Aquaculture Canada^{OM} 2004

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C.I. Hendry, editor


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Sustainable AQUACULTURE

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Algal Cultivation / Production des Algues

Aquaculture Engineering / Génie Aquacole

Integrated Multitrophic Aquaculture / Aquaculture Multi-trophique Intégrée

Aquaculture Risk Communications / La Communication des Risques en Aquaculture

Socioeconomics of Aquaculture / Socio-économie de l'Aquaculture

President's Report

The 21st Annual Meeting of the Aquaculture Association of Canada (AAC) marked the close of an exciting and challenging year as President. Having little knowledge of what I was truly agreeing to when I was asked to stand for this august position, I can honestly confess that it brought a whole new appreciation of the day-to-day activities of our Association and of the stalwart souls that keep the Association and our annual meetings on track. Despite having been a member for many years, I had a very naïve concept of what is required to keep AAC healthy and relevant to the dynamic issues that challenge and push our industry as it continues to grow and mature.

As I return to 'normal' life within the Association and come towards the end of my term on the Board of Directors, I would like to encourage all members (students, professors, industry, government, and associated interests) to seriously consider playing a more active roll in the AAC. Board membership certainly takes sporadic time and effort, but it also provides an excellent opportunity to share ideas with a diversity of expertise spanning all realms interested and involved in Canadian Aquaculture spanning marine, freshwater, finfish, shellfish, and algae production.

This being said, membership in the Association and Board activities also increases our exposure to the scope of challenges that face aquaculture as it strives to develop in Canada. Aquaculture has fought hard to gain the leases and sites needed to produce affordable high-quality fish and seafood. This fight has certainly taken its toll in the face of seemingly inexhaustible criticism, challenges and regulatory inertia. 2004 provided little respite for the socio-economic casualties associated with these challenges. Despite this, however, progress continues with new species, new government commitments dedicated to aquaculture, and ever-increasing market opportunities. Canadians love producing, eating, and marketing home-grown seafood. Few permanently discard this passion in the face of spurious science and anti-aquaculture campaigning.

Our Association takes pride in its historic inclusion of all aquaculture interests to provide a forum for open and transparent debate of issues challenging aquaculture development and survival. This years meeting was no exception with an excellent plenary presentation by Dr. Jason Clay of the World Wildlife Fund. What struck me as I listened to him was, despite many similarities between terrestrial farming and aquaculture, there is a fundamental difference that appears to fuel much of the angst aimed at aquaculture. The public rarely feels ownership of a farmer's field or the wind blowing through it. Many do, however, feel a natural tie to the oceans and rivers that promotes close attention to all the activities they see taking place therein. Since we are unlikely to lose this interest (and nor should we want to!), we need to develop the ways to keep this interest engaged, but in a positive manner. Community involvement in siting decisions, development of local as well as remote markets, among others, were ideas presented and discussed at AC04. I would just like to re-iterate the value of engaging community support. Although it can be a costly investment effort-wise, it pays significant dividends when the community emerges to protect aquaculture from unwarranted attack by 'outsiders'. Examples of such positive intervention exist in certain rural parts of Canada, but there remains scope for spread.

Your new AAC President, Dr. Thierry Chopin, brings personal enthusiasm and experience to this forward momentum. In 2004 we actively sought more freshwater involvement in national activities – with resounding success and thanks to the Association des Aquaculteurs du Québec and Eric Gilbert of the Aquaculture Management Directorate of Fisheries and Oceans Canada, among *many* others - and 2005 is likely to see marine plants gain a higher profile as a new and promising opportunity for the Canadian entrepreneurial spirit.

With sincere thanks to everyone who made my Presidency such an enjoyable experience, I look forward to continuing to actively support your Association as it continues to evolve. Don't hesitate to join me – it's an excellent (and achievable!!) New Year's Resolution…

Sharon McGladdery
AAC President 2003-2004

Rapport de la présidente

La 21e réunion annuelle de l'Association aquacole du Canada (AAC) a marqué la fin d'une année excitante et stimulante en tant que présidente. Je ne savais pas vraiment ce qui m'attendait quand on m'a demandé d'occuper ce noble poste, et je peux vous dire honnêtement que mon expérience m'a permis de mieux comprendre les activités quotidiennes de notre association et des membres vaillants qui s'assurent que notre association et nos réunions annuelles restent sur la bonne voie. Malgré le fait que j'étais membre de l'Association depuis plusieurs années, j'avais une perception très naïve des éléments nécessaires pour maintenir la santé de l'AAC et veiller à ce qu'elle tienne compte des enjeux dynamiques auxquels notre industrie est confrontée à mesure qu'elle progresse et se stabilise.

Mon mandat à titre de membre du conseil d'administration tire à sa fin, et je me prépare à reprendre un rôle « normal » au sein de l'Association. Je voudrais encourager tous les membres (étudiants, professeurs, membres de l'industrie, représentants gouvernementaux et aux parties intéressées) à envisager sérieusement de jouer un rôle plus actif dans l'AAC. Le temps et l'effort qu'exige la participation au conseil d'administration sont sporadiques, mais il s'agit d'une excellente occasion d'échanger des idées avec toute une gamme de spécialistes dans tous les domaines liés à l'aquaculture au Canada, notamment la production de poisson, de mollusques et crustacés et d'algues en eaux marines et douces.

Ceci dit, la participation aux activités de l'Association et du conseil d'administration nous sensibilise également davantage à l'étendue des défis que doit relever le secteur aquacole à mesure qu'il se développe au Canada. Le secteur aquacole a déployé de grands efforts pour obtenir les baux et les sites nécessaires en vue de produire des poissons et des fruits de mer de grande qualité et abordables. Cette lutte a certainement été difficile en raison des critiques incessantes, des défis et du manque d'avancement de la réglementation. L'année 2004 a accordé peu de répit en ce qui a trait aux répercussions socioéconomiques associées à ces défis. Toutefois, malgré cela, nous continuons de réaliser des progrès relativement à de nouvelles espèces, à de nouveaux engagements gouvernementaux visant l'aquaculture et aux perspectives du marché de plus en plus nombreuses. Les Canadiens aiment produire, manger et commercialiser des produits de la mer bien de chez nous. Ils ne sont pas nombreux à laisser tomber cette passion à cause de données scientifiques trompeuses et de campagnes anti-aquacoles.

