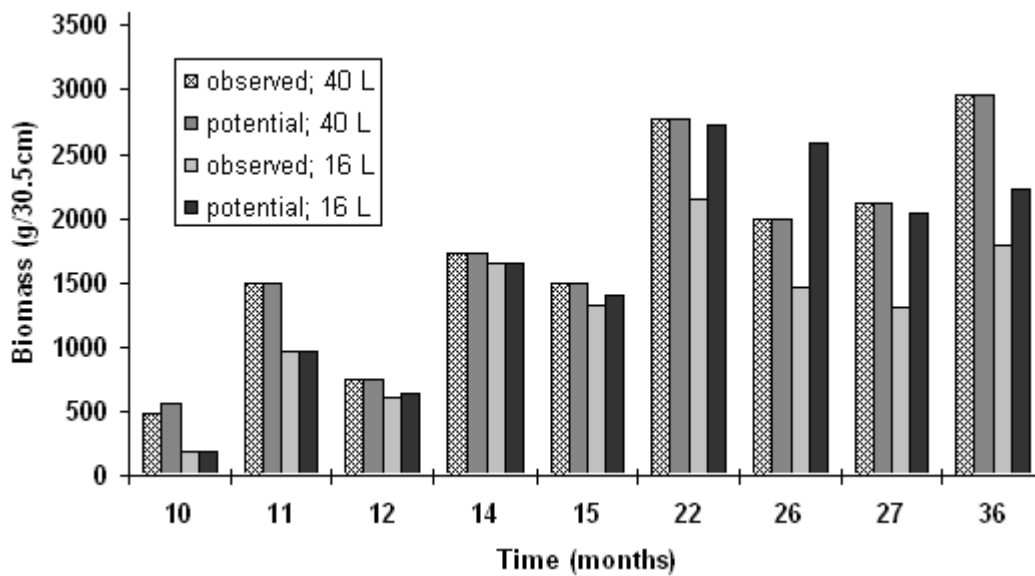


Figure 1. Biomass yield with Techniques A (16 L buoys) and B (40 L buoys) at site 2 as a function of time since nominal date of spat collection (July 1, 2003). Observed yield includes all samples, irrespective of whether stripping of mussels occurred. Potential yield takes into account only those samples which showed no evidence of stripping.

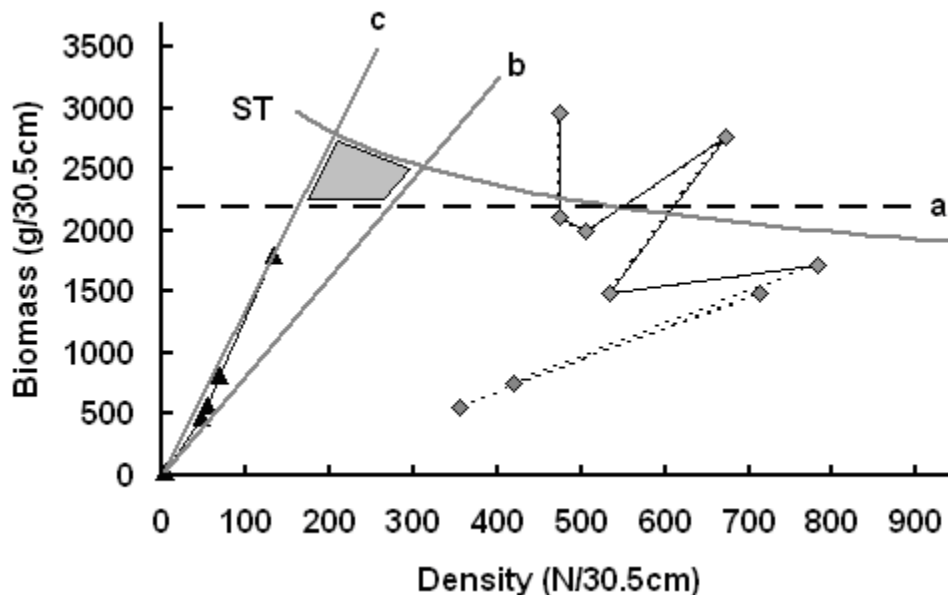


Figure 2. Relationship between yield and population density using Technique B. Self-thinning curve (ST) shows the upper limit to yield. Line **a** shows breakeven point 36 months after spat settlement. It is assumed that price is constant through time and that all mussels have reached commercial size. Line **b** shows the relationship between population density and biomass (yield) for mussels exactly 5 cm long, which is the limit for commercial size. Commercial-size mussels lie above this line in the biomass-density space. Line **c** shows the relationship between commercial yield and population density at harvest. In practice, lines **a** and **c** are not fixed because they vary with length of the production cycle. The elevation of line **a** is expected to rise through time because a longer production cycle increases production costs. Line **c** was convex because growth continued once mussels had reached commercial size. The grey area shows that profitable combinations of density and yield of commercial-size mussels may be achieved without self-thinning constraining production. This indicates that technique B is profitable, in spite of the fact that this conclusion is based on an extrapolation of yield. Diamonds indicate yield-density relationship for undersized and commercial-size mussels.

## Results and Discussion

Data from the depth recorders showed that the longlines performed as expected at the beginning of the study, but that the main lines made contact with the bottom repeatedly towards the end of the trial. Temperature showed a typical annual cycle, with large variations in the summer. Technique A produced a total harvest of 1.6 kg/30.5 cm of collector after 40 months of growth. Observed biomass was lower than potential after the 15<sup>th</sup> month (Fig. 1), indicating that contact with the bottom partially or completely stripped collectors

from this time on. With Technique B, the total harvest averaged 2.1 kg/30.5 cm of collector after 36 months, and we did not observe any stripping from bottom contact (Fig. 1). Results published elsewhere clearly show that production dynamics were governed by self-thinning<sup>1</sup>, as expected in high-density situations<sup>2,3</sup>. An economic breakeven point is reached at 2.2 and 2.1 kg/30.5 cm of collector for Techniques A and B, respectively<sup>4</sup>. Our analyses indicate that overpopulation and individual growth are not obstacles in obtaining profitability, because the self-thinning curve lies

above the breakeven point (grey area in Fig. 2). If there is an obstacle, it will be at the level of the performance of the lines: Technique B allows better flotation of the collectors and avoids stripping due to contact with the bottom.

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## Organic footprint and composition of particles from marine finfish aquaculture operations



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### **Abstract**

Finfish aquaculture operations release food particles and faecal pellets to the benthos. We are studying the particle field associated with finfish aquaculture and how benthic organisms interact with it in land-based and cage site facilities. Measurements were made of dissolved and particulate matter in the inflow and outflow of six, 6000 L land-based juvenile Atlantic cod (*Gadus morhua*) tanks. Lipid composition results show significantly more free fatty acid (FFA) in the outflow indicative of lipid breakdown and faecal matter. Fishmeal-derived copepod fatty acids and the essential fatty acid 22:6 $\omega$ 3 (DHA), were also significantly higher in the outflow. In a separate study, Atlantic salmon (*Salmo salar*) cage sites in Clayoquot Sound and the Broughton Archipelago, British Columbia, were sampled by retrieving invertebrates in transects surrounding the farms. Mollusc lipid composition correlated linearly with distance from the farms and FFA composition decreased significantly with distance. Fishmeal-derived copepod markers were significantly higher in mussels collected within 400 m of the farm than in those collected further away. Among the molluscs, blue mussels (*Mytilus edulis*) had significantly higher proportions of the essential fatty acids which would relate to their potential use in integrated multi-trophic aquaculture.

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### **Introduction**

The release of food particles and faecal pellets to the benthos is one of the major concerns with finfish aquaculture operations. Increased sedimentation due to farming can overload the local ecosystem, leading to anoxic conditions and a reducing environment producing ammonia, hydrogen sulphide and methane<sup>1</sup> and subsequently causing eutrophic conditions. In addition, lipid content in the surface sediment directly under cages increases with decreasing distance<sup>2</sup>. Given the pressure to provide adequate seafood products<sup>3</sup>, quantification of finfish farm outputs is a major requirement to understand increasing effects on the local ecosystem as well as the potential benefits of an enriched growing environment for other organisms. This research describes the particle field associated with finfish aquaculture and benthic organisms' interactions within this field

by measuring dissolved and particulate matter in the inflow and outflow of six 6000 L land based juvenile Atlantic cod (*Gadus morhua*) tanks, followed by the uptake of organic particles by invertebrates surrounding Atlantic salmon aquaculture operations in British Columbia.

### **Materials and Methods**

Sampling from land-based tanks took place inside the Dr. Joe Brown Aquatic Research Building (JBARB). Samples were taken from six 6000 L tanks containing juvenile Atlantic cod (*Gadus morhua*) maintained by the JBARB staff. There was a 13-day collection period where samples were taken of the inflow and outflow. Inflow water was passed through a sand-bed filter, removing particulates < 50  $\mu$ m. Outflow samples alternated between passive flow as well as flushed output from pulling the

standpipe. The Samples for the field study were taken from the periphery of three Atlantic salmon aquaculture sites in Clayoquot Sound and the Broughton Archipelago, British Columbia, to determine lipids in the surrounding marine invertebrates.

Samples were homogenised manually with a metal rod or with a Polytron homogenizer. Lipid class composition was determined using a three step development system method<sup>4</sup> with silica coated Chromarods and an Iatroscan. Fatty acid methyl esters (FAME) were obtained using 14% BF<sub>3</sub>/MeOH for 1.5 h at 85°C with agitation at 45 min and were analyzed in a GC-FID equipped with an autosampler.

### Results and Discussion

There was significantly more free fatty acid (FFA) in the outflow (46% total lipid) than the inflow (13%) to the land-based cod tanks, indicative of lipid breakdown and faecal matter<sup>5</sup>. There was also significantly more of the essential fatty acid 22:6 $\omega$ 3 (DHA) in the outflow compared to the inflow. Along with this, the fishmeal-derived copepod marker fatty acids were significantly higher in the effluent than the influent.

In order to estimate the output of larger aquaculture facilities, these data were used to calculate the amount of material released per kilogram of fish biomass (Table 1). The amount resulting from the presence of the fish alone (i.e., excess feed and faecal material) was obtained by subtracting material supplied in the tank inflow. Dry weight calculations indicated 1.69 g/day/kg exited the tanks. As a first approximation, applying a 1:1 scaling operation to a Newfoundland-size cod farm (1880 t) gives 3170 kg/day dry mass supplied to the surrounding ecosystem. It is important to note that the throughput of the land-based tanks is based on juvenile fish and the farm sizes relate to harvest size fish. With an 1880 t farm, 108 kg/day FFA would be produced. Fatty acids in the free form have the potential to cause problems for marine animals near aquaculture sites. Nevertheless, certain individual fatty

acids in fish farm effluent can be nutritionally enhancing. For instance, DHA is highly valuable in terms of nutritional quality and is one of the limiting factors for animal development<sup>6</sup>. Here it made up approximately 50% of the total essential fatty acids, and 0.16 g/day was provided by the land-based tanks. A working size (1880 t) cod farm would provide 4.7 kg/day of DHA to organisms surrounding the marine net pens. The availability of DHA to surrounding organisms requires investigation. In addition, the levels of FFA, as well as feed additives, pesticides, antibiotics and other feed- and faecal-associated contaminants, must be considered to fully understand the value of this enrichment to the environment.

An average Newfoundland mussel farm produces about 200 t of mussels (38 million mussels at 5.31g/mussel). Based on data from a Newfoundland research project which tracked essential fatty acid and mussel weight over a 500-day growing period<sup>7,8</sup>, the 3170 kg/day dry weight produced from the scaled cod farm could sustain a 210 t (40 million) mussel farm. This assumes that all output is available for mussel consumption<sup>9</sup>. In terms of DHA output, 1400 t (260 million) mussels could be maintained. This shows that a single 1880 t Atlantic cod farm could theoretically sustain a substantial portion of the mussels reared in Newfoundland in terms of DHA requirements, demonstrating the large potential of multi-trophic, co-culturing systems.

In a separate study, Atlantic salmon cage sites in British Columbia were sampled by retrieving invertebrates in transects surrounding the farms to investigate their uptake of aquaculture-derived organic particles. Mollusc lipid composition correlated linearly with distance from the farms. Specifically, regression analysis with distance showed a significant decrease (slope =  $-1.69 \times 10^{-3}$ ;  $p = 0.018$ ;  $n = 179$ ) in FFA with distance from the farm for all molluscs. In addition, DHA was significantly lower nearer to the farm sites for all molluscs (slope =  $1.70 \times 10^{-3}$ ;  $p = 0.007$ ;  $n = 179$ ) as well as for mussels alone (slope =  $1.10 \times 10^{-3}$ ;

Table 1: Estimated output rates from a cod aquaculture operation based on data obtained from tank trials

	Tank outflow (all size fractions) (g/day)	Corrected outflow <sup>1</sup> (g/day)	Corrected outflow <sup>1</sup> (g/day/kg biomass)	1880 t farm (kg/day)
Dry Mass	186.8	96.8	1.69	3170
FFA <sup>2</sup>	3.26	3.19	0.06	108
DHA	0.16	0.14	0.0025	4.7

<sup>1</sup> Minus inflow

<sup>2</sup> FFA: free fatty acids; DHA: docosahexaenoic acid, 22:6 $\omega$ 3

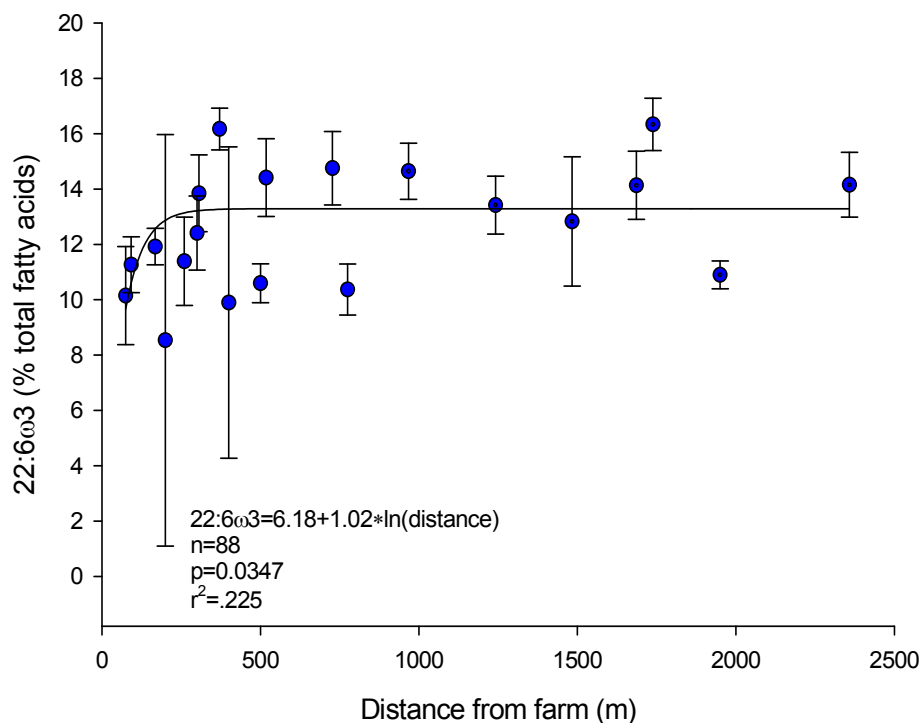


Figure 1: Docosahexaenoic acid (DHA; 22:6 $\omega$ 3) proportions in mussels surrounding B.C. Atlantic salmon farms. (Mean  $\pm$  SD; regression line is plotted through raw data.)

p = 0.006; n = 93) (Fig. 1). Salmon feed is supplemented with essential fatty acids; however, the preferential retention of the essential fatty acids in the feed by the finfish and the abundance of other lipids and fatty acids exiting the netpens could account for the depletion nearer to the farms by overloading the surrounding invertebrates with less nutritious fatty acids. As distance increased, DHA leveled off to background values. Despite lower DHA, mussels were found to be heavier closer to the farm, indicating greater food availability nearer the farm. Among the molluscs, blue mussels also had significantly higher proportions of the essential fatty acids which would relate to their potential use in integrated multi-trophic aquaculture.