L'Association est fière d'avoir regroupé pour la première fois tous les intérêts aquacoles afin de servir de tribune où tenir des débats ouverts et transparents sur les enjeux qui compromettent le développement et la survie de l'aquaculture. La réunion de cette année n'a pas fait l'exception grâce à la présentation en plénière de M. Jason Clay, du Fonds mondial pour la nature. Pendant que je l'écoutais, quelque chose m'a marqué : malgré les nombreuses similitudes entre l'agriculture et l'aquaculture, il semblerait qu'une différence fondamentale soit à l'origine des inquiétudes concernant l'aquaculture. La population se sent rarement touchée par le champ d'un fermier et le vent qui souffle sur lui. Un grand nombre de Canadiens ressentent cependant un attachement naturel aux océans et aux rivières et se préoccupent ainsi de toutes les activités qui y sont menées. Puisque cet intérêt ne se dissipera sûrement pas (et nous ne voulons pas qu'il se dissipe!), nous devons élaborer des manières de le maintenir, mais d'un point de vue positif. La mise à contribution des collectivités dans les décisions sur les sites et le développement de marchés locaux et éloignés, entre autres, ont été proposés lors de la conférence Aquaculture Canada 2004 et ont fait l'objet de discussions. Je tiens à souligner l'importance du soutien communautaire. Bien qu'il faille parfois déployer des efforts immenses pour obtenir ce soutien, les retombées sont considérables lorsque la collectivité se manifeste pour défendre l'aquaculture contre des attaques injustifiées. De telles interventions positives ont déjà eu lieu dans certaines régions rurales du Canada, mais il faudrait les étendre à d'autres régions.

Votre nouveau président de l'AAC, M. Thierry Chopin, contribuera son enthousiasme et son expérience à cet avancement. En 2004, nous avons travaillé activement en vue d'apporter un point de vue axé davantage sur les eaux douces aux activités nationales. Nous avons remporté un franc succès grâce à l'Association des aquaculteurs du Québec et à Eric Gilbert, de la Direction générale de l'aquaculture de Pêches et Océans Canada, parmi *bien* d'autres, et l'année 2005 verra sûrement les plantes marines prendre une place importante en tant que nouvelle perspective prometteuse pour les entrepreneurs canadiens.

Je voudrais remercier sincèrement tous ceux et celles qui ont fait de mon mandat à titre de présidente une expérience extraordinaire, et je me réjouis à l'idée de continuer d'appuyer activement notre association à mesure qu'elle évoluera. N'hésitez pas à vous joindre à moi – c'est une excellente résolution du Nouvel An (que nous pourrons tenir de surcroît!!)…

Sharon McGladdery
AAC Présidente 2003-2004

2004 Honourary Lifetime Achievement Award

Ovila Daigle

Ovila Daigle grew up in Pointe-Sapin a small coastal community in New Brunswick. He has a degree in Forestry from UNB where he learned his trade of land surveyor. He started his career in Newcastle N.-B. in 1959 working with the province of NB, surveying forest lots to mark their boundaries and assess wood production. He went on to work with what was then the minister of Environment and Fisheries at the Ellerslie Fisheries Research Station in PEI. Ovila's skills at surveying were extensively used to develop the shellfish leasing program and policy of the day.

Mr. Daigle and his team were responsible to define and survey the new leases, assess their potential, produce the maps and maintain a database for the hundreds of sites around the Maritime Provinces. Before the days of GPS and GIS, this type of work required lugging heavy surveying equipment through the woods, long hours of triangulation on water followed by animated discussions on wharves about oyster culture. Anyone who knows Ovila is bound to share is enthusiasm for shellfish culture and to appreciate his kind nature.

Part of his work at the Ellerslie station was to carry out extension programs to promote oyster culture. Because each new site had to be individually surveyed, Mr. Daigle developed an intimate knowledge of each bay. To this day, people still call him to find information about specific sites.

Following the onset of the Malpeque disease in the 50s and 60s, Mr. Daigle was called upon to re-stock all bays in New Brunswick, Nova Scotia and PEI with seeds resistant to the disease. Again, Ovila's intimate knowledge of each bay proved

valuable in ensuring the success of this program. Most oysters cultivated or harvested today (except in Bras d'Or Lake) in the Maritime Provinces are direct descendant of this seed stock. In total, 11 000 barrels of 3-inch oysters and 272 barrels of 1-inch oysters were re-introduced in our waters by Mr. Daigle and his team between 1960 and 1970.

Following this, Mr. Daigle became manager of the Shellfish Leasing Program for Fisheries and Oceans where he was instrumental in setting up the burgeoning mussel aquaculture industry, especially in PEI. He retired from DFO in 1992.

Mr. Daigle is still very active in the family shellfish aquaculture enterprise, Aquaculture acadienne Ltée owned and operated by his son Maurice Daigle since 1982.

The shellfish aquaculture industry of the three Maritime provinces is indebted to Mr. Daigle for this lifetime contribution.

Sharon McGladdery, AAC President, presents the Honourary Lifetime Achievement Award to Ovila Daigle.

Sharon McGladdery, Présidente de l'AAC, présente le Prix d'excellence pour l'ensemble des réalisations à Ovila Daigle.

2004 Research Award of Excellence

Edward M Donaldson PhD DSc FRSC

Dr. Donaldson is Scientist Emeritus at the West Vancouver Laboratory of the Department of Fisheries and Oceans. Originally, from Cumbria in the UK, he completed his BSc (Hon's) in Zoology at the University of Sheffield in 1961 and a PhD in Zoology from the University of British Columbia in 1964. Funded by a NIH Postdoctoral Fellowship, he spent a year in the Department of Medical Biochemistry at the University of Minnesota. He joined DFO 's Vancouver Laboratory in 1965 and moved to the West Vancouver Laboratory in 1968 where he was a Research Scientist and became Head of the Biotechnology, Genetics and Nutrition Section. His research covered a range of topics including the development of techniques for induced ovulation and spermiation, production of monosex and sterile populations, growth acceleration and evaluation of stress in wild and cultured salmonids. This research would not have been possible without the efforts of many graduate students, postdoctoral fellows, and colleagues at DFO, UBC, SFU, UVic, the aquaculture industry, and research institutes around the world. Dr. Donaldson has served on the Editorial Advisory Board for *Aquaculture* since 1983 and as Section Editor of Physiology and Endocrinology for *Aquaculture* since 1999. He has sat on numerous DFO committees including serving as chair of the Deputy Minister's Science Advisory Committee and has lectured or consulted on aquaculture research in over 30 countries. He currently serves (2001-2004) on the Life Sciences Fellowship Selection Committee, Academy of Science, Royal Society of Canada. He has been a member of the Board of Directors of the Vancouver Aquarium Marine Science Centre since 1992, serves as an Adjunct Professor at UBC and founded Ed Donaldson & Associates Ltd., aquaculture and fisheries consultants in 2001. Awards received include the American Fisheries Society, 1977 Most Significant Paper Award, Fisheries and Oceans Canada, 1989 Ministerial Merit Award, Science Council of British Columbia, 1992 Gold Medal in Natural Sciences, Royal Society of Canada, 1995 Thomas W. Eadie Medal, Fisheries and Oceans Canada, 1997 Deputy Minister's Commendation.

Chris Hendry, AAC Vice President, presents the Research Award of Excellence to Dr. Edward Donaldson.

Chris Hendry, Vice Président de l'AAC, présente le Prix d'excellence en recherche à Dr. Edward Donaldson.

2004 Prix d'excellence pour l'ensemble des réalisations

Ovila Daigle

Ovila Daigle grandit à Pointe-Sapin, une petite collectivité côtière du Nouveau-Brunswick. Il détient un diplôme en foresterie de l'Université du Nouveau-Brunswick, où il a appris son métier d'arpenteur. Il débute sa carrière en 1959 à Newcastle, au N.-B., où il travaille pour la province à l'arpentage des terres boisées afin d'en définir les limites et d'évaluer leur production de bois. Il travaille par la suite pour le ministre de l'Environnement et des Pêches d'alors à la Ellerslie Fisheries Research Station, à l'Î.-P.-É. Ovila y met grandement à contribution ses compétences en arpentage afin de mettre sur pied le programme de baux de secteurs coquilliers et la politique connexe.

M. Daigle et son équipe ont alors la responsabilité de définir et d'arpenter les nouvelles concessions, d'évaluer leur potentiel, de concevoir des cartes et de mettre à jour une base de données portant sur des centaines de sites dans les provinces des Maritimes. Étant donné que les GPS et GIS n'ont pas encore fait leur apparition, ce type de travail nécessite de transporter du matériel d'arpentage très lourd dans les bois, d'effectuer de la triangulation durant de longues heures sur l'eau et de tenir par la suite de vives discussions au sujet de l'ostréiculture sur les quais. Tous ceux qui connaissent Ovila sont tenus de partager son enthousiasme pour la conchyliculture et d'apprécier son amabilité.

Une partie du travail qu'il effectue à la Ellerslie Fisheries Research Station consiste à mettre en œuvre des programmes d'appoint sur l'ostréiculture. Étant donné que chaque nouveau site doit être arpenté individuellement, M. Daigle apprend à connaître en détail chaque baie. Des gens qui cherchent de l'information sur des sites particuliers l'appellent encore aujourd'hui pour le consulter.

Après l'apparition de la maladie de Malpèque dans les années 50 et 60, on fait appel à M. Daigle pour qu'il rétablisse les stocks dans toutes les baies du Nouveau-Brunswick, de la Nouvelle-Écosse et de l'Île-du-Prince-Édouard à l'aide de stocks reproducteurs immunisés contre cette maladie. La connaissance approfondie qu'a Ovila de chaque baie s'avère d'une grande utilité pour faire de ce programme une réussite. La plupart des huîtres cultivées ou pêchées aujourd'hui dans les provinces des Maritimes (sauf dans le lac Bras d'Or) descendent directement de ces stocks reproducteurs. En tout, M. Daigle remet à l'eau le contenu de 11 000 barils d'huîtres de trois pouces et de 272 barils d'huîtres d'un pouce entre 1960 et 1970.

Par la suite, M. Daigle devient gestionnaire du Programme de baux de secteurs coquilliers à Pêches et Océans Canada, où il joue un rôle de premier plan dans l'éclosion de l'industrie florissante de la mytiliculture, surtout à l'Î.-P.-É. Il prend sa retraite du MPO en 1992.

M. Daigle demeure très actif au sein de l'entreprise conchylicole familiale, Aquaculture acadienne Ltée, que son fils Maurice Daigle possède et exploite depuis 1982.

L'industrie conchylicole des trois provinces Maritimes est reconnaissante à M. Daigle pour son énorme contribution dans le domaine.

2004 Prix d'excellence en recherche

M. Edward M. Donaldson, docteur ès sciences, MSRC

M. Donaldson est scientifique émérite au Laboratoire de Vancouver-Ouest du ministère des Pêches et des Océans. De Cumbria, au Royaume-Uni, il obtient un baccalauréat en sciences (avec distinction) en zoologie à l'Université de Sheffield en 1961 et un doctorat en zoologie à l'Université de la Colombie-Britannique en 1964. Titulaire d'une bourse de recherche postdoctorale du NIH, il passe une année au Département de biochimie médicale de l'Université du Minnesota. Il se joint au Laboratoire de Vancouver du MPO en 1965 et au Laboratoire de Vancouver-Ouest en 1968 à titre de chercheur et il y devient par la suite chef de la section Biotechnologie, génétique et nutrition. Ses recherches portent sur divers sujets, dont les techniques de provocation de l'ovulation et de l'émission du sperme, de production de populations monosexuées et stériles, d'accélération de la croissance et d'évaluation du stress chez les salmonidés sauvages et d'élevage. Ces recherches n'auraient pas pu être réalisées sans la collaboration de nombreux étudiants diplômés, de détenteurs d'une bourse de perfectionnement postdoctoral ainsi que de collègues du MPO, de l'Université de la Colombie-Britannique, de l'Université Simon Fraser, de l'Université de Victoria, de l'industrie de l'aquaculture et d'instituts de recherche de partout dans le monde. M. Donaldson siège au comité consultatif de rédaction d'Aquaculture depuis 1983 et il est rédacteur en chef de la section Physiologie et endocrinologie d'Aquaculture depuis 1999. Il siège à de nombreux comités du MPO; il est notamment président du Comité consultatif du sous-ministre des Sciences et il a donné des conférences et mené des consultations sur la recherche en aquaculture dans plus de tente pays. Il siège actuellement (2001-2004) au sein du comité de sélection des récipiendaire de bourse en sciences de la vie, Académie des sciences, Société royale du Canada. Il est membre du conseil d'administration du Vancouver Aquarium Marine Science Centre depuis 1992, professeur auxiliaire à l'Université de la Colombie-Britannique et il a fondé en 2001 Ed Donaldson & Associates Ltd., une firme de consultants dans le domaine de l'aquaculture et des pêches. Il a reçu le prix décerné par l'American Fisheries Society pour l'article le plus important en 1977, le prix d'excellence de Pêches et Océans Canada en 1989, la médaille d'or en sciences naturelles décernée par le Conseil des sciences de la Colombie-Britannique en 1992, la médaille Thomas W. Eadie décernée par la Société royale du Canada en 1995 et la mention du sous-ministre de Pêches et Océans Canada en 1997.