### Acknowledgements

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## Phototherapy: applications for growth enhancement and maturation delay in farmed Bay of Fundy Atlantic salmon (*Salmo salar*)



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### **Abstract**

Maturation (grilsing) rates in farmed Atlantic salmon steadily increased from less than 1% in 1978 (average among the first salmon farms) to greater than 30% at some Bay of Fundy farming sites in 2001. This resulted in significantly reduced farm gate sales and prompted the industry to give high priority to research into methods to decrease grilsing. In 2001, a study on the effect of artificial photoperiod on grilsing rates was initiated at two commercial salmon farms. During the first month, specific growth rates decreased in the November-lit cages. However, by the end of the following May, fish in November-lit cages showed significantly higher growth rates than those in control cages. At the first farm, only 1% of the salmon in the November-lit cages matured compared to 11% and 22% of the fish in the February-lit and unlit control cages, respectively. On the second farm, 5% of the salmon from the October-lit cages matured compared to 17.5% in the control cages. An economic benefit analysis of the improved growth and delayed maturation due to the use of an artificial photoperiod showed a saving of up to \$100,000 per cage (based on the November photoperiod adjustment and assuming a cost of lighting equipment purchase and operation of \$5,000 per cage). A more recent study involving four farms confirmed the effectiveness of light treatment for reducing grilsing in salmon and that initiation of constant lighting as late as December is as effective as initiation in October.

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### **Introduction**

One of the main challenges to the long term sustainability of the Atlantic salmon farming industry in Canada is the early sexual maturation of fish following their first winter in sea cages<sup>1,2</sup>. Early maturation reduces growth rates, affecting the size and quality of the final product and, as a result, farmers incur significant financial loss (4-9% of gross income). This research investigated the potential of artificially increasing day length (photoperiod) during the grow-out period as a way to overcome early sexual maturity and increase the profitability of the industry. This research provides a cost-effective tool that will directly improve the productivity and sustainability of the Canadian Atlantic salmon farming industry.

### **Methods**

Immature salmon were subjected to either continuous light from October/November to May or natural light conditions (control) at two farm sites in South Western New Brunswick. Site #1 contained twelve 70m circular cages. Two artificial lights, simulating the natural light spectrum, were placed in each of six cages at depths of 5 m and 7 m, respectively. Three of these cages were lit on November 21, 2001, and the other three on February 15, 2002. Lights remained on until May 31, 2002. The remaining six cages were maintained under natural light conditions. Site #2 consisted of four 50m circular cages. Two of these cages were exposed to continuous light from October 31, 2001,



to May 31, 2002, while the two control cages received only natural light.

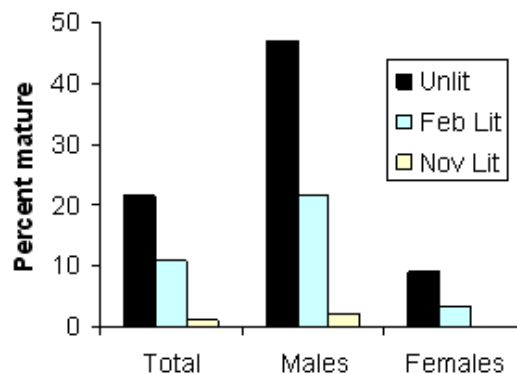
Fish were filmed within each cage using a synchronized dual video camera that, when lowered into the cage, videotaped the fish at 1, 2, 3, 4 and 5 m below the water surface. Fish body mass, length and girth were estimated from still images taken from the video using mathematical formulae. Twenty fish were measured from each depth, for a total of 100 fish per cage, with measurements made on three occasions (November 15 and December 21, 2001, and May 29, 2002). February-lit cages were not sampled by video imaging, but were sampled at harvest.

On July 12, 2002, 59-74 fish were sampled from each cage at Site #1. Sex, round weight, fork length, girth, dressed weight, mean fat content and gonad weight were recorded. Samples were also retrieved between August 12, 2002, and February 6, 2003, and length, weight, sex, maturity and muscle total fat content (leanness) recorded. The number of fish per cage sampled ranged between 91-153 fish. At Site #2, 98-100 fish per cage were sampled from the lit cages on August 19, 2002, and from the control cages on September 4, 2002. Weight, sex, maturity, and muscle fat content were recorded. Data were analyzed to compare the growth characteristics and sexual maturation in salmon between lit and unlit treatments and among light period treatments.

## Results

Fish in the continuously lit cages initially had reduced growth rates compared to controls but this was temporary: from December 2001 to May 2002 fish from cages lit in November (Site #1) had higher growth rates (0.32% body mass per day) than controls (0.29%). In July, maturing fish in control cages were growing at a faster rate than immature fish (as determined from harvest weight samples). However, in the months preceding harvest (harvest from August 2002 through to February 2003) this was reversed, with mature fish growing very slowly. Twenty-two percent of the sampled fish in the control cages were mature (47% of the males and 9% of the females; Figure1). For February-lit cages

this was reduced to 11% (22% of males and 3% of females) but results were variable among cages (2-19% total maturation rate). In contrast, only 1% of November-lit fish were mature (2% of males and no females). Harvest data collected during August and September from Site #2 showed similar trends of reduced maturation rates with the application of light starting a month earlier, in October: 17.5% in control cages (22.5% of males and 3% of females) compared to only 5% in lit cages (8.5% of males and 1% of females). There was no detectable difference among treatments in total lipid (fat) levels during the preliminary harvest.



**Figure 1.** Grilse maturation rate in lit and unlit Atlantic salmon cages averaged over all cages for each photoperiod.

## Conclusions

Increasing the perceived day length of farmed Atlantic salmon through exposure to continuous artificial light in the fall results in significant increases in their overall growth by the end of the following May and significantly lowers the rates of sexual maturation of both sexes. Artificially increasing the day length starting in February does not have the same effect on reducing maturation rate, and outcomes are less predictable. The extended photoperiod did not affect harvested salmon leanness (fat levels), an important factor in defining carcass quality.

A simple cost analysis of the use of light treatment equipment was conducted to estimate the financial gains to farmers should they employ this treatment on their farms. The cost of purchasing, wiring and

operating the lights was less than \$5,000 per cage (2002 dollars). Based on November results for each 70 m sea cage, the potential net financial gain from maintaining high production rates and flesh quality by delaying sexual maturity would be greater than \$100,000 per cage.

Follow-up experiments conducted in 2007<sup>3</sup> have confirmed that continuous light has a significant effect in reducing grilising rates in cages. These experiments have also shown that light treatment beginning as late as December is as effective as in early autumn. This reduction in required light treatment duration represents a reduction in the cost of artificially lighting the cages and represents an additional cost savings to salmon farmers.

#### **Acknowledgements**

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## Enhancing sustainable mussel industry production and growth through assessment and removal of constraints in seed supply



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### Abstract

This report updates a mussel seed project which has been ongoing for four years in Placentia, Notre Dame and Bonavista Bays, NL. The focus of the 2009-2010 field season was to evaluate five sites in Bonavista Bay and three sites in the Harry's Harbour area of Notre Dame Bay. This past year also included mussel seed transfers from two Bonavista Bay sites,

the Harry's Harbour sites, existing commercial seed sources and local Placentia Bay seed into an existing Placentia Bay mussel farm site.

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### Introduction

The Newfoundland Aquaculture Industry Association (NAIA) requested assistance from the Marine Institute's Centre for Aquaculture and Seafood Development (CASD) to evaluate the performance of mussel seed collected from sites in Bonavista and Notre Dame Bays, NL, in 2008 and 2009. The objective was to determine each site's potential as a source of good mussel seed to aid future mussel aquaculture industry development.

### Materials and Methods

Mussel cohorts were analyzed by PCR, using the diagnostic genetic markers Glu, ITS, to identify *Mytilus edulis*, *M. trossulus* and their hybrids. Before any collectors were deployed in Bonavista Bay or the Harry's Harbour area, larval monitoring was completed at selected sites in Bonavista Bay. This consisted of vertical plankton tows at each site in early July to determine presence or absence of mussel larvae, as well as the collection of site-specific environmental, CTD/SONDE and chlorophyll data to assist in determining deployment orientation, depth, etc.

Based on seed abundance (including genetic confirmation of *M. edulis* presence) and

morphometric results from the 2008 study, five sites were selected in Bonavista Bay for the 2009 study (Fig. 1): Lockers Bay (Site 2), Gut Cove (site 3), Tumblers Reach (Site 9), Rocky Bay (Site 12) and Cat Bay (Site 13). Three sites were selected in the Harry's Harbour area (Fig. 2): Southern Arm (Site 1), Middle Arm (Site 2) and Western Arm (Site 3). All 2008 mussel collector arrays (Fig. 3) that were submerged for overwintering were surfaced in July 2009. Those at sites excluded from the 2009 study were removed and NWPA were notified in mid-July. Thermographs deployed in 2008 were also retrieved and their data collected, and they were then redeployed on the 2009 study sites. Seed samples from 2008 single-drop deployments were collected in summer 2009 by sampling triplicate 30-cm pieces of collector from each site for genetics and morphometrics. New collector arrays were deployed at the selected sites where they remained until after the seed transfer study was completed in fall 2009.

The 2008 seed collectors in the selected sites were sampled in November 2009 before the transfer study occurred. The new collector arrays deployed in 2009 were also sampled by collecting triplicate 30-cm pieces of collector

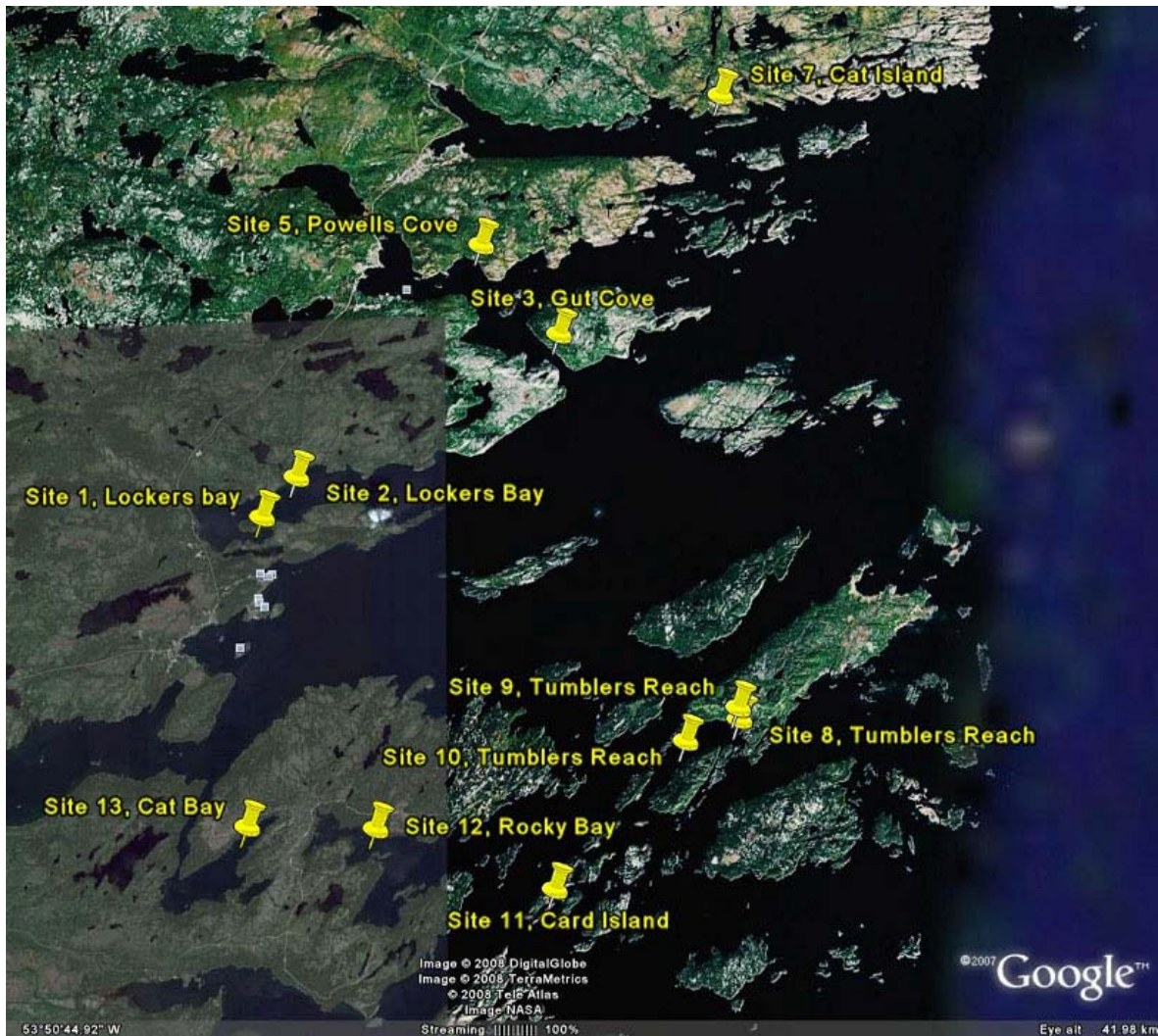


Figure 1: Bonavista Bay sites where mussel seed collectors were deployed in 2009.

from each site. Many of the collector arrays deployed in the summer of 2009 were missing by the fall. It is believed that fishing activity by herring seiners was responsible for their disappearance in Bonavista Bay. The new collector arrays deployed in 2009 at the Harry's Harbour sites were also all missing. There has been a great deal of public displeasure with the activities in the area and it is believed that local residents removed the collectors. Remaining collector arrays were overwintered by submerging them below the surface; Transport Canada was advised of this overwintering.

A seed transfer to an existing Placentia Bay farm site was completed in fall 2009, using seed from the 2008 and 2009 deployments in

Rocky Bay and Cat Bay and from the 2008 deployments in the Harry's Harbour area (approximately 18 months old), as well as commercially grown seed from the Green Bay area and local seed from the Placentia Bay farm site as controls for comparison. All transferred seed was sampled and measured (length; mm) prior to transfer. Seed from the recipient experimental sites were socked and also deployed in pearl nets at the same time. All seed samples were analysed by collection of triplicate 30-cm sections of the collector, which were transferred to the Marine Institute on ice for morphometric (length) analysis. Total wet weight (field weight), fouling weight, mussel wet weight and mussel counts of entire 30-cm

samples were determined, as were individual lengths of 50 mussels from each collector sampled.

**Results and Discussion**

Many sites in both bays had missing collector arrays, possibly the result of user conflicts rather than environmental factors. Education efforts in these areas would be a positive venture before any extensive seed collection efforts continue. Many of the Bonavista Bay sites experienced poor or inconsistent collection since 2007; only Cat Bay and Rocky Bay have shown some consistency. Additional research should be completed on larval monitoring,

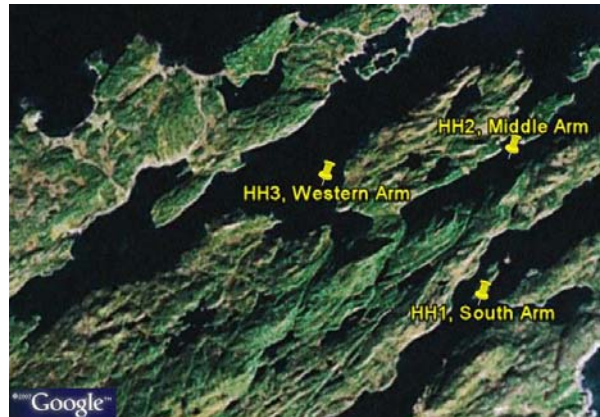


Figure 2: Harry's Harbour area sites where mussel seed collectors were deployed in 2009.