AC04 Student Affairs Report

Another year has passed, and another great conference! Aquaculture Canada[OM] 2004 was a wonderful time in Quebec City, and support for and from students was again very strong.

This year was record-breaking with respect to student oral and poster presentations; 34 oral presentations and 35 poster presentations were judged among 33 judges, the most of any AAC conference in history! After the evaluations were finished among the superior student presentations, **Carla Walbourne** (Dalhousie University) was awarded for the best oral presentation (sponsored by the Aquaculture Centre, University of Guelph), entitled: "Effects of dietary lipid on prevalence of fatty liver condition in juvenile haddock, *Melanogrammus aeglefinus*." Additionally, **Spencer Russell** (University of Guelph) was awarded for best poster presentation (also sponsored by the Aquaculture Centre, University of Guelph), entitled "Plasma proteomic analysis of the acute phase response of rainbow trout (*Oncorhynchus mykiss*)." Papers of both of these award-winning presentations may be found in this publication. Thanks to all the judges and congratulations to all the outstanding student presenters.

The student BBQ was unique this year, occurring onboard the MV Louis Joliet on the St. Lawrence River. This offered a great waterfront view of Old Quebec, as well as providing a feast of seafood and other delicacies, a quality the Student BBQ is known for every year. Thanks to all the musicians who entertained into the wee hours of the morning. Another reason the BBQ is so popular is because of the silent auction. With hundreds of donated items available for bidding, and all proceeds benefiting the AAC Student Endowment Fund (SEF), this has become one of the annual conferences favorites. The silent auction would not be nearly as successful without the tireless efforts of AAC students soliciting local businesses for donations, particularly Louis Bourque and Amélie Bélanger-Lamonde before and during the auction. At the AC04 silent auction, $1838 was raised for the SEF, which will help future students attend Aquaculture Canada[OM] meetings. The following sponsors are gratefully thanked:

Aquaculture Association of Canada, Advantage Delivery Service, AquaHealth, Canadian Aquacuture Industry Alliance (CAIA), Debbie Martin-Robichaud, Département de l'Agro-economie – Université Laval, Département de la Biologie – Université Laval, Département des Sciences Animales – Université Laval, Fisheries and Oceans Canada, Domaine Orléans, EWOS, Fruit and Passion du Petit Champlain, Galerie Denis Laroche, Galerie du Petit Champlain, Hoskin Scientific, Laura Halfyard, Le Roquet, Marine Institute, Musée d'art Inuit, National Shellfisheries Association, Ocean Sciences Centre, PEI Aquaculture Alliance, PerOS, Pot en Ciel, ShurGain, Souffleur de Verre Petit Champlain, Springer, University of Guelph, US Trout Farmers Association

While on the topic of the SEF, which funds AAC Student Travel Awards (this year sponsored by the Atlantic Provinces Council on the Sciences (APICS), Société de développement de l'industrie maricole (SODIM) inc., CAIA, the University of Guelph, and the Science Branch of Fisheries and Oceans Canada), the following eleven students were partially funded to attend AC04:

Cyr Couturier (AAC Past President) and Sharon McGladdery (AAC President) prepare oysters onboard the MV Louis Joliet at the Student BBQ.

Cyr Couturier (AAC Président sortant) et Sharon McGladdery (AAC Présidente) préparent les huîtres à bord du MV Louis Joliet pour le BBQ des Étudiants.

Zeng Duan (Ocean University of China); Andre Dumas and Ling Yang(University of Guelph); Cris Jenkins, Keith Sullivan, and Stephanie Synard (Marine Institute); Leah Lewis and Carla Walbourne (Dalhousie); Daphne Munroe (UBC); Robyn O 'Keefe and Jeff Piercey (UNB); and Yi Pan (Universit du Quebec Rimouski).

Finally, the efforts of Terralynn Lander must be applauded for her organization of AV logistics and student coordination at the meeting, allowing sessions and presentations to run smoothly.

I hope to see you all at AC05 in St. John's, NL, where there is sure to be significant social activity on George Street!

Chris Hendry
AAC Vice President 2003-2004

Rapport des Affaires étudiantes sur Aquaculture Canada 2004

Une autre année s'est écoulée et une autre excellente conférence a eu lieu! Aquaculture Canada^{OM} 2004 (AC04) s'est avéré un événement fort agréable à Québec, et le soutien accordé aux étudiants et par eux a été très grand. Le nombre d'exposés et de présentations d'affiches d'étudiants a été sans précédent cette année; 34 exposés et 35 affiches ont été évalués par 33 juges, du jamais vu dans toutes les conférences de l'AAC! Une fois l'évaluation des présentations exceptionnelles d'étudiants terminées, **Carla Walbourne** (Université Dalhousie) a reçu le prix du meilleur exposé (parrainé par le centre d'aquaculture de l'Université de Guelph) intitulé « Effects of dietary lipid on prevalence of fatty liver condition in juvenile haddock, *Melanogrammus aeglefinus* ». **Spencer Russell** (Université de Guelph) a reçu le prix de la meilleure affiche (parrainée par le centre d'aquaculture de l'Université de Guelph) intitulée « Plasma proteomic analysis of the acute phase response of rainbow trout (*Oncorhynchus mykiss*) ». Ces deux présentations sont incluses dans la présente publication. Un grand merci à tous les juges, et félicitations à tous les étudiants pour leurs présentations formidables.

Le barbecue des étudiants a été unique cette année. Il a eu lieu à bord du NM Louis Joliet sur le fleuve Saint-Laurent. Les participants se sont réjouis de la vue du Vieux Québec du bord de l'eau en se régalant d'un festin de fruits de mer et d'autres mets délicats, un exploit reconnu du barbecue des étudiants chaque année. Merci à tous les musiciens qui nous ont divertis jusqu'aux petites heures du matin. L'encan silencieux contribue également à la popularité du barbecue. Des centaines d'articles avaient été donnés, et toutes les recettes de l'encan ont été remises au fonds de dotation des étudiants de l'AAC. Il s'agit de l'une des activités les plus courues de la conférence annuelle. L'encan silencieux n'aurait pas été aussi réussi sans les efforts inlassables des étudiants de l'AAC qui ont contacté des entreprises locales pour leur demander de faire des dons avant et pendant l'encan, notamment Louis Bourque et Amélie Bélanger-Lamonde. L'encan silencieux d'AC04 a rapporté 1 838 $ pour le fonds de dotation des étudiants,

Rich Moccia (University of Guelph) presents the award for Best Student Oral Presentation to Carla Walbourne (Dalhousie).

Rich Moccia (Université de Guelph) présente le prix du meilleur exposé à Carla Walbourne (Dalhousie).

Rich Moccia (University of Guelph) presents the award for Best Student Poster Presentation to Spencer Russell (University of Guelph).

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qui aidera des étudiants à assister aux réunions d'Aquaculture Canada[OM] futures. Nous tenons à remercier nos commanditaires :

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En ce qui concerne le fonds de dotation des étudiants, qui finance les bourses de voyage d'étudiants de l'AAC (parrainées cette année par l'APiCS, la SODIM, l'AICA, l'Université de Guelph et les Sciences du MPO), voici les onze étudiants qui ont reçu des fonds afin qu'ils assistent à AC04 :

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Au plaisir de vous revoir à AC05 à St. John's (Terre-Neuve et Labrador), où nous saurons certainement apporter notre contribution à la vie sociale sur la rue George!

Chris Hendry
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Effect of Size and Diet on Seawater Mortality of Triploid Brook Charr (*Salvelinus fontinalis*)

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We investigated the effect of diet and size as a strategy aimed at the reduction of sea-water (SW) post-transfer mortality in triploid brook charr. During two consecutive years, triploid (3n) fish of two sizes (0^+, 50 g, 2003 and 1^+, 100 g, 2004) were fed experimental diets containing NaCl and betaine in order to stimulate the activation of Na^+K^+ATPase and increase the survival and growth performances of fish population reared in SW. In 2004, T_3 (12 mg/kg) supplementation in addition to NaCl (10%) and betaine (3%) was also tested. We also investigated the possibility of modulating the window of SW introduction of this species using a pre-transfer conditioning on different experimental diets one month prior to the SW transfer in late October. Significant effect of feed supplementation on the survival of 3n (0^+, 50 g) fish was observed in 2003 with a significant improvement of the order of 50% in survival compared to 3n (0^+, 50 g) fish fed a non-supplemented feed. In 2004, 3n (1^+, 100 g) fish did not respond to the feed supplementation (no difference among the experimental groups). Mortality was significantly lower on the basis of size: 3n (1^+, 100 g) fish displayed less than 5% of mortality compared to 32% and 23% in non-supplemented and supplemented 3n (0^+, 50 g) fish, respectively.

Nous avons étudié l'effet de la taille et du régime alimentaire à titre de stratégie pour réduire la mortalité chez l'omble de fontaine triploïde après son transfert en eau de mer. Durant deux années consécutives, nous avons nourri des ombles triploïdes (3n) de deux tailles différentes (0^+ an, 50 g, 2003 et 1^+ an, 100 g, 2004) d'aliments expérimentaux contenant du NaCl et de la bétaïne en vue de stimuler l'activation de la Na^+K^+ATPase et d'accroître le taux de survie et la performance de croissance des stocks élevés en eau de mer. En 2004, nous avons aussi fait des essais d'alimentation par apport complémentaire de T_3 (12 mg/kg), en plus de NaCl (10 %) et de bétaïne (3 %). Nous avons en outre étudié la possibilité de moduler la période d'introduction dans l'eau de mer en conditionnant les ombles un mois avant leur transfert à la fin octobre par apport de différents régimes alimentaires expérimentaux. Nous avons observé en 2003 un effet significatif de l'enrichissement des aliments sur la survie des triploïdes du groupe (0^+ an, 50 g), leur taux de survie ayant connu une augmentation significative de l'ordre de 50 % en comparaison des triploïdes du groupe (0^+ an, 50 g) nourris d'aliments non enrichis. En 2004, les triploïdes du groupe (1^+ an, 100 g) n'ont pas réagi à l'enrichissement des aliments (aucune différence entre les groupes expérimentaux). Le taux de mortalité selon la taille était significativement moins élevé chez les individus du groupe (1^+ an, 100 g), qui ont connu un taux de mortalité inférieur à 5 % en comparaison de 32 % et de 23 % respectivement chez les individus nourris d'aliments non enrichis et les individus du groupe (0^+ an, 50 g) nourris d'aliments enrichis.

Introduction

The mariculture of brook charr (*Salvelinus fontinalis*) in Quebec is regarded as a priority axis of development. An experimental program was launched in order to evaluate the social, environmental and biological constraints prior to any development of this activity.

Within this program, a previous project conducted in the summer of 2002 by Le François and Lamarre[(1)] assessed the potential of increasing survival of 3n (0^+, 50g) brook charr after direct transfer to seawater (SW). The main findings were that the gill Na^+K^+ATPase activity was higher in the groups treated with supplemented food and a significant reduction of mortality post-transfer was found for groups fed 10% NaCl and 3% betaine as compared to the control group (without supplements).

However, mortality in 2002 was still relatively high. A second project, this time evaluating 3n (1^+, 100g) juvenile brook charr in order to significantly reduce the high mortality, was performed, coupled with the evaluation of the possibility of prolonging the SW window of introduction in late October-December[(2-3)].

Juvenile brook charr 3n (1^+, 100g) were fed experimental diets containing sodium chloride[(4)], betaine[(5)], 3,5,3'-triiodo-L-thyronine (T_3)[(6)], and different combinations for one month prior to SW transfer. Comparison of survival, growth, and osmoregulatory performances among the different experimental groups was realized.

Materials and Methods

The experiment was carried out from mid May to July 2004 (67 days) at the Centre Aquacole Marin facilities (Grande–Rivière, Quebec). 1400 triploids of brook charr (*Salvelinus fontinalis)* of initial average weight of 100 g were obtained from a commercial aquaculture centre (Marinard, Gaspé, Quebec) and were randomly assigned to each eight experimental groups. 75 fish were

used per replicate per experiment and fish were acclimated for 9 days before the start of experiment. The diets were prepared in the Centre Aquacole Marin (Gaspé, Quebec). Treatments were divided into nine replicated groups: FW control, SW control, betaine (B), 3,5,3'-triiodo-L-thyronine (T_3), NaCl (N), BT_3, BN, NT_3, BNT_3. All of them had the same basal mix prepared according to a common commercial formula. The control groups for FW and SW were fed with the same basal diet. The others groups received the same basal diet supplemented with B (3%), N (10%), and T_3 (12 mg/kg). Throughout the trial (FW and SW experiments), experimental diets were kept frozen at -20ºC and were thawed daily before feeding fish.

Freshwater experiment (FW)

The field trial was carried out under quasi-natural conditions from mid May to mid June (4 weeks).

Saltwater experiment (SW)

After 30 days from the start of the experiment, the fish that survived the FW experiment were directly transferred to SW in the same experimental units, held from mid June to the end of July (5 weeks) at an average salinity of 26.8±1.4‰. In both experiments oxygen, temperature, salinity, and mortality were recorded daily.