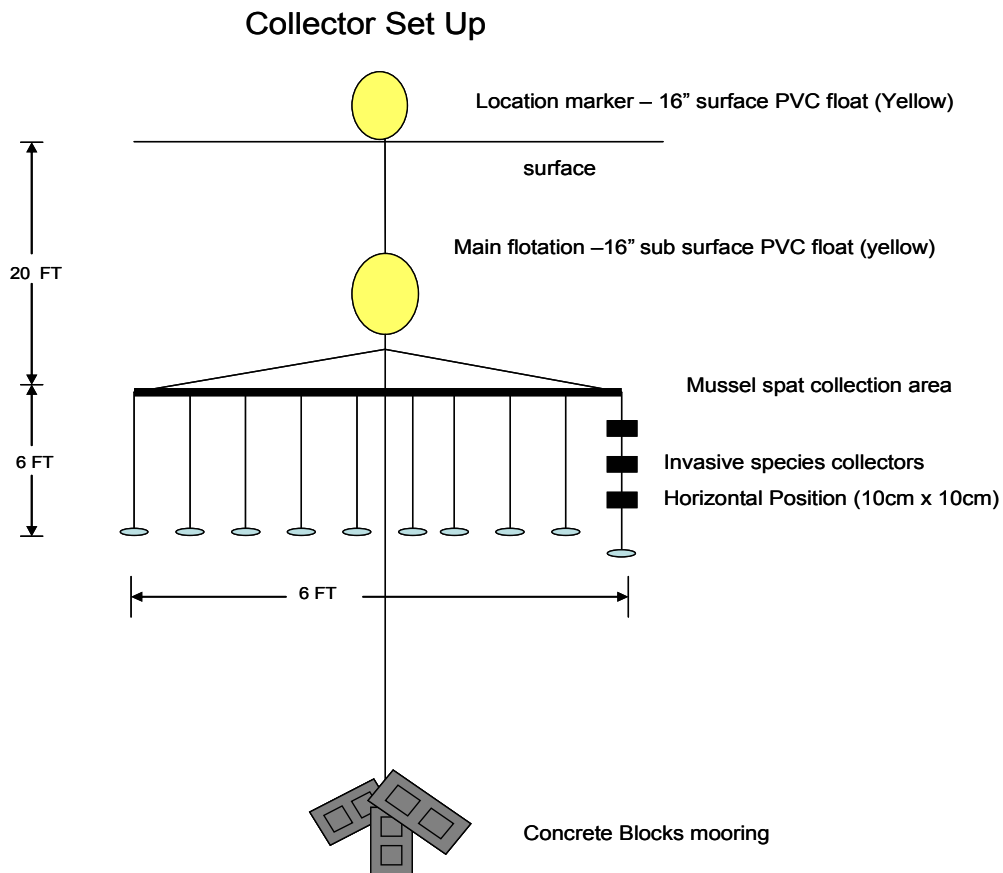


Figure 3: Mussel seed collector array design.

The sites near Harry's Harbour were sampled in 2009 because of interest from existing mussel farmers. Unfortunately, seed collectors were missing from two of the three sites containing 2008 seed, as were all of the 2009 collectors.

The one site that was sampled for 2008 seed, Southern Arm (HH1), did provide promising results for seed collection with ideal genetics, and continued research is encouraged.

In 2007 some changes were made to the work plan for 2008, including more extensive larval monitoring, a staggered deployment schedule and a near-surface collector setup.

These changes resulted in much better collection on all sites in 2008. Unfortunately, due to budget constraints, staggered larval monitoring and deployments were not used in 2009 and the Bonavista Bay seed collection was not as good as in 2008. Staggered collector deployment is recommended because of the mixed results we have gotten from year to year. Only Cat Bay and Rocky Bay showed seed collection to any extent this past year.

The four years of research into the seed collection potential of several areas in Placentia, Bonavista and Notre Dame Bays has led to a better understanding of what each area has to offer. Several sites in Placentia Bay have proven to be very good for seed collection and grow out, but the genetics in those sites are not ideal with a mixed population of *M. edulis* and *M. trossulus*. Bonavista Bay has proven to have

a very desirable genetics profile on most sites but has been plagued with inconsistent collection and user conflicts. Notre Dame Bay also has very good genetics but a true picture of its seed collection potential has not been ascertained due to lost experimental equipment. Information gathered under this research should be used as a guide only and more information should be gathered by the interested parties before formal activities are planned. The 2009 project marked the end of the seed supply surveys, however, a mussel seed transfer study was initiated in Placentia Bay. The continuation of this seed transfer into 2010-2011 is dependent upon project funding.

#### **Acknowledgements**

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## Program for aquaculture regulatory research: Implementation and future directions



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### **Abstract**

The Program for Aquaculture Regulatory Research (PARR) was developed in 2008 under the Aquaculture Regulatory element of the New Aquaculture Program, which was funded in Budget 2008. It is a Fisheries and Oceans Canada (DFO) initiative that is internal to DFO with a research agenda designed to support new and emerging priorities and identify those areas that require knowledge in the medium and long term. The goal is to support informed DFO ecosystem-based environmental regulation and decision making for aquaculture. Research priorities are identified through consultation with internal DFO clients, Aquaculture Management Directorate and Habitat Management Directorate and through Canadian Science Advisory Secretariat (CSAS) peer review, aquaculture-related workshops. In 2008 and 2009, 13 and 8 projects were funded, respectively. In 2010, a targeted call for proposals was adopted and a National PARR Advisory Committee was established to help guide the program.

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### **Introduction**

The Program for Aquaculture Regulatory Research (PARR) was launched by Fisheries and Oceans Canada (DFO) in 2008 under the Aquaculture Regulatory Science element of the New Aquaculture Program. It is now entering the third of its five year research agenda. The New Aquaculture Program was funded to support the Framework for Aquaculture Environmental Management (FAEM), which provides the basis for a coherent national approach to the regulation of the aquaculture sector. PARR is an internal DFO program with a research agenda designed to support new and emerging priorities and to identify those areas that require knowledge development in the medium and long term. PARR will help fulfil the goals of FAEM and the Aquaculture Regulatory Science element by increasing the science knowledge to support and advise informed DFO ecosystem-based environmental regulation and decision making.

In addition to the PARR, the Centre for Integrated Aquaculture Science (CIAS) and the jointly funded DFO-NSERC Canadian Integrated Multi-Trophic Aquaculture Network (CIMTAN), which also involves university partners, were also developed to provide science support for the Aquaculture Regulatory element of the New Aquaculture Program. CIAS is a “virtual” DFO Centre of Expertise that leads and implements a national integrated aquaculture research program in support of ecosystem-based approaches. This program addresses DFO’s aquaculture research priorities and supports sustainable aquaculture management and development within Canada. To meet this goal, the CIAS coordinates the delivery of research by leveraging expertise and capacities in finfish (marine and freshwater), shellfish, and marine plant aquaculture science across research facilities. CIMTAN objectives are to further develop integrated multi-trophic aquaculture approaches in Canada by strategically enhancing economically sustainable seafood production systems and

developing environmentally friendly aquaculture systems. These systems can then be adopted by the industrial partners to, improve growth rates of co-cultured species while reducing the organic and inorganic nutrient load into the surrounding ecosystem.

### **Structure of the Program**

The PARR is centrally coordinated for administration and accountability reporting from the DFO Aquaculture Science Branch in Ottawa. Unlike the industry-driven Aquaculture Collaborative Research and Development Program (ACRDP) that partners industry with DFO scientists, PARR is an internal DFO research program. In the first two years of the PARR, research priorities were identified after extensive consultation with clients, Aquaculture Management Directorate and Habitat Management Directorate.

In 2009, DFO scientists developed eight Pathways of Effects (POEs) research documents and diagrams, which map the potential stressors of various aquaculture activities (finfish and shellfish) on the environment, and the potential effects of those stressors on different components of the ecosystem. In October 2009, a Canadian Science Advisory Secretariat (CSAS) meeting was held to peer review the eight draft documents. The finalized documents will inform the development of policy documents used by government regulators responsible for managing aquaculture, and identify PARR research priorities for 2010, the third year of the program. In 2010, a PARR National Advisory Committee was created to recommend research priorities and evaluate and recommend research proposals. The National Advisory Committee membership is composed of one DFO Science representative from each DFO region as nominated by the Regional Director (Science), one representative each from the Aquaculture and Habitat Management Directorates (AMD and HMD), and DFO Science Ottawa. Research proposals are provisionally approved by the PARR National Advisory Committee; however,

DFO's Departmental Aquaculture Management Committee and the National Science Directors Committee confirm final approval.

### **Program Overview**

In 2008, the first year of the PARR, 13 projects were approved and funded. The three priorities for this year were: genetic and ecological interactions of wild and cultured fish – mitigating the effects of escapes; ecosystem carrying capacity; and ecosystem and far-field indicators of aquaculture effects on fish habitat. In the second year, 8 projects were funded under the priorities fish health management in aquaculture and siting requirements (Table 1).

The focus of the third year of the program was to target research to address specific regional and national knowledge gaps that were identified through the CSAS POE process and through extensive consultation with national and regional AMD and HMD staff. A targeted call for proposals to address these knowledge gaps was developed and issued. By region, the identified priorities were: Pacific - wild-farmed interactions and sea lice management; and the characterization of the environmental impacts to hard bottom substrate; Central and Arctic - environmental regulation for farm siting; environmental impacts of deposition; Québec and Gulf - environmental carrying capacity for shellfish culture; Maritimes - evaluation and monitoring of therapeutants for the development of a sea lice Integrated Pest Management Plan and evaluation of environmental impacts for guidance in determining HADD (harmful alteration disruption and destruction of fish habitat) Authorizations for farming operations; Newfoundland - establishment of Bay Management Areas for the south coast of Newfoundland for site and fish health management.

### **Moving Forward**

The research needs of DFO clients, AMD and HMD will continue to evolve as each directorate moves forward in developing and



Table 1. The number of PARR projects and Aquaculture Regulatory research funding in 2008-2010.

Year	Number of projects funded	Yearly funding
2008-09	13	\$616,000
2009-10	8	\$528,000
2010-11	11	\$880,000

implementing aquaculture regulations and siting requirements. The PARR priorities will continue to be re-evaluated and identified each year through discussions with our clients and through follow-up CSAS workshops and other knowledge dissemination forums. The targeted call for proposals approach adopted this year

will continue as the best way to strategically respond to clients' specific needs.

To date, the PARR has been a very successful program that will sunset in two years. However, based on results to date, it will be critical for the program to be extended in order to continue meeting the regulatory research needs of our clients and to support informed DFO ecosystem-based environmental regulation and decision making. In the coming years, DFO will be submitting a request for an extension to Treasury Board for the long term funding of the New Sustainable Aquaculture Program, which will include PARR. This will ensure that the successes of the program will continue in the future to benefit the sustainability of aquaculture in Canada.

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## Aquaculture Canada<sup>OM</sup> 2010 and Northern Harvest<sup>TM</sup> 2010

### *Aquaculture product donors / Commanditaires de produits aquacoles:*



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## Determination of the lethal dissolved oxygen threshold in spotted wolffish (*Anarhichas minor*) of Quebec origin according to two methods: LC<sub>50</sub> and PO<sub>2crit</sub>



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### Abstract

This study presents preliminary results of hypoxia tolerance in the spotted wolffish (*Anarhichas minor*) of Quebec origin according to two methods: LC<sub>50</sub> and PO<sub>2crit</sub>. The LC<sub>50</sub> after 96 h exposure to varying dissolved oxygen (DO) concentrations established from 180 fish (average weight ~2 kg) was 20.9% (95% CI = 20.1 – 21.7) of air saturation. Average PO<sub>2crit</sub> for a sample of 20 similarly-sized fish was 16.9% (95% CI = 15.3 – 18.4) air saturation. From these results it is apparent that DO levels below 21% will result in a high mortality rate in spotted wolffish (up to 50% within 4 days).

### Introduction

The spotted wolffish is a promising new species for fish farming in Quebec<sup>1,2</sup>. However, the recommended rearing method for this species (land-based water recirculation system<sup>3</sup>) can involve rapid and significant fluctuations in dissolved oxygen (DO) due to low water volume and high fish density<sup>4</sup>. Such DO fluctuations can have negative impacts on growth and sometimes even result in death. It is therefore important to determine the lethal DO threshold for this species to prevent major losses in production. We compared two measures of hypoxia tolerance, the Critical Oxygen Pressure (PO<sub>2crit</sub>) frequently used in respiratory physiology research, and the lethal concentration for 50% of subjects (LC<sub>50</sub>) common in toxicology studies.

### Materials and Methods

The main experiment for determining LC<sub>50</sub> used sixteen 800 L tanks. A follow-up experiment used 2 of these tanks to expose the fish to 2 new DO levels to improve the analysis. Overall, there were

16 hypoxic DO levels (14.7–27.2 % sat. during the main experiment, and 29.1 and 35.7 in the follow-up experiment) and 2 tanks at normoxia. DO was monitored every 5 min using custom-designed computer software and a galvanic oxygen probe (OxyGuard, Denmark, Model 420) in each tank, connected to the computer. DO was controlled by adjusting the injection of air and nitrogen through a gas-exchange column. The probes were calibrated before the experiment and their performance verified repeatedly by Winkler titrations. Temperature was maintained at 8°C and salinity at 28 PSU. Ten fish (average weight ~ 2 kg) were transferred to each tank directly to its preset DO level at t=0. As there were no pre-lethal correlates found that predicted impending death, death was used as the endpoint. Dead fish, if any, were counted and removed after 1, 3, 6, 12, 24, 30, 48, 54, 72, 78 and 96 h of exposure, but only results for 96 h are presented here. LC<sub>50</sub> was determined by logit analysis using R software<sup>5,6</sup>.

Four cylindrical respirometers were used for determining PO<sub>2crit</sub>. Software and equipment from

Loligo Systems (Denmark) was used to control DO and measure oxygen consumption ( $MO_2$ ) in real time. Temperature was maintained at 8°C and salinity at 28 PSU. Twenty fish (average weight ~ 2 kg) were tested in groups of 4 fish in a total of 5 sessions.  $MO_2$  was measured by intermittent-flow respirometry<sup>7</sup>. Cycles of 15 minutes were used during which respirometers were flushed with new water for 5 min. The  $MO_2$  was calculated from the slope of the decrease in oxygen concentration during the last 8.5 min when the respirometer was closed. Fish spent  $\geq 3$  d in the respirometer: 1 d of acclimation,  $\geq 2$  d to estimate standard metabolic rate ( $SMR = \text{quantile } 0.15 \text{ of } MO_2 \text{ values recorded after the acclimation period}$ ). On day 4, fish were exposed to decreasing levels of DO for 1 h each (~40, 30, 25, 20, 16, 12, 10, 8, 6, 4 % air sat., ending the experiment when  $MO_2$  was clearly proportional to DO).  $PO_{2crit}$  was estimated by finding the intersection of the regression line for  $MO_2$  values below  $SMR$  (fitted visually) and the horizontal line representing  $SMR$ .

## Results

The 96 h  $LC_{50}$  was 20.9% of air saturation (95% CI = 20.1 – 21.7), with 5% mortality ( $LC_{05}$ ) at 26.1% (95% CI = 24.3 – 27.9) and 95% mortality ( $LC_{95}$ ) at 15.7% (95% CI = 13.9 – 17.6) (Fig. 1). The average  $SMR$  was 24.3 mg  $O_2/(kg \cdot h)$  (95% CI = 22.3 – 26.3) and average  $PO_{2crit}$  of 16.9% (95% CI = 15.3 – 18.4) (Fig. 2).

## Discussion and Conclusions

The  $LC_{50}$  results agree well with those of Le François and *al.*<sup>8</sup> for the related Atlantic wolffish (*Anarhichas lupus*): no survivors after 96 h below 16% air saturation and no mortality above 22%, compared with 16 and 26%, respectively, in the present study. However, there was a difference between our two measures of hypoxia tolerance, with the 96-h  $LC_{50}$  suggesting a greater sensitivity to hypoxia (20.9 % air sat.) than the  $PO_{2crit}$  (16.9 % air sat.). One possible reason for this is that  $PO_{2crit}$  represents the average tolerance for all individuals tested, whereas with the  $LC_{50}$ , the true tolerance of half of the fish is still unknown. The  $LC_{95}$  (15.7 %

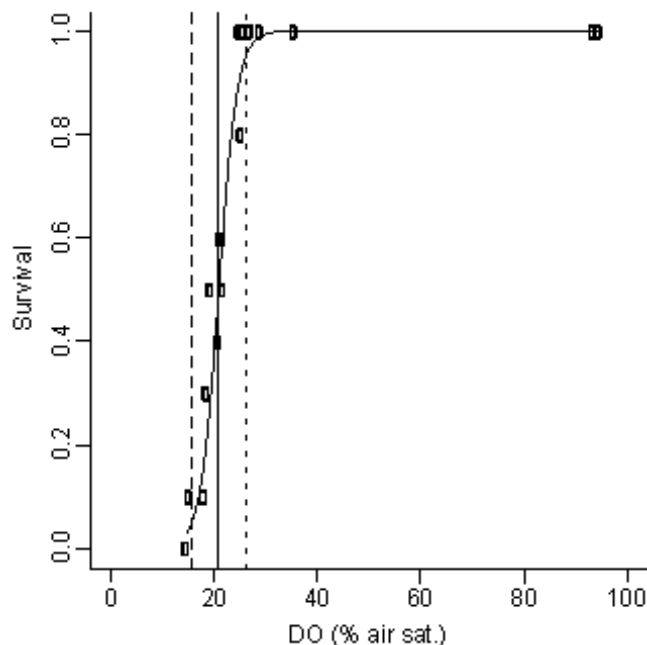


Figure 1. Survival after 96 h as a function of dissolved oxygen (DO) concentration ( $LC_{50} = \text{—}$ ,  $LC_{05} = \text{⋯⋯⋯}$ ,  $LC_{95} = \text{- - - -}$ ).

air sat.) was closer to the average  $PO_{2crit}$ . The difference in sample size between the two methods (180 vs. 20) may also result in a greater range of tolerances being present in the LC study, as suggested by the fact that the greatest  $PO_{2crit}$  value was 22.9 % air sat., yet 5% of the fish died after 96 h exposure at 26.1 %. Furthermore, fish may be unable to survive 96 h when their metabolic scope is reduced to nearly zero.

Although these are still preliminary analyses, it appears that wolffish incur a high risk of mortality when DO levels fall below ~ 20% air saturation. The industry must take every precaution to avoid such drops in DO to reduce the risk of massive mortality. Levels above 20% air saturation are not necessarily without negative effects, as growth may be reduced and vulnerability to disease, parasites or poor water quality (nitrates, unfavorable temperature, etc.) may increase. These results are also of interest in understanding constraints for survival and growth in wild spotted wolffish, a threatened species.

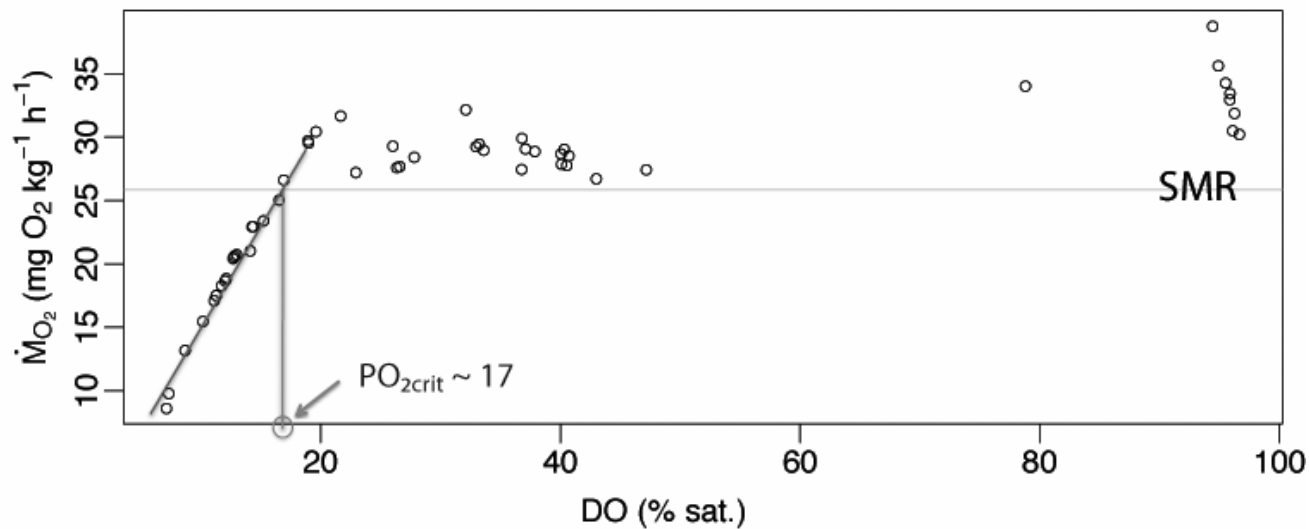


Figure 2. Example of determination of  $PO_{2crit}$  from oxygen consumption rate as a function of dissolved oxygen (DO) concentration.

### Acknowledgements

A thought to Donald Thomas, my first director, who passed away last year. An enormous thanks to the entire team at the IML, without which this project would never have been possible, in particular to Jérôme Gagnon for his valuable help in the experimental setup and Tanya Hansen for her help in running the experiments.

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## Effect of chronic sublethal hypoxia on growth in the spotted wolffish (*Anarhichas minor*) and its hybrid with Atlantic wolffish (*A. lupus*)



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### Abstract

The overall aim of this study was to determine the impact of 4 different dissolved oxygen (DO) levels (40, 50, 60 and 100% air saturation) on growth, condition, food intake, hematocrit and the activity of key enzymes of aerobic metabolism (citrate synthase, pyruvate kinase), anaerobic metabolism (lactate dehydrogenase), antioxidant activity (glutathione reductase) and oxidative stress damage (Tbars, aconitase) of the spotted wolffish (*Anarhichas minor*) and its hybrid with Atlantic wolffish (*A. lupus*). This paper presents preliminary results on length and mass gain as a function of DO. DO levels of  $\leq 50\%$  saturation appear to slow down growth in mass in these wolffish.

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### Introduction

Spotted wolffish (*Anarhichas minor*) are demersal marine fish that are particularly well suited for cold-water aquaculture<sup>1</sup>. Their farming-friendly behaviour and high-density tolerance makes shallow raceways the best growing system for a land-based facility<sup>2</sup>. This system has the advantage of reducing the quantity of water used and optimizing the terrestrial space for the producer, but the resulting low water volume to fish biomass ratio makes it particularly vulnerable to the deterioration of water quality. Following the feeding period, or equipment failure problems, oxygen levels can be the first parameter affected. Thus, it is essential for the producer to know the low dissolved oxygen (DO) tolerance of the species produced, and the physiological impacts on health and growth of long-term exposure to sublethal levels of factors such as DO.

Hypoxia (low DO) is also a common phenomenon in aquatic environments. Aquatic organisms have consequently developed a wide range of physical and physiological adaptations to survive unfavorable DO levels<sup>3,4</sup>. If DO is not sufficient to support their standard metabolic rate (SMR), fish

will attempt to maintain their oxygen supply through different mechanisms. Generally, the first line of adaptive response relates to oxygen uptake and its redistribution. Mechanisms such as increasing gill ventilation frequency or amplitude and the number of lamellae perfused are frequently observed<sup>4,5</sup>. Oxygen distribution in the organism can also be improved by increasing erythrocyte counts or haemoglobin-oxygen affinity<sup>5</sup>. Behavioural adaptations, such as moving to oxygen favorable areas or lowering active metabolic rate by reducing activity level, can also help to maintain sufficient oxygen delivery to tissues<sup>6,7</sup>. Food acquisition, digestion and conversion are also energetically costly processes and consequently directly related to oxygen consumption, making them growth-limiting factors<sup>8</sup>. Many authors have observed that reduced feed intake is the main factor responsible for growth depression in moderately hypoxic conditions<sup>9-12</sup>. Indeed, Chabot and Dutil<sup>11</sup> reported that 97% of the variation in growth rate between different hypoxia treatments in Atlantic cod (*Gadus morhua*) was explained by feed intake. Reduced appetite can be explained by the metabolic increment involved in digestion, absorption and nutrient utilization. This post-prandial energetic cost is called “specific dynamic

action of food” (SDA) and is associated with a rise in oxygen consumption within minutes or hours following food ingestion<sup>13</sup>. Finally, in severely hypoxic conditions, other mechanisms are involved such as aerobic/anaerobic ATP shifts and adaptations associated with hypo-metabolism allowing fish to survive below SMR<sup>14-16</sup>. As part of a larger study to determine the impact of DO on wolffish culture and basic physiology, this paper presents preliminary results on growth as a function of DO.

## Materials and methods

The experimental fish (n=63 *A. minor* and 135 hybrids, ranging in mass from 0.4 to 2kg) hatched during 2002-2007 at Centre Aquacole Marin (Grande-Rivière, QC) from a broodstock of 10-14 year old spotted and Atlantic wolffish. The experiment was carried out at the Maurice-Lamontagne Institute (Mont-Joli, QC). In the summer of 2009, 198 individually PIT-tagged fish were distributed among 16 circular tanks (800L, ~ 8 hybrids and 4 spotted wolffish per tank). Following a 1-month acclimation period (100% air sat., hereafter “sat.”), different DO levels (40, 50, 60 and 100% sat.) were randomly assigned to the tanks in 4 replicates. The experiment lasted 3 months. The desired oxygen levels were obtained by mixing air and nitrogen in a gas exchange column connected to the tanks. Oxygen probes (Oxyguard, model 420 galvanic electrode, Bikerød, Denmark) were linked to a central computer monitoring the DO levels. Oxygen levels were also frequently measured with a portable oxygen probe (YSI Professional plus model 605000 galvanic probe, Yellow Springs, USA). The temperature was maintained at 8°C and water salinity at ~ 28 PSU. The fish were exposed to the natural photoperiod for the Institute, which is close to 18:6 in summer. Fish were fed to satiation 3 times/week with Corey Aquafeeds.

On July 5<sup>th</sup>, two weeks following transfer to their tanks, all fish were anesthetized with Aquacalm<sup>tm</sup> (Western Chemical, USA) and individually measured (body length and mass). Tank acclimation ended 2 weeks later (day 0) and DO levels in all experimental tanks were adjusted on

the same day to the target values (40, 50, 60 or 100% sat.). At the same time, a control group (9 hybrid and 9 spotted wolffish) was anesthetized and sacrificed for morphological measurements and tissue sampling. Blood, liver, heart, white muscle and gill samples were immediately frozen at -80 °C. All fish were measured at days 49, 73 and 102 of the experiment. All manipulations on live fish were made in accordance with the recommendations of the Institute’s Animal Care Committee.

## Results and Discussion

Although fish increased in size under all treatments, growth in mass was depressed in the two most severe hypoxia treatments (40% and 50% sat.), as shown by non-overlapping 95% CI (Fig. 1), especially during the last month of the experiment. However, growth in length was little influenced by hypoxia (Fig. 2). Although changes in condition have not been examined yet, a slower growth in mass than in length must have resulted in a lowering of fish condition at 40 and 50% sat. In

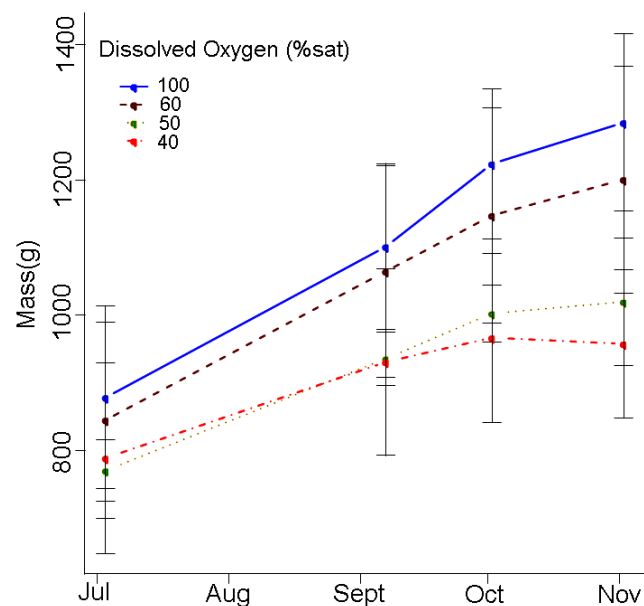


Figure 1. Spotted and hybrid (spotted X Atlantic) wolffish increase in body mass during rearing at various dissolved oxygen (DO) levels. Values are averages from 4 replicate tanks for each DO level, with both species combined. Error bars represent 95% confidence intervals.

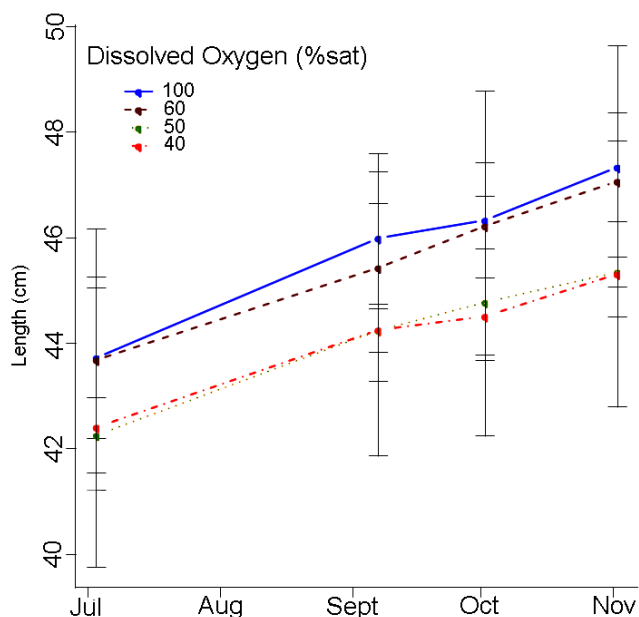


Figure 2. Spotted and hybrid (spotted X Atlantic) wolffish increase in body length during rearing at various dissolved oxygen (DO) levels. Values are averages from 4 replicate tanks for each DO level, with both species combined. Error bars represent 95% confidence intervals.

agreement with these observations, food intake was proportional to DO, although these results have not been analyzed statistically yet. Unfortunately, we had to include a wide size range of fish in each tank, which increased variability in size at each measurement date, and likely also variability in growth.