Sampling procedure

Initially (time zero) twenty fish from the lot were collected, and on days 15 and 29 three fish from FW conditions and five fish from SW conditions on days 43, 52, and 67 were randomly collected from each experimental tank. Maximal blood volume was collected and immediately centrifuged at 7000×*g* for 10 min at 4ºC. The plasma fraction was collected and immediately frozen at -80ºC. The second gill arches from the left side and middle and posterior intestine were dissected out and immersed in SEI buffer. Samples of of liver and white muscle from the dorsal region were also rapidly frozen and then stored at -80ºC until analysed. White muscle water content[7] were measured, and branchial and intestinal Na^+K^+ATPase (U mg prot.$\cdot h^{-1}$) activities[8] were measured using a Lambda 40 (Perkin Elmer) equipped with a thermostated cell holder. Plasma osmolality (mOsm$\cdot kg^{-1}$) was measured using a single-chamber micro-osmometer and ionic concentrations of sodium (Na^+), chloride (Cl^-), and calcium (Ca^{2+}) were measured using an ion analyser. Condition factors (CF) for all growth measurements, hepatosomatic index (HSI), viscerasomatic index (VSI), and specific growth index (G) were calculated.

Results and Discussion

Supplemented feed had a positive effect on survival for 3n (0^+, 50 g) fish but not for 3n (1^+, 100g) fish (Fig. 1). In 2004, 100-g triploid fish in the FW control group suffered 4% mortality during the first 29 days compared with 0-2.6% in the groups fed with the experimental feeds. Following SW transfer, mortality was higher in groups fed with supplemented diets (2-9%). Increased size reduced mortality to 4.9% for 3n (1^+, 100 g) fish compared to 32% and 23% in non-supplemented and supplemented 3n (0^+, 50 g) fish, respectively. Previous studies have shown that fish age and body size are factors that influence the ability of *Salvelinus* spp. to survive exposure to seawater[9].

Supplemented diets had no significative effect on weight, length, growth rate (approx. 1.2× initial size and 0.4% bodyweight/day), osmolality values during the entire experiment, and serum Na^+ and Cl^- during pre-transfer. On day 67, the FW control group showed a significant (P<005) hyponatremia and hypochloremia compared to the BT_3 and BN groups (Table 1).

Continuing studies will investigate the possibility of modulating the window of SW introduction of brook charr 3n (1^+, 100g) using pre-transfer conditioning with different experimental non-supplemented and supplemented diets (N, B, and T_3) one month prior to the SW transfer in late October.

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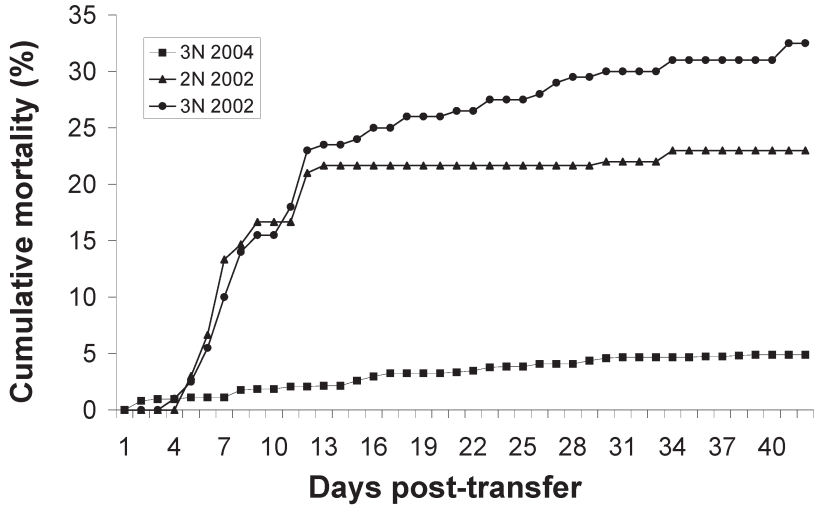

Figure 1.
Comparison of cumulative mortality data in relation to size (50 g vs. 100 g) following direct transfer into seawater.

	Condition Factor	Muscle water content (%)	Osmolality ($mOsm \cdot kg^{-1}$)	Plasmatic Na^+ ($mM \cdot L^{-1}$)	Plasmatic Cl^- ($mM \cdot L^{-1}$)
FW					
0 days (17 May)	0.91 ± 0.08	74.61 ± 1.23	326.83 ± 12.3	157.3 ± 10.20	119.4 ± 4.31
15 days (1 June)	0.94 ± 0.2	75.66 ± 0.69	315.83 ± 10.51	152.0 ± 8.29	118.0 ± 4.1
29 days (15 June)	0.99 ± 0.13	75.47 ± 1.10	323.92 ± 11.05	154.33 ± 15.83	118.17 ± 3.43
SW					
14 days (29 July)	0.96 ± 0.05	75.8 ± 0.91	337.25 ± 9.34	153.9 ± 5.86	120.2 ± 4.21
24 days (8 July)	0.91 ± 0.07	76.74 ± 0.85	329.45 ± 6.16	151.6 ± 4.95	119.3 ± 3.59
38 days (23 July)	0.98 ± 0.08	76.48 ± 1.05	342.55 ± 44.28	160.53* ± 20.3	122.95 ± 9.79

Table 1.
Condition factor, muscle water content, plasma osmolality, and plasma Na^+ and Cl^- during experimental trials in SW introduction. Asterisk indicate significant differences from CFW values ($P = 0.11$). FW values are means ± SE of 3 fish/experiment. SW values are means ± SE of 5 fish/experiment.

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Phytoplankton Early Warning Approaches for Salmon Farmers in Southwestern New Brunswick

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Phytoplankton blooms can cause severe economic losses at salmon farms due to mortalities and/or reduced growth as a result of direct impacts such as toxicity and gill damage, or indirect impacts such as reduced or elevated dissolved oxygen levels. As a result of the economic consequences, salmon farmers in the lower Bay of Fundy would like to have a monitoring approach that warns of potentially harmful phytoplankton events. This project investigates the feasibility and cost-effectiveness of several potential early warning approaches and estimates concentration thresholds for production losses in harmful algal species. The components of this 3-year project include: training farm personnel on the sampling and identification of harmful algal species; implementation of daily phytoplankton monitoring by farm staff at selected salmon farms; statistical analyses of the high frequency monitoring data; retrospective analyses of existing monitoring data; laboratory experiments to determine threshold concentrations of selected harmful algae which cause problems for farmed salmon; water circulation studies to determine the spatial and temporal origin of water entering salmon farms; evaluation of the effectiveness of a light sensor array for bloom detection; and evaluation of the usefulness of satellite imagery for bloom detection. The project began in July 2004, and updates on activities to date will be presented.