In summary, these preliminary results suggest that hypoxia levels of  $\leq 50\%$  slow the growth in mass of these wolffish, but more analyses are clearly required. In this preliminary examination of the results, spotted wolffish and its hybrid were not distinguished and the statistical power of having 4 replicate tanks per treatment was not taken advantage of. Furthermore, future analyses will use individual specific growth rates. Together, these improvements should increase the sensitivity of the final analyses. We also plan to compare food consumption among treatments and to look at our biochemical data.

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## Pilot-scale cultivation of the spotted wolffish (*Anarhichas minor*) in Québec, Canada



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### Abstract

A pilot-scale growth trial with spotted wolffish (*Anarhichas minor*) is currently underway at the facilities of the Centre Aquacole Marin (Grande-Rivière, QC; reproduction and early-stages) and the Maurice Lamontagne Institute (Fisheries and Oceans, Mont-Joli, QC; juvenile on-growing to commercial size) in collaboration with UQAR, SODIM, MAPAQ, MDEIE and the Biodôme de Montréal. It is one of the largest marine aquaculture R&D projects in Québec, and its success is in large part due to collaborative research with Norway, Iceland and Newfoundland. The wolffishes are a group of promising candidates for aquaculture diversification in cold environments due to the quality of their flesh, domestication attributes and conformity with sustainable aquaculture production characteristics. In collaboration with Norwegian and Icelandic researchers, research efforts on these species have been conducted in Québec since 1999, focused on reproduction, early-life stages and market outlooks of the spotted wolffish. This project is aimed at applying state of the art rearing practices in order to reach optimal growth of spotted wolffish to commercial market size, while taking into consideration fish welfare. Our large-scale trial will also be examining family effects and the impact of grading on growth and hierarchy. Our current estimations indicate that our captive populations display similar performance as those in Iceland and Norway.

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### Introduction

Spotted wolffish cultivation displays interesting potential for diversifying cold-water mariculture efforts in eastern Canada. Eastern Québec and the west coast of Newfoundland, in particular, experience serious constraints for the development of a sustainable, practicable and competitive commercial off-shore aquaculture industry based on current “aquaculture species” such as Atlantic cod (*Gadus morhua*) or Atlantic halibut (*Hippoglossus hippoglossus*). Wolffish species present very high tolerance to the prolonged presence of ice coverage and sub-zero temperatures during the winter months at these locations. Recently, Canadian wolffish populations have become the subject of a species recovery plan due

to serious declines of their natural populations<sup>1</sup>, giving increased relevance to eco-physiology research and aquaculture initiatives aimed at the *Anarhichadidae*.

Wolffishes are promising aquaculture species based on multiple criteria that mostly relate to their strong domestication attributes as well as freeze tolerance capacities<sup>2,3</sup> (allowing the consideration of cage culture) and their strong suitability for cultivation in intensive land-based recirculation systems (i.e., stocking density<sup>4,5</sup>, stress response<sup>6,7</sup>, niche market<sup>8,9</sup> and disease tolerance<sup>10,11</sup>). In light of current environmental awareness and regulations, on-growing using recirculation technologies will likely be favoured for marine aquaculture development in Québec, Canada.

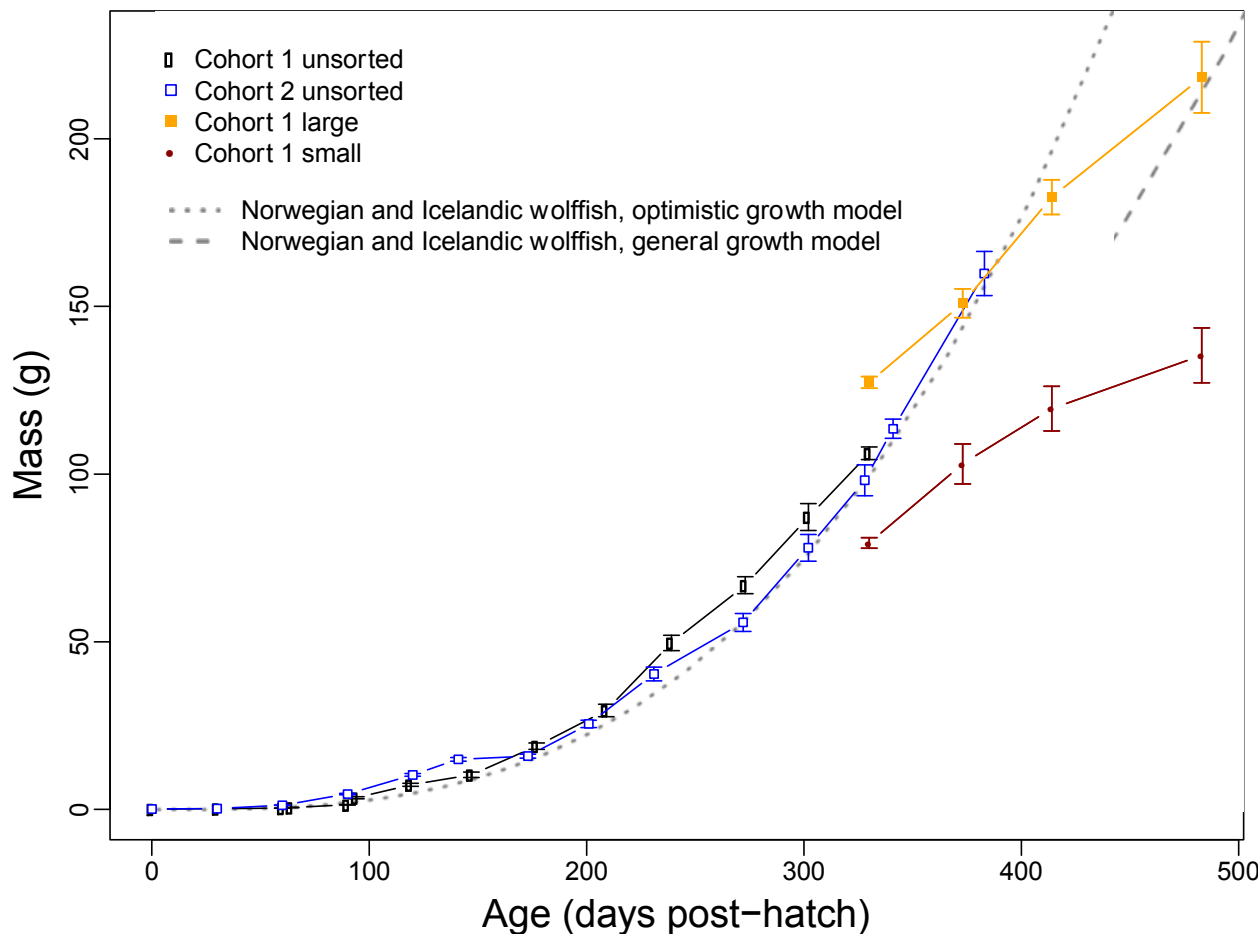


Figure 1. Mass at age for 2 cohorts of Québec-source spotted wolffish hatched in winter 2009. Cohort 1 was split (large fish > 100 g, small fish < 100 g) at 330 days post-hatch to evaluate the effect of grading. Also shown are two Gompertz growth curves fitted to data from Norwegian and Icelandic studies on wolffish. The general model was fitted using all available data. The second (“optimistic”) model was fitted to a subset of the data that included only the largest fish at age.

The objective of this two-year project is to apply “state of the art” rearing practices in order to realize maximal growth capacities of the spotted wolffish (15g-1.25Kg) within a pilot-scale setting. Secondary objectives include the identification of fast growing families and the evaluation of the impact of size grading on growth. Fully detailed information on spotted wolffish cultivation and current knowledge is provided in Le François *et al.* (2010)<sup>12</sup>.

### Material and Methods

Approximately 20 families are represented in the pilot study, which began with production at the Centre Aquacole Marin de Grande-Rivière

followed by transfer of 10-15g juveniles to the Maurice Lamontagne Institute for on-growing to market size (1.0-1.5Kg). Fish have been reared in low-level raceways with 50% water recirculation. Culture conditions have been maintained at optimal growth temperature<sup>13,14</sup> and rearing densities, using feed with < 18% lipid content.

### Results and Discussion

Growth rates achieved during the first year of this trial are comparable to Norwegian and Icelandic growth data. Average mass of the different cohorts compared to the Scandinavian research results are shown at Fig. 1. The effects of a *Trichodina* and *Gyrodactylus* infection on growth of Cohort 1 is detectable after 350 d. Given these positive results,

the pilot-scale project has entered its second year of realization. The outcomes of the growth trial will provide production data including growth rate in “state-of-the art” rearing conditions enabling the full bio-economic evaluation of wolffish cultivation.

### Acknowledgments

Thanks to Tony Grenier (UQAR) and Jérôme Gagnon (DFO-MPO) for their skilled technical assistance rearing the fish and to the regular staff at the CAMGR and IML facilities. This project was funded by MDEIE, ACRDP, MAPAQ and SODIM.

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## Indigenous and non-indigenous ascidian tunicates of Newfoundland and Labrador



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### Abstract

Ascidian tunicates (sea squirts) can be aggressive invaders that grow over shellfish (e.g., mussels) and consequently out-compete them for space, and perhaps for food. The recent discovery of non-indigenous ascidians in Newfoundland and Labrador warrants the compilation of a comprehensive checklist of ascidian species to provide insight into the diversity of local ascidians and a basis for future comparisons with neighbouring marine environments. We have conducted a thorough literature review, search of online databases, and our own field collections to assemble the first comprehensive checklist of ascidian species for insular Newfoundland and mainland Labrador. A total of 38 ascidian species have been reported from the cold ocean waters of Newfoundland and Labrador since 1860. Among them are two non-indigenous, colonial ascidians, *Botrylloides violaceus* (Oka, 1927) and *Botryllus schlosseri* (Pallas, 1766), which have had an impact on aquaculture sustainability in Atlantic Canada. Our field collections are the first reports of these invasive, encrusting, marine invertebrates to have survived the winter as established resident populations on Newfoundland's south coast.

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### Introduction

Circa 2,300–2,500 ascidian species (Ascidiacea, Tunicata) are known to science<sup>1</sup>. About 100 ascidian taxa inhabit the Pacific coastal waters of western Canada and United States<sup>2</sup>, while 88 species are reported from the Atlantic Continental Shelf of the eastern United States<sup>3</sup>, and only 37 species from the Gulf of St. Lawrence<sup>4</sup>. Currently, there are 6 confirmed non-indigenous ascidians documented in both Atlantic and Pacific waters of Canada: *Botrylloides violaceus*, *Botryllus schlosseri*, *Ciona intestinalis*, *Didemnum vexillum*, *Molgula manhattensis*, and *Styela clava*<sup>5, 6, 7, 8, 9, 10</sup>. Marine ecosystems are threatened by the invasion of ascidians, which has become an emerging global problem<sup>9, 11</sup>. In response, a growing number of studies aim to understand the process of ascidian invasions, towards the goals of minimising their detrimental ecological and economic damage and of controlling their potential spread. The recent discovery of two non-indigenous, colonial

ascidians, *Botrylloides violaceus* (violet tunicate) and *Botryllus schlosseri* (golden star tunicate), on Newfoundland's south coast<sup>10</sup> warrants an examination of local biological diversity of ascidians. In particular, there is a serious knowledge gap in the general description of indigenous ascidian taxa and an overall lack of taxonomic expertise<sup>12</sup>. Up-to-date, baseline information on species richness and the extent of biological invasion are needed to better inform the management of invasive ascidians in Newfoundland and Labrador.

### Materials and Methods

*Field collections.* SCUBA divers collected specimens during the rapid assessment surveys (RAS, September 2006–October 2008)<sup>10</sup> and additional field surveys (September 2009–March 2010) from insular Newfoundland. Specimens from the RAS were preserved in ethanol. Indigenous specimens collected in September 2009 and

February 2010 were returned to our laboratory where they were maintained alive in tanks containing flowing unfiltered sea water and identified and photographed within a few days. In January 2010, samples of non-indigenous *Botrylloides violaceus* (n=2) and *Botryllus schlosseri* (n=5), along with several indigenous ascidian specimens, were sent to Lambert<sup>13</sup> for taxonomic identification.

*Published literature and online database records.* In addition to ascidian occurrence records interpreted from published scientific literature, occurrence data were downloaded from 2 online databases: the Ocean Biogeographic Information System (OBIS, <http://www.iobis.org/>) and the Global Biodiversity Information Facility (GBIF, <http://www.gbif.org/>). All taxonomic names were updated to be consistent with the World Register of Marine Species (WoRMS, <http://www.marinespecies.org/>) and the Integrated Taxonomic Information System (ITIS, <http://www.itis.gov/>). If available, a qualitative description of abundance (i.e., rare, uncommon, common, abundant, or very abundant) was also noted. *Botrylloides violaceus* and *Botryllus schlosseri* are indigenous to the Mediterranean Sea and north-western Pacific Ocean, respectively<sup>14</sup>. *B. violaceus* was likely introduced to eastern North America via transport of aquaculture equipment<sup>15</sup> and *B. schlosseri* via transport on fouled ship hulls<sup>16</sup>; both are considered non-indigenous to Newfoundland and Labrador. We assumed all other records of ascidian taxa to be indigenous to the north-western Atlantic Ocean and to Newfoundland and Labrador.

## Results

*Field collections.* Eight indigenous and two non-indigenous ascidian species were collected from insular Newfoundland (Table 1). In addition to those previously described<sup>10</sup>, we collected *Dendrodoa carnea* (average body length:  $0.47 \pm 0.20$  cm, n=3) in Salmonier Arm at a depth of 6–11 m, and *Aplidium glabrum* ( $2.20 \pm 1.37$  cm, n=4), *Ascidia callosa* ( $4.87 \pm 2.89$  cm, n=8), *Boltenia echinata* ( $2.08 \pm 0.48$  cm, n=3), *Didemnum albidum* (2.01 cm, n=1), and *Halocynthia pyriformis* ( $5.09 \pm 2.41$  cm, n=4) in Logy Bay at a depth of 11 m. Returning to Arnold's Cove and Belleoram, we collected overwintering colonies of *Botryllus*

*schlosseri* (n=4, depth of 1–2 m) and *Botrylloides violaceus* (n=9, depth of 2–8 m), respectively.

*Published literature and online database records.* We found 12 published papers and 3 datasets reporting the occurrence of ascidians in Newfoundland and Labrador (Table 2). The earliest paper describes ascidians collected in 1860<sup>17</sup>. There are 156 ascidian records (collected in the years 1879–1971) in the National Museum of Natural History Invertebrate Zoology Collections<sup>18</sup>, 58 records (collected in August 2004) in the North American Sessile Marine Invertebrate Survey<sup>19</sup>, and 25 records (undated) in the Yale Peabody Invertebrate Zoology DiGIR Service<sup>20</sup> that are geo-referenced to Newfoundland and Labrador. Considering all sources together (including diver collections), a total of 38 ascidian species (36 indigenous and 2 non-indigenous) have been reported either from insular Newfoundland, or Labrador (Table 2). Ten (26%) belong to the sub-order Aplousobranchia, 5 (13%) to Phlebobranchia, and 23 (61%) to Stolidobranchia. Overall, 13 (34%) are colonial and 25 (66%) are solitary species. Thirty-three species were clearly referenced to insular Newfoundland, 25 clearly to Labrador and 20 common to both, giving a Sørensen's similarity coefficient of 0.69. Thus, we conclude that the ascidian fauna of Newfoundland and Labrador is relatively diverse (i.e., 38 species in total), with a high similarity between the Province's island and mainland portions.

## Discussion

Accurate checklists of native species are important tools for protecting biodiversity, identifying knowledge gaps and monitoring the status of non-indigenous species<sup>21</sup>. A checklist of indigenous and non-indigenous ascidian species provides essential information needed for managing invasive ascidians, which includes (a) not misidentifying indigenous species as non-indigenous, (b) identifying potential new invaders, (c) determining future changes to local species richness, and (d) determining distribution and extent of biological invasions. A description of indigenous ascidian species for Newfoundland and Labrador was previously difficult to access in the literature; in the most comprehensive compilation<sup>18</sup>, descriptions are spread throughout the text since it is organised by species rather than region. Reports of non-indigenous ascidians come primarily from insular

**Table 1** Geographic location, latitude and longitude, collection date, and number of specimens (n) of ascidian species that were collected from dive surveys of insular Newfoundland

<b>Species</b>	<b>Location</b>	<b>Latitude</b>	<b>Longitude</b>	<b>Collection date</b>	<b>n</b>			
<i>Aplidium glabrum</i>	Bauline	47.7232	-52.8348	June 7, 2007	1			
	Gadd's Harbour	49.5089	-57.8719	April 26, 2007	1			
	Logy Bay	47.6253	-52.6646	August, 2007	1			
				February 2, 2010	4			
<i>Ascidia callosa</i>	Bauline	47.7232	-52.8348	June 7, 2007	1			
	Logy Bay	47.6253	-52.6646	August, 2007	1			
				February 2, 2010	8			
<i>Ascidia prunum</i>	Harbour Breton	47.4736	-55.8270	October 23, 2007	1			
	Hermitage	47.5563	-55.9259	October 23, 2007	1			
<i>Boltenia echinata</i>	Bauline	47.7232	-52.8348	June 7, 2007	1			
	Belleoram	47.5272	-55.4092	November 1, 2007	15			
				September 20, 2007	1			
	Harbour Breton	47.4736	-55.8270	October 23, 2007	8			
				August, 2007	7			
				February 2, 2010	3			
	Logy Bay	47.6253	-52.6646	July 16, 2007	2			
				November 6, 2006	1			
	Port-Aux Basques	47.5751	-59.1402	September 19, 2007	3			
				Southern Harbour	47.7122	-53.9699	September 19, 2007	3
<i>Botrylloides violaceus</i>	Belleoram	47.5272	-55.4092	October 24, 2007	18			
				October 28, 2007	6			
				November 1, 2007	12			
				March 11, 2008	10			
				March 10, 2010	9			
<i>Botryllus schlosseri</i>	Argentia	47.2920	-53.9904	December 7, 2006	4			
				Arnold's Cove	47.8747	-54.1682	September 16, 2007	1
	Hermitage	47.5563	-55.9259	September 19, 2007	1			
				October 13, 2009	6			
				October 27, 2009	7			
				November 24, 2009	25			
				February 15, 2010	4			
				September 19, 2007	1			
				October 23, 2007	4			
				Long Harbour	47.4407	-53.7874	February 22, 2007	1
				North Harbour	47.8590	-54.1000	September 11, 2007	14
				<i>Dendrodoa carnea</i>	Salmonier Arm	47.1487	-53.4693	September 22, 2009
<i>Didemnum albidum</i>	Bauline	47.7232	-52.8348	June 7, 2007	1			
	Gadd's Harbour	49.5089	-57.8719	April 26, 2007	1			
	Logy Bay	47.6253	-52.6646	August, 2007	3			
				February 2, 2010	1			
<i>Halocynthia pyriformis</i>	Bauline	47.7232	-52.8348	June 7, 2007	3			
	Logy Bay	47.6253	-52.6646	August, 2007	4			
				February 2, 2010	4			
	Port-Aux Basques	47.5751	-59.1402	November 6, 2006	4			
				July 16, 2007	3			
				November 6, 2006	22			
<i>Molgula griffithsi</i>	Gadd's Harbour	49.5089	-57.8719	May 5, 2009	1			

**Table 2** A checklist of 38 species from indigenous and non-indigenous ascidian records of insular Newfoundland (Nfld.) and Labrador (Labr.). 0 = absent, 1 = rare, 2 = uncommon, 3 = common, 4 = abundant, 5 = very abundant, black shading = indigenous, hatched shading = non-indigenous

Species	Location																								
	Caribou Island, Labr.	Strait of Belle Isle, Labr.	Strait of Belle Isle, Labr.	Labr.	Nfld.	Labr.	Nfld.	Nfld.	Nfld.	Bonne Bay, Nfld.	West Coast of Nfld.	Near St. John's, Nfld.	Conception Bay, Nfld.	Nfld.	Labr.	South Coast of Nfld.	Port Burwell, North of Labr.	Grand Banks, Nfld.	South Coast of Nfld.	Bonne Bay, Nfld.	Near St. John's, Nfld.	South Coast of Nfld.			
<i>Aplidium glabrum</i>																								3	3
<i>Aplidium pallidum</i>											3														
<i>Aplidium translucidum</i>																									
<i>Ascidia callosa</i>	4	4																						4	
<i>Ascidia obliqua</i>																									
<i>Ascidia prunum</i>							4				2														3
<i>Ascidia dijmphiana</i>																									
<i>Bostrichobranchus pilularis</i>																									
<i>Boltenia echinata</i>											4													4	4
<i>Boltenia ovifera</i>																									
<i>Botrylloides aureum</i>							1																		
<i>Botrylloides violaceus</i>																									
<i>Botryllus schlosseri</i>																									
<i>Chelyosoma macleayanum</i>																									
<i>Cnemidocarpa finmarkiensis</i>																									
<i>Dendrodoa carnea</i>							3																	3	
<i>Dendrodoa grossularia</i>																									
<i>Dendrodoa pulchella</i>																									
<i>Didemnum albidum</i>			3								3												3	3	
<i>Distaplia clavata</i>																									
<i>Eudistoma vitreum</i>																									
<i>Eugyra glutinans</i>																									
<i>Halocynthia pyriformis</i>			3								3													5	5
<i>Leptoclinides faerøensis</i>																									
<i>Lissoclinum aureum</i>																									
<i>Microcosmus glacialis</i>																									
<i>Molgula citrina</i>											3														
<i>Molgula complanata</i>																									
<i>Molgula griffithsii</i>																									
<i>Molgula retortiformis</i>																									
<i>Molgula siphonalis</i>																									
<i>Pelonaia corrugata</i>	2																								
<i>Polycarpa fibrosa</i>																									
<i>Rhizomolgula globularis</i>																									
<i>Styela coriacea</i>																									
<i>Styela rustica</i>																									
<i>Synoicum pulmonaria</i>																									
<i>Trididemnum tenerum</i>																									
<b>Publication year</b>	1863	1867	1870	1922	1922	1945	1945	1964	1966	1975	1998	1999	2005	2006	2009	2009	2009	2009	2009	2010	Present study	Present study	Present study		
<b>References</b>	17	24	25	23	23	16	16	26	27	22	4	28	19	6	18	18	20	20	10	Present study	Present study	Present study			

Newfoundland's south coast, with only 2 reports from the island's west coast and none from Labrador. *Botryllus schlosseri* was initially detected in Bonne Bay and St. Paul's Inlet, on Newfoundland's west coast, in the 1970s<sup>22</sup>. It was subsequently observed in several harbours on Newfoundland's south coast for the first time in 2006. These include harbours at Argentia, Arnold's Cove, Fox Harbour, Garden Cove, Hermitage and North Harbour<sup>10</sup>. *Botrylloides violaceus* was first reported in 2007 from Belleoram, on Newfoundland's south coast<sup>10</sup>. Currently, *B. violaceus* and *B. schlosseri* are both locally abundant in the harbours that they are fouling on Newfoundland's south coast.

We are doubtful of the identification of *Didemnum granulatum* (undated record from Hopedale, Labrador) and *Molgula pugetiensis* (2004 record from Port de Grave, Newfoundland) listed in the databases. These 2 species were not included in our checklist because they are not known to this region of the world and were possibly misidentified. We are uncertain of *Aplidium translucidum* (1950 record from Kongulaksiarvik, Labrador), which typically occurs in Alaska and may be a misidentification of *Aplidium glabrum*<sup>16, 23</sup>. Our checklist included *Ascidia dijmphniana* (1921 record from Labrador), as it is a rare Arctic species in North America which was positively documented in Baffin Bay<sup>16</sup>. We included *Chelyosoma macleayanum* (1950 record from Hamilton Inlet, Labrador), as it is a rare species which has a known circumpolar Arctic distribution<sup>16</sup>. Also, we included *Molgula siphonalis* (1880 record from Grand Banks, Newfoundland), as it is an Arctic species which was positively identified in Foxe Bay<sup>16</sup>.

### Conclusion

Subsequent to Callahan et al.<sup>10</sup>, we returned to several harbours and discovered that populations of *Botrylloides violaceus* and *Botryllus schlosseri* have survived the winter and are well established on Newfoundland's south coast. Despite the relatively high similarity in ascidian faunal composition between insular Newfoundland and Labrador, there are currently no reports of non-indigenous ascidians from Labrador. Table 2 is the first comprehensive checklist of ascidian species from Newfoundland and Labrador, and addresses the formerly limited information of indigenous ascidians in this Province. In addition, the checklist

provides a baseline to monitor for potential ascidian invaders. In the advent of global climate change, the discovery of established populations of *B. violaceus* and *B. schlosseri* in the cold waters of Newfoundland is unusual for these temperate species. This raises concerns for the potential biological invasion of non-indigenous ascidians such as *Ciona intestinalis*, *Didemnum vexillum*, and *Styela clava* in insular Newfoundland, which have been affecting aquaculture industries in other parts of Atlantic Canada.

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## Temporal variations in sediment sulfide levels under marine salmon farms in southwestern New Brunswick, Bay of Fundy, during the annual environmental monitoring period



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### **Abstract**

Sediment sulfide levels were measured every week during September-October 2008 and every two weeks during August-October 2009 at two salmon farms and a reference site in southwestern New Brunswick. The reference site had low sulfide levels at all sample locations on all dates. Mean sulfide levels increased over time at both farms during September-October 2008 coincident with increasing fish biomass and feeding rates. During August-October 2009, mean sulfide levels initially increased and then decreased at both farms; during this time, fish biomass and feeding rates also decreased due to harvesting. There was considerable variation in sulfide levels among locations sampled at the same site on the same date, as well as between successive samples from the same location.

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### **Introduction**

The purpose of this study was to investigate if there are temporal variations in sediment sulfide levels under salmon farms in southwestern New Brunswick (SWNB), Bay of Fundy, during the months when annual regulatory environmental monitoring occurs (August-October). Previously collected data indicated that there are seasonal changes in sediment sulfide levels under salmon farms in SWNB<sup>1,2</sup> but the frequency of data collection was insufficient to describe changes within the regulatory monitoring period. Other data collected at two locations near fish farms in SWNB, but not in the immediate vicinity of the cages, found mostly oxic conditions, with no clear seasonal trends in sulfide levels<sup>3</sup>. The present study measured sediment sulfide levels weekly in September-October 2008 and every two weeks in August-October 2009 at two salmon farms and a reference site in the Letang area of SWNB.

### **Materials and Methods**

Sediment samples were collected at two fish farms (sites A and B) in the Letang area in SWNB, as

well as a reference site (C) located in the same area but away from fish farms. Site A had ten 70-m circumference circular cages (approximately 150,000 Atlantic salmon in total) and site B was an integrated multitrophic aquaculture (IMTA) farm growing approximately 500,000 Atlantic salmon in twenty 100-m circular cages, plus mussels and kelp. Both farms were stocked in the fall of 2007. Harvesting at site A started on 23 July 2009 and was completed by 3 October 2009. Harvesting at site B started on 31 August 2009 and was completed at the end of November 2009.

Sediment samples were collected weekly at six locations at each farm site (at the four corners of the cage array plus two locations near the middle) and at six similarly spaced locations at site C from early September to late October in 2008 and every two weeks from mid-August to late October in 2009, using a Hunter-Simpson grab which collected 0.024 m<sup>2</sup> of sediment (16 × 15 cm). One grab was taken at each location on each sampling date, except on 27-28 October when triplicate grab samples were taken per location. From each grab

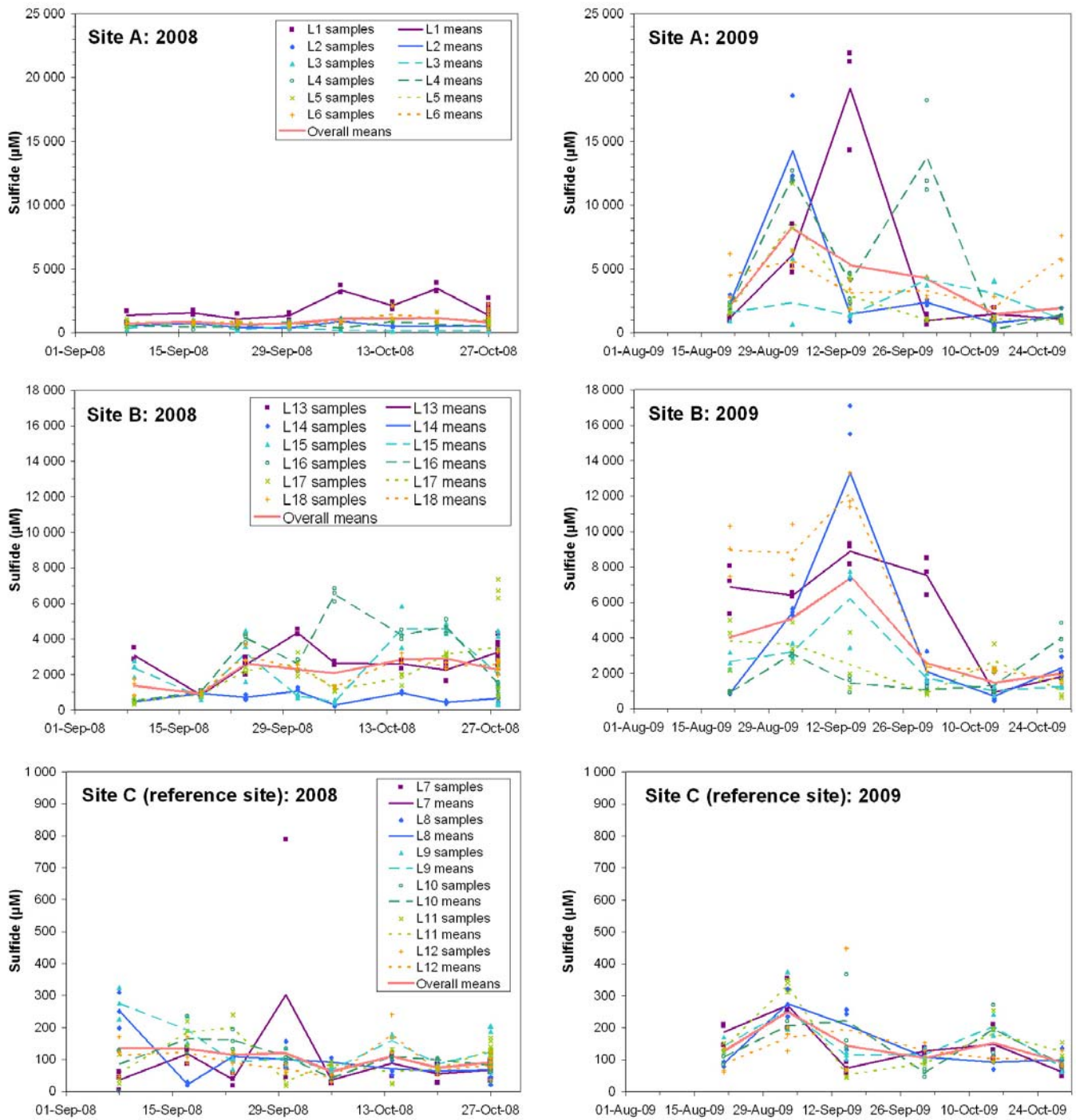


Figure 1. Sediment sulfide levels measured under two salmon farms (Sites A and B) and a reference site (C) in southwestern New Brunswick in 2008 (left) and 2009 (right). Six locations were sampled at each site: L1-L6 at site A, L13-L18 at site B, and L7-L12 at site C.

sample, three spatially scattered 5-ml syringe subsamples were collected from the top 2 cm of sediment. The subsamples were analyzed for total sulfides within 1 d of sampling.