Les poussées phytoplanctoniques peuvent causer de graves pertes financières aux salmoniculteurs, imputables aux mortalités et/ou à la croissance réduite résultant d'impacts directs, comme la toxicité et les dommages aux branchies, ou d'impacts indirects, comme des teneurs en oxygène dissous réduites ou élevées. En raison des conséquences sur le plan économique, les salmoniculteurs de l'entrée de la baie de Fundy aimeraient disposer d'un mécanisme de surveillance qui leur permettrait de détecter les poussées phytoplanctoniques potentiellement nuisibles. Ce projet visait à établir la faisabilité et la rentabilité de plusieurs mécanismes potentiels de détection rapide et à estimer les seuils de concentration d'algues nuisibles qui résulteraient en des pertes au niveau de la production. Les éléments de ce projet de trois ans sont les suivants : formation du personnel des salmonicultures en échantillonnage et en identification des algues nuisibles; exécution par le personnel de certaines salmonicultures d'activités de surveillance quotidienne du phytoplancton; analyses statistiques des données de surveillance fréquemment observées; analyses rétrospectives des données de surveillance existantes; expériences de laboratoire en vue d'établir les seuils de concentration de certaines algues nuisibles pour le saumon d'élevage; des études sur la circulation de l'eau en vue d'identifier l'origine spatiale et temporelle de l'eau entrant dans les salmonicultures; évaluation de l'efficacité d'un réseau de détecteurs optiques pour déceler les poussées; et évaluation de l'utilité de l'imagerie satellitaire pour déceler celles-ci. Le projet a débuté en juillet 2004. Des bilans réguliers des activités seront présentés.

Introduction

Phytoplankton blooms occur naturally throughout the world, including the lower Bay of Fundy. They may originate offshore and be transported by water to inshore areas where salmon farms are located or they may originate inshore. When phytoplankton blooms occur in areas where salmon farming is conducted, the health of the caged salmon may be compromised. This has happened several times in southwestern New Brunswick (SWNB) within the past decade, especially within the Passamaquoddy Bay area (Fig. 1). Blooms occur less frequently in other areas of SWNB, but a bloom in 2003 caused severe economic losses at several farms at eastern Grand Manan Island. Phytoplankton blooms can affect dissolved oxygen levels, cause physical damage to the gills of fish, and/or introduce toxins into the fish. The result may be mortality or loss of growth in caged farmed salmon.

As a result of the economic consequences, the salmon farmers would like to have a monitoring approach that warns them of an upcoming potentially harmful phytoplankton event. Warnings of hours to days are useful since farmers could act on the information by adjusting harvesting schedules, delaying the entry of smolts, and/or adjusting feeding schedules and medication treatments. The purpose of this project is to investigate the feasibility and cost-effectiveness of several potential early warning approaches and to estimate concentration thresholds (for causing losses in farmed salmon production) of some of the dominant harmful algal species in SWNB.

The present understanding of phytoplankton dynamics and patterns in SWNB (Bay of Fundy) suggests that:

- higher-frequency sampling programs are needed at critical locations and at critical times of the year;
- statistical approaches need to be applied to time series of individual phytoplankton species to determine the potential for forecasting bloom events;
- the usefulness of satellite imagery and other new technology for detecting offshore phytoplankton blooms in the Bay of Fundy needs to be investigated; and
- information is needed on the critical threshold levels of harmful algae which cause harm to farmed salmon.

The project is a collaborative effort involving scientists from Fisheries and Oceans Canada (DFO) and salmon farmers including Aqua Fish Farms Ltd., Cooke Aquaculture Inc., Heritage Salmon Ltd., Stolt Sea Farm Inc., and the New Brunswick Salmon Growers' Association. Funding is provided by the DFO Aquaculture Collaborative Research and Development Program (ACRDP), DFO Science, and the participating companies.

The project is to be carried out over a three-year period. The majority of the work will be conducted during the first two years of the project. The third year is to enable completion and publication of technical reports and scientific manuscripts. Project activities began in July 2004. This report describes the seven project components and provides updates on activities during the first 4 months of the project.

Description of Project Components

1: Training and communication of information on harmful algal blooms between DFO Science and the SWNB salmon aquaculture industry

A meeting was held on 28 May 2004 to introduce the project to the participants, provide input on the implementation of the project at their farms, and to provide a forum for information exchange between fish farmers and scientists on the subject of harmful algal blooms. A workshop was held on 7 July 2004 to train industry staff in the use of phytoplankton sampling equipment, phytoplankton identification, and record keeping. Subsequent meetings and workshops will be held to review project progress and results, review plans for the subsequent field season, and refresh and update trainees on phytoplankton identification.

An efficient mechanism for rapidly and frequently communicating results has been established. Industry partners can report results to DFO via telephone or e-mail and DFO scientists can send messages, briefly summarizing key progress and findings of the project, on a regular basis to participating farmers.

2: Retrospective analyses of abundance patterns in harmful algal species

This component will analyze existing phytoplankton monitoring data to estimate bloom characteristics of dominant harmful algal species. The phytoplankton community in SWNB has been monitored at several locations at weekly to monthly intervals since 1989[(1)]. The data will be analyzed for bloom characteristics such as the time of onset, duration, maximum cell concentration, frequency per year, spatial extent, and inter-annual time trends. Work completed to date on *Alexandrium* is described in a separate report[(2)]. Analyses on other algal species are in progress.

3: Enhanced phytoplankton, environmental and fish sampling at fish farms

Daily sampling of phytoplankton and environmental parameters is to be conducted by staff at participating salmon farms, following training (see Component 1). In addition, occasionally 12-13-hour periods of intensive sampling are to be conducted by DFO staff at hourly or half-hourly intervals at selected farms and time periods. When combined with statistical time series analyses, this sampling is meant to test the hypothesis that the onset of a bloom in an area can be detected and forecasted by daily phytoplankton monitoring at the farms.

It was proposed that daily phytoplankton sampling would be conducted at 4-6 locations: 2-3 in Passamaquoddy Bay and 2-3 in the eastern Grand Manan area. Unfortunately, due to an outbreak of infectious salmon anemia (ISA) in Passamaquoddy Bay in 2004, it was not possible for farms in that area to participate. As a result, in 2004 the project was limited to 4 farms in the eastern Grand Manan area (Fig. 2).

For the daily sampling, duplicate samples are to be taken and preserved. One set is to be analyzed immediately by farm staff. Data (species and cell counts) are to be recorded and forwarded to the DFO St. Andrews Biological Station (SABS). Both samples are to be sent to SABS, for later examination by phytoplankton experts at DFO St. Andrews.