### Results

Consistent with previous data<sup>3</sup>, the sulfide levels at reference site C remained low throughout the sampling periods in both years (Fig. 1). At both farm sites, the mean sulfide levels increased over time in September-October 2008, although at site A the increase was small and mainly due to the trend at one location (L1). During the same period, the fish biomass at both sites increased, and feeding rates at both sites increased initially and then decreased in late October. During August-October 2009, sulfide levels at both farm sites initially increased and then decreased, with much variability among sampling locations and dates. Fish biomass and feeding rates decreased during this period at site A due to harvesting, and there were no fish remaining after early October. Fish biomass and feeding rates increased initially at Site B and then decreased due to harvesting, but there was still about half of the maximum fish biomass remaining at the end of October 2009. The highest sulfide values at both farms in 2009 occurred after the times of maximum fish biomass and feeding rates. At site A, some high sulfide levels were observed after feeding had ceased in 2009, and at the end of the 2009 sampling period sulfide values were still considerably higher than at the reference site even though there had been no farmed fish or feeding at the farm for several weeks.

### Discussion and Conclusions

Our results suggest that at farms where biomass levels and feeding rates are increasing, sulfide

levels may also increase during the 3-month window for regulatory monitoring. Sulfide levels will likely decrease once harvesting has started, although there appears to be a lag behind the decreases in fish biomass and feeding rates. Sediment sulfide levels at farm sites after fish had been harvested did not return to background levels within the sampling period. Our results also showed considerable variability among locations at the same farm, as well as between successive samples at the same location.

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## Seaweed farming in Chaleurs Bay (Québec): Results from 4 years of research and development activities

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### Abstract

There are currently 18 companies involved in seaweed harvesting, processing and sales in Québec. There is also a growing interest from the mussel industry for diversification and several seaweed cultivation projects have been initiated with mussel producers. In 2006, one kelp harvesting company started a kelp farm in Chaleurs Bay and several experiments on *Saccharina longicruris* cultivation were carried out in the lab and on the farm. Through manipulation of photoperiod and water temperature, out of season sporogenesis was induced in *S. longicruris* blades kept in indoor basins. *In vitro* cultivation and multiplication of gametophytes allowed the seeding of culture ropes that were successfully out-planted on submerged longlines. In the kelp nursery, ropes seeded with spores currently yield 4 mm plantlets within 4 weeks and an experiment was carried out to determine optimal plantlet density. During the first attempts to cultivate kelp at sea, they were invaded by colonies of the non-indigenous bryozoa *Membranipora membranacea*, which resulted in the loss of most plants in the autumn. Modifying the culture schedule avoided bryozoan settlement on the kelp fronds. Efforts are now oriented towards increasing kelp culture yields and assessing new seaweed species such as *Alaria esculenta* and *Palmaria palmata*.

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### Introduction

Farming seaweed is uncommon in Eastern Canada. However, global marine aquaculture is dominated by marine plants, not fish. Annually, the total sales of cultivated seaweeds are worth US\$ 6 billion. Cultivating seaweed has advantages over harvesting natural beds. Culture often translates into high yields and constant high quality products. It gives the opportunity to gain control over the kelp life cycle, providing year-round access to seedlings. Finally, the culture of marine plants can be considered as sustainable extractive aquaculture. However, production costs are generally higher because of the necessity to maintain onshore facilities. Cultivation activities require also the development of new skills. Results are highly dependant on meteorological conditions, as storms can adversely affect production. But the main problem is user conflicts since kelp farming requires large areas that are in many cases already exploited by fishermen.

There are currently 18 companies involved in seaweed harvesting, processing and sales in Québec<sup>1</sup>, most of them working with wild brown algae. Seaweeds are processed into fertilizers, cosmetics, nutraceuticals, animal food and fine foods. However, resource availability and accessibility are limited, and currently only 150 tonnes are harvested annually on a total of 1000 tonnes allowed by Fisheries and Oceans Canada (DFO).

In 2006, *Les Gaspésiennes*, a kelp harvesting company, started a kelp farm in open water at Paspébiac, Chaleurs Bay, Québec. The objective was to cultivate *Saccharina longicruris* and *Saccharina latissima* or Kombu royal to produce fine food products. There is also a growing interest from the mussel industry for diversification and several seaweed cultivation projects have been initiated with mussel producers. In collaboration with these companies, we focused our efforts on

three main R&D themes: 1) spore availability, 2) gametophyte cultivation and 3) improvement of the culture yields on the marine farm.

### Methods, Results and Discussion

In order to gain control on spore production, we have adapted the approach of Pang and Lüning<sup>2</sup>. By removing the blade meristematic area and by keeping immature blades of *S. longicuris* under a constant short-day regime (8L:16D) at 15 °C, we can now accelerate the maturation of the blade and obtain viable spores in four weeks, at any time of the year. We also adapted the technique developed by IFREMER in France for *Undaria pinnatifida* to keep a stock of living seeds (in this case seeds are

gametophytes) in a liquid medium<sup>3</sup>. When cultivated under red light, the gametophytes of *S. longicuris* cannot mature sexually and can only grow vegetatively. By fragmenting them regularly, it is possible to multiply their numbers. When required, the seeds are sprayed on culture ropes and exposed to white light to promote sexual maturation and reproduction, in order to finally produce sporophytes. Using this method we were able to keep viable cultures of gametophytes during 10 months and these cultures were successfully used to produce kelp on the marine farm<sup>4,5</sup>. In the future, this method will be used to work on hybridisation and strain selection.

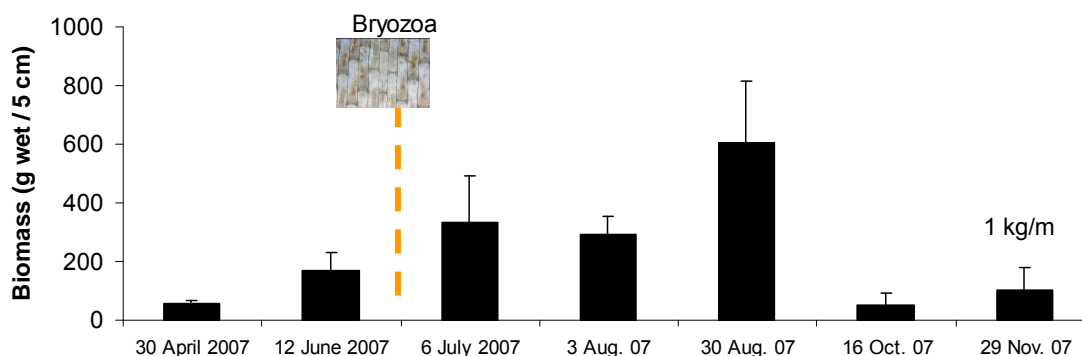


Figure 1. Seasonal changes in the biomass (wet weight) of *S. longicuris* cultivated on a submerged longline at Paspébiac (Québec) between April and November 2007. In this trial, 5 cm-long Nylon strings seeded with kelp, were inserted at 10 cm intervals through 1 m-long vertical culture ropes, suspended below the longline. The dashed line indicates when the first colonies of the bryozoan *Membranipora membranacea* were observed.

Experiments were made to test the effect of seedling density on the growth rate of kelp plantlets in the hatchery<sup>6</sup>. Results indicate that by artificially decreasing the plant density on the culture ropes, growth rate can be doubled over a 3 to 4 month period. Further experiments are planned to examine if density control in the hatchery can translate into higher harvest yields on the marine farm.

During our first trial, the kelp were cultivated on a subsurface longline at 2 m of depth<sup>6</sup>. During the summer, it became covered with a non-indigenous bryozoan (*Membranipora membranacea*). It started to form colonies on the blades in July and in August, almost 100% of the blade surface was covered. The blades then lost their flexibility and during the autumn storms, almost all the cultivated kelp were broken and lost. So, in November the

final biomass was extremely low, ca. 1 kg wet weight per meter of vertical ropes (Fig. 1). In this trial, it was also observed that the kelp blades had a pale yellow colour. This was probably due to the exposure to UV radiations or to excessive light combined with lower nutrient concentrations in the surface water during the summer.

In the second trial, the possibility of making more than one harvest per year using submerged longlines was investigated<sup>5</sup>. Longlines were kept 7 m below the surface to protect the plants and culture gear from winter drift ice. From these trials, it was learned that when transferring the 2mm-long seedlings on the farm in November or in April, kelp reached only a length of 60 cm after a 4-month growth period (November-March and April-August).



Figure 2. July harvest of *S. longicruris* cultivated during 8 months on a submerged longline at Paspébiac (Québec).

However, kelp left on the farm from November to July reached nearly 2 m long and mean yield reached 3.3 kg per meter of rope (min. 1.9 kg/m; max. 4.5 kg/m), which is a 3 fold increase from the initial trial (Fig. 2). Furthermore, there were almost no bryozoa on the blades. The blades were clean and very thin, which makes them suitable for food products.

To summarize, it is now possible to get viable spores of *S. longicruris* at any time of the year and to keep a large stock of seeds in the lab to quickly re-seed culture ropes in case of a loss caused by a gale or by a disease. Furthermore, in order to avoid encrusting bryozoa, blade discoloration and drift ice, transfer of the seedlings at sea should be done in November and harvesting in July, while keeping the longlines below 5 m of depth. These preliminary results show high potential to improve

harvest yields and help reach commercial objectives.

### Acknowledgments

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## Stress response of the spotted wolffish (*Anarhichas minor*) to rearing density and a stressful event reveals a true farming-friendly species

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### Abstract

The spotted wolffish (*Anarhichas minor*) is a benthic species of the northern Atlantic Ocean that has been identified as a promising species for cold-water aquaculture. Advantages of raising this species include high growth rate, low disease susceptibility, tolerance to large water quality variations and a very calm and non-aggressive behavior in captivity in contrast to their aggressive reputation in the wild. Determining the optimal culture conditions and their impact on fish welfare is of primary importance for reducing stress in aquaculture. Juvenile wolffish of two sizes (50-100 g and 100-150 g) were individually tagged and submitted to three different densities. At the end of the growth trial, the second group (100-150 g) was then submitted to a stressful event (held out of the water for 1 minute in a net). Parameters measured included body mass and length, haematocrit, hepatosomatic index, muscle and liver water content and plasma concentration of Na<sup>+</sup>, K<sup>+</sup>, protein, lysozyme and cortisol. A second experiment on adult wolffish measured the time course of cortisol response to the same stressor. Results show that spotted wolffish has a good tolerance to crowding and stress.

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### Introduction

An optimal rearing density is of primary importance for intensive cost-efficient aquaculture operations. Stress is unavoidable in aquaculture (netting, sorting, crowding, etc.)<sup>1-2</sup>. Exposure to such stressors causes a series of biochemical and physiological changes, many of them mediated via cortisol secretion during the primary stress response. Rearing fish at high density can cause cumulative stress or homeostatic acclimation. Optimal rearing density is highly species specific and varies with age and life stage<sup>3-4</sup>. The objectives of this study were therefore, a) to determine optimal rearing density and b) investigate stress response to both density and an acute stressor in

the spotted wolffish (*Anarhichas minor*), a promising cold-water aquaculture species<sup>5-6</sup>.

### Material and Methods

*Experiment 1 – Increasing rearing density: 50-100 g fish*

Individually tagged juvenile wolffish (n=199) were randomly distributed among six tanks for duplicate initial rearing densities of 10, 20 and 40 kg/m<sup>2</sup>. Densities were allowed to increase as the fish grew and the fish were measured (body mass) on days 0, 39, 59 and 120. Blood samples were taken from three fish per tank on days 0, 15 and 30 for measurement of plasma Na<sup>+</sup> and K<sup>+</sup> concentrations.



### Experiment 2 – Fixed rearing density: 100-150 g fish

Juvenile wolffish (n=189) were randomly divided into nine groups for triplicate rearing densities of 20, 30 and 40 kg/m<sup>2</sup>. Individual body mass measurements were made on days 0, 29, 59 and 90, and rearing densities readjusted to initial values at each sampling date by moving adjustable screens. At day 186 (6 months) of the experiment, four fish per tank were netted and held in the air for 1 min<sup>7</sup>. After 60 min, blood samples were taken and the fish killed by a blow to the head and the following parameters measured: haematocrit, plasma cortisol, total plasma proteins (TP) and lysozyme activity, hepatosomatic index and water content of muscle and liver.

### Experiment 3 – Time course of the stress response

Two groups of adult spotted wolffish were used (mean body mass: 857.3 g ± 225.0), with one serving as the control (n=43) to establish baseline cortisol values for unstressed wolffish. Fish of the second group (n=53) were netted in eight different groups, held in the air for 1 min. and released in eight identical tanks. Blood samples were taken at 0.5, 13, 37 and 168 hours post-stress from five fish per tank, with duplicate tanks for each sampling time, for the measurement of plasma cortisol concentration.

## Results and Discussion

### Experiment 1 and 2 -Rearing density

The range of rearing densities covered by our experiments was within previously published limits for spotted wolffish<sup>8-9</sup>. Holding wolffish at up to 56.3 kg/m<sup>2</sup> decreased the final body mass of 50-100 g fish compared to fish held at lower densities (Fig. 1, p=0.001 for effect of density but post-hoc multiple comparison test was not significant). However, the growth of 100-150 g fish was not affected by stocking density up to 40 kg/m<sup>2</sup> (final mean mass of 160.2±5.9, 159.7±3.7 and 163.7±11.5 g at 20, 30 and 40 kg/m<sup>2</sup>, respectively; F<sub>2,6</sub>=0.048, p=0.953). Muscle and liver water content, haematocrit and plasma concentrations of Na<sup>+</sup>, K<sup>+</sup>, total proteins and lysozyme were not affected by density. Hepatosomatic index was significantly higher at the higher density,

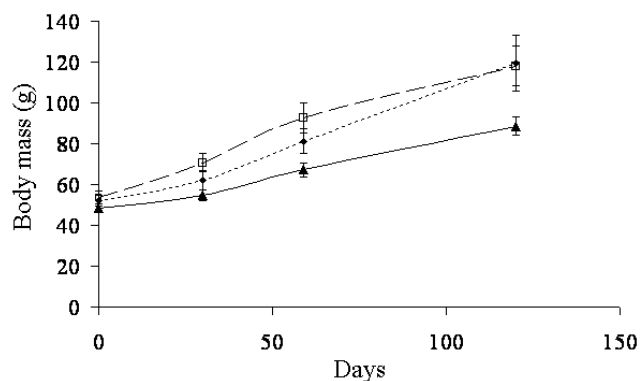


Figure 1. Growth (mean body mass ± standard error) of wolffish held at different rearing densities with no adjustment of densities as the fish grew; starting densities were 10 kg/m<sup>2</sup> (diamonds), 20 kg/m<sup>2</sup> (clear squares) and 40 kg/m<sup>2</sup> (triangles).

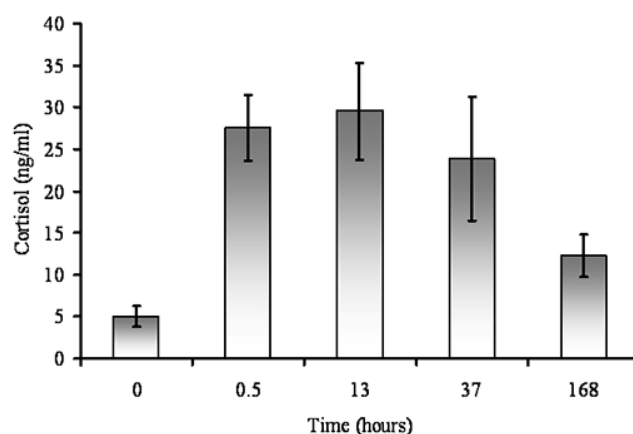


Figure 2. Plasma cortisol concentrations in wolffish before (0 h) and at different times after being held in a net out of the water for 1 min (mean ± standard error).

suggesting an overall decrease in swimming activity<sup>10</sup>. Plasma cortisol concentrations increased with increasing density (effect of density p=0.04 but post-hoc multiple comparison test not significant); values were 14.8 ± 3.9, 31.1 ± 8.7 and 45.1 ± 7.0 ng/ml at densities of 20, 30 and 40 kg/m<sup>2</sup>, respectively). The cortisol value obtained at 20 kg/m<sup>2</sup> is generally considered representative of unstressed fish<sup>11</sup>. When compared with the same unit as for pelagic species (kg/m<sup>3</sup>), density of 30 kg/m<sup>2</sup> is equivalent to approximately 215 kg/m<sup>3</sup> for

fish of that size<sup>12</sup>. Another experiment<sup>9</sup> on 3.5 kg wolffish suggests an optimum rearing density of  $\geq 90$  kg/m<sup>2</sup> with respect to growth rate, feed conversion and productivity.

#### *Experiment 3 - Time course of the stress response*

Time had a significant effect on plasma cortisol values (Fig. 2). Cortisol reached maximal values between 0.5 and 13 hours post stress (27.5 and 29.5 ng/ml, respectively), and returned to values non-significantly different from pre-stressed values after 168 hours. Another study on wolffish<sup>12</sup> showed that cortisol did not increase before 4 hours post-stress (tank emptying for 10 min.) and reached maximal values of 30 ng/ml. Cortisol response is influenced by previous life-history of the fish<sup>13</sup>, which may explain why we obtained a different time response. The highest cortisol values recorded post-stress (29.5 ng/ml) are comparable to what was observed in a similar experiment on haddock<sup>14</sup> but below what is observed for other fish species (cod (*Gadus morhua*): 75 ng/ml<sup>15</sup>; European seabass (*Dicentrarchus labrax*): 800 ng/ml<sup>15</sup>; three-spined sticklebacks (*Gasterosteus aculeatus*): 80 ng/ml<sup>16</sup>). Therefore, unless a stronger response was missed between 0.5 and 13 hours post-stress, wolffish response to an acute stress could be qualified as relatively weak.

#### **Conclusion**

Spotted wolffish of 50-100 g should be raised at density of 30 kg/m<sup>2</sup> and 100-150 g fish at density of 40 kg/m<sup>2</sup>. A stressful event provoked an elevation of cortisol that is indicative of a weak response and it returned to initial value by 168 hours post-stress. Tolerance to high densities along with their remarkably calm behaviour, lack of escape response to people in captive conditions<sup>12</sup> and low stress response makes wolffish a perfect candidate for farming.

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## Aquaculture Canada<sup>OM</sup> 2010 and Northern Harvest<sup>TM</sup> 2010

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## Effect of finfish aquaculture operations on biochemical composition and growth of algae

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### **Abstract**

Overloading of nutrients in effluent from finfish aquaculture operations leads to various environmental impacts. We are studying the dissolved inorganic nutrients associated with finfish aquaculture and their effects on algae. Three species of microalgae, *Isochrysis* sp., *Nannochloropsis salina* Hibberd and *Chaetoceros muelleri*, were cultured in the effluent from land-based juvenile Atlantic cod (*Gadus morhua*) tanks. Three methods were chosen to sterilize the effluent in preliminary experiments: autoclaving, filtration, and ultraviolet light. The algae grown in filtered effluent had the highest growth rate, followed by ultraviolet light and autoclave processing, suggesting filtration and ultraviolet light are practical methods for sterilizing effluent in large scale culture. In a separate study, Atlantic salmon cage sites in the Broughton Archipelago, British Columbia, were sampled by retrieving macroalgae with 20 µm net tows in transects surrounding the farms. Levels of dissolved inorganic nutrients at 10 m depth surrounding cage sites were significantly higher than those at 1 m depth. Total lipid concentration in net tows was significantly higher than that in *Fucus* and eelgrass. Bacterial fatty acids and PUFA in *Fucus* were negatively correlated with distance from farms. In addition, principal component analysis indicated different biochemical compositions among species.

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### **Introduction**

The worldwide growth in aquaculture has increased awareness of its environmental impacts, which are mostly the result of nutrient overloading in the effluent from finfish aquaculture operations. This can cause hypernutrification and eutrophication<sup>1,2</sup> that lead to concomitant changes in aquatic community structure and function by favoring a few tolerant species while decreasing biodiversity and altering the structure of the food web in marine and coastal areas<sup>3</sup>.

To mitigate the impacts of intensive aquaculture, studies have been carried out on developing integrated aquaculture systems, recently known as Integrated Multi-Trophic Aquaculture (IMTA), in which finfish culture is combined with culture of organisms that extract either dissolved inorganic nutrients or particulate organic matter, establishing a balance between aquaculture needs and environmental impacts<sup>4,5</sup>. We are studying the dissolved inorganic nutrients associated with

finfish aquaculture and their effects on algae. The objectives of this research are to: (i) identify the influence of fish aquaculture on phytoplankton, as well as seaweed in the surrounding environments, (ii) determine the growth performance and biochemical qualities of typical diatom and flagellate species grown in fish effluent, (iii) determine inorganic nitrogen and phosphorus extraction capabilities of these species, as well as principal controlling factors affecting these capabilities, and (iv) model the growth and biochemical compositions of these species in the context of land-based IMTA systems.

### **Methods**

Three species of microalgae, *Isochrysis* sp., *Nannochloropsis salina* Hibberd and *Chaetoceros muelleri*, were cultured in the effluent from land-based juvenile Atlantic cod (*Gadus morhua*) tanks. Three methods were chosen to sterilize the effluent in preliminary experiments: autoclaving, filtration and ultraviolet light. Growth rates of each

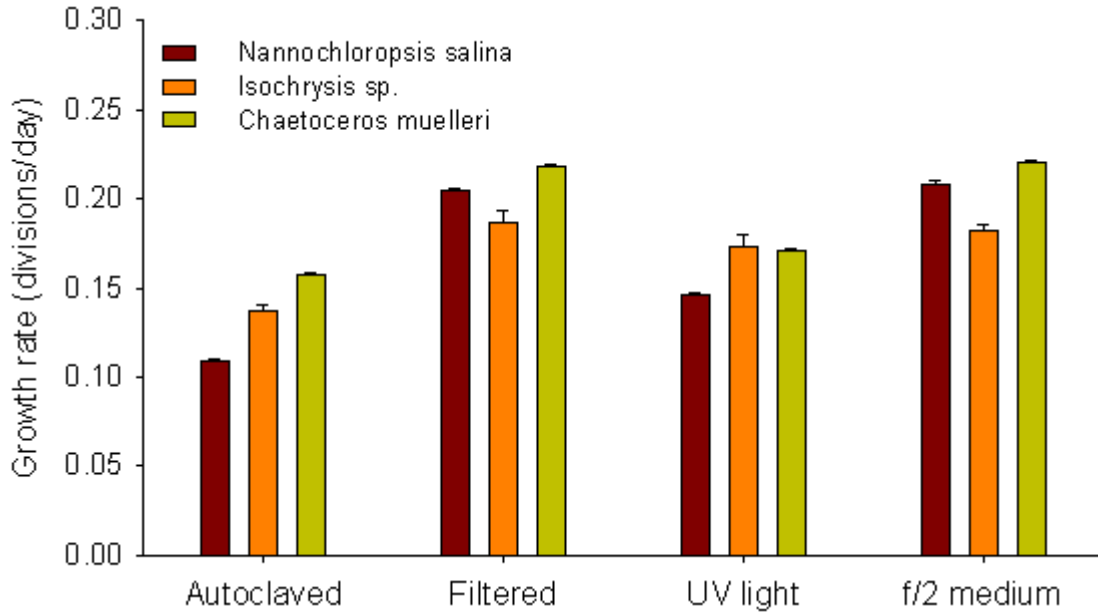


Figure 1. Growth rates of each species grown in autoclaved fish effluent (*autoclaved*), filtered fish effluent (*filtered*), ultraviolet light (*UV light*), and f/2 enriched seawater (*f/2 medium*), respectively, during the first ten days (mean ± S.D.).

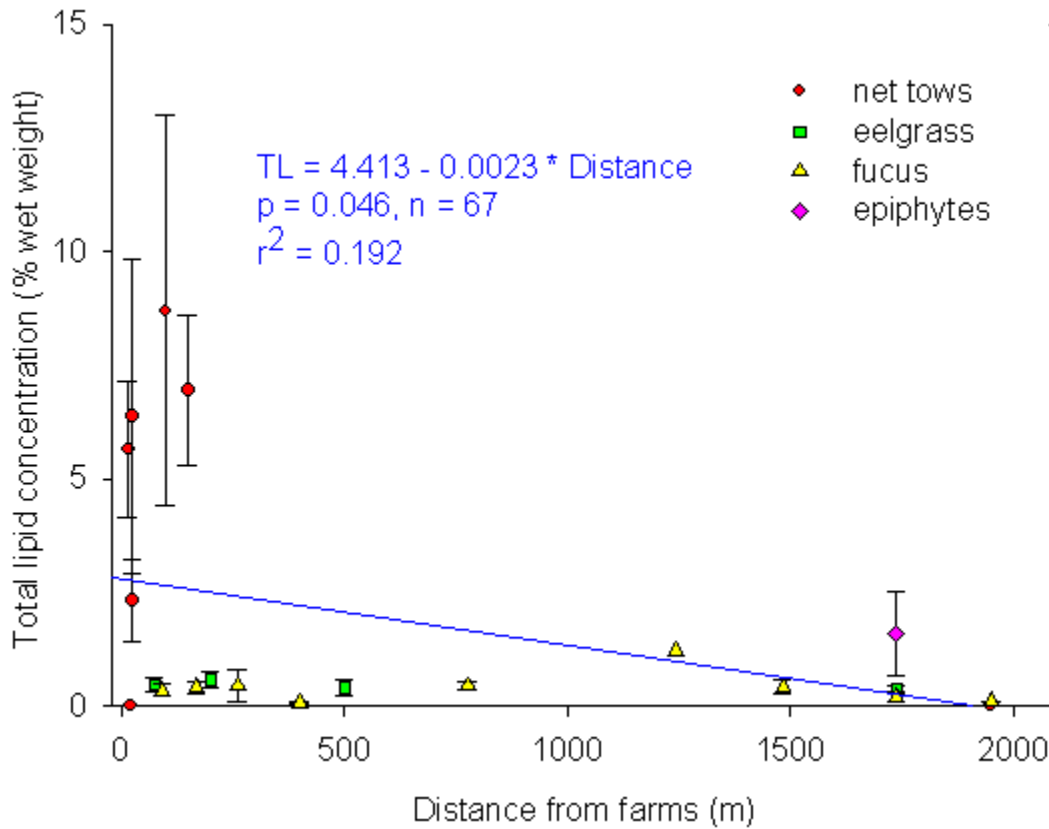


Figure2. Total lipid concentrations in net tow, *Fucus*, eelgrass, and epiphyte samples collected at Atlantic salmon cage sites in British Columbia. The linear regression was calculated from raw data.

species cultured in effluent that was sterilized by different methods were measured to determine the best method for effluent sterilization. In a separate study, Atlantic salmon cage sites in the Broughton Archipelago, British Columbia, were sampled by retrieving macroalgae and by performing 20 µm net tows in transects surrounding the farms. Dissolved inorganic nutrients, lipid and fatty acid compositions were measured<sup>6</sup>.

### Results and Discussion

The algae grown in filtered effluent had the highest growth rate, followed by those with ultraviolet light and autoclave treatments (Fig. 1). This suggests filtration and ultraviolet light are practical methods for sterilizing effluent in large scale culture and supports the potential use of microalgae in land-based IMTA systems.

The levels of dissolved P, Si and N around fish cages at 10 m depth were significantly higher than those at 1 m depth. Total lipid concentrations in net tows were significantly higher than in *Fucus* and eelgrass (Fig. 2). Bacterial fatty acids and PUFA in *Fucus* correlated negatively with distance from farms. Principal component analysis (PCA) was conducted to determine the relationship among the lipid composition and species, as well as the locations in the entire data set. This allows for a great deal of information to be presented in a few graphs and shows the correlation of cases (species and locations) and variables (lipids and fatty acids). PCA also reduced the number of response variables for further analysis. PCA results suggested that

finfish aquaculture had different effects on the biochemical composition of different species.

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Association Aquacole du Canada

**The objectives of our association are:**

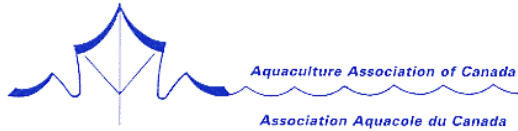
- a) *To foster an aquaculture industry in Canada, to promote the study of aquaculture and related science in Canada, to gather and disseminate information relating to aquaculture, and to create public awareness and understanding of aquaculture;*
- b) *To promote, support, and encourage educational, scientific, and technological development and advancement of aquaculture in Canada;*
- c) *To gather and disseminate technical and scientific information on aquaculture development in Canada and throughout the world;*
- d) *To conduct seminars for the presentation, exchange, and discussion of information, findings, and experiences on all subject and techniques related to aquaculture;*
- e) *To encourage the teaching of all phases of aquaculture and the training of aquaculture and the training of aquaculturists in accredited colleges and universities in the field of aquaculture; and*
- f) *To encourage private industry and government agencies, both provincial and federal, to support education, research and development.*

AAC carries out these objectives primarily through our annual meeting Aquaculture Canada<sup>OM</sup> where we hold workshops, seminars, contributed papers and posters and discussions. We also publish the Bulletin of the AAC. Proceedings of the annual general meeting are published under the Special Publications series.

# Aquaculture Canada<sup>OM</sup> 2010 & Cold Harvest<sup>TM</sup> 2010

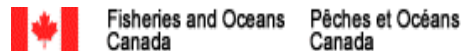
*Co-hosts / Hôtes conjoints*

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## *Diamond/ Platinum contributors / Commanditaires diamant:*

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## *Gold contributors / Commanditaires or:*

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AQUACULTURE  
CANADA<sup>OM</sup>

2011

CULTURING DIVERSITY AND SUCCESS  
CULTIVER LE SUCCÈS ET LA DIVERSITÉ

QUÉBEC CITY MAY 8<sup>TH</sup> TO 11<sup>TH</sup>, 2011  
VILLE DE QUÉBEC 8 AU 11 MAI 2011  
LOEWS HÔTEL LE CONCORDE QUÉBEC

[WWW.AQUACULTUREASSOCIATION.CA](http://WWW.AQUACULTUREASSOCIATION.CA)

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