The species chosen for identification and counting include 4 species which have previously been associated with problems at salmon farms in SWNB: *Alexandrium fundyense*, *Chaetoceros socialis*, *Ditylum brightwellii,* and *Eucampia zodiacus*. In addition, *Corethron criophilum*, *Chaetoceros convolutus*, *Leptocylindrus minimus*, and total *Pseudo-nitzschia* spp. are included, since these species have been known to cause problems for salmon farms elsewhere, and have been observed in the Bay of Fundy, but have not yet caused problems at salmon farms there. When species other than those listed above are observed at very high densities, these species will also be identified and counted. It is not proposed to do complete community structure analyses on these samples.

As part of this sampling, participating farmers will keep records of observations on fish behaviour (e.g., fish not feeding, fish swimming in unusual patterns, etc.) to give an indication as to whether the fish may be suffering some negative consequences that might be associated with the phytoplankton. If the fish show signs of stress, they will be collected so they can be examined for evidence of toxins in tissues or other phytoplankton-related insults.

Water temperatures will be monitored continuously at several depths in order to give some insight into water stratification and the association of phytoplankton with changes in the local water mass structure. Vertical profiles of the water temperature, salinity, dissolved oxygen, and fluorescence will be taken occasionally to provide insights into the vertical stability of the water column, vertical structure of the phytoplankton, and dissolved oxygen.

Statistical analyses of each time series will be conducted to determine if there is predictive power in the time series and, if so, whether the predictions require daily or less frequent sampling. Statistical comparisons of the plankton time series will give an indication of the spatial scale of the phytoplankton blooms and whether an ongoing sampling program would benefit from sam-

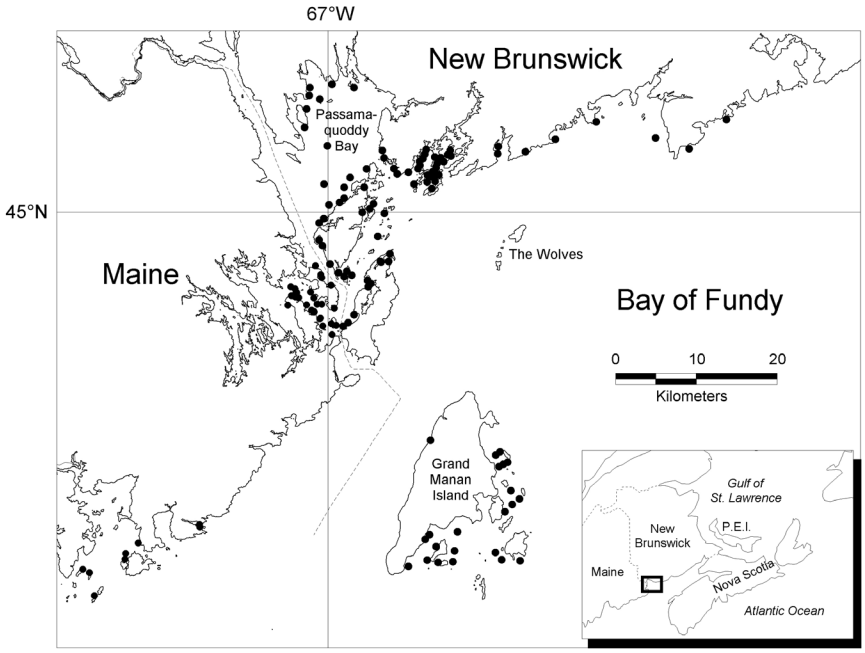

Figure 1.
Map of southwestern New Brunswick and adjacent Maine showing licensed finfish aquaculture sites in 2004.

pling at more than one farm in an area or at a frequency of more than once a day.

In 2004, samples were collected by staff at the 4 participating farms from 8 July to the end of September. A total of 198 samples were collected (range: 21 to 66 samples per site). Some of the participating companies also conducted sampling and counting at other farms in SWNB. One 12-hour-duration high frequency sampling event was conducted by DFO staff.

During the summer of 2004, elevated levels of *Alexandrium* were reported, with cell counts reaching greater than 100 000 cells per litre, peaking in mid-August. There were no elevated mortalities or reduced growth rates reported from the farms participating in this project. However, elevated salmon mortalities, believed to be due to elevated *Alexandrium* levels, were reported in at least 5 farms in other areas of SWNB in July 2004. These farms were in Passamaquoddy Bay, Lime Kiln Bay, Bliss Harbour, and Beaver Harbour. As a result of these problems, farmers in those other areas have expressed interest in participating in the 2005 field program of this project.

4: Determine the threshold concentrations of harmful algae which are harmful to farmed salmon

This component is meant to determine the concentrations (threshold levels) at which harmful algae will cause problems for farmed salmon. This work is necessary to help determine trigger points for farmers to initiate husbandry strategies on their farms to mitigate the impacts of the phytoplankton.

Species of potentially harmful algae commonly found in the SWNB area will be isolated and cultured in the laboratory and salmon will be exposed to different concentrations of these algal monocultures. Four species (*Alexandrium fundyense*, *Chaetoceros socialis*, *Ditylum brightwellii*, and *Eucampia zodiacus*) were selected. These were selected as they have been suggested as having caused problems to salmon at cage sites in SWNB in recent years. During the study period, if another species is deemed to be causing problems with fish, efforts will be made to isolate and produce cultures and determine threshold levels as well (although *Mesodinium rubrum* has been implicated to have caused problems, it is not included in the list due to the fact that it has not, to date, been possible to culture.)

Salmon smolts will be tested for lethality following 24-h exposure to different concentrations of harmful algae. The fish will be tested in static seawater in 200-L aquaria, with 5 smolts per aquarium. Five different concentrations, plus a control, will be tested for each of the 4 selected algal species. It is proposed to do 5 replicates (for each algal species), resulting in sample sizes of 25 fish for each algal concentration and the controls. If possible, we will also try to examine changes in feeding responses and gill damage in fish exposed to different concentrations of harmful algae.

Trials with *Alexandrium fundyense* started on 18 October 2004 and trials with *Ditylum brightwellii* will also be conducted in 2004. Trials with the other two species will be conducted in 2005.

5: Water circulation

The spatial and temporal origin of water that flows through a farm will be estimated on tidal time scales using an existing numerical model of the mean tidal circulation in the SWNB area. The influence of deviations from the mean tidal circulation on the origins of the water will also be investigated. This may be useful for estimating the likelihood of a bloom being transported to a farm within a tidal cycle. This work will begin in the winter of 2004-2005.

6. Test the usefulness of a real-time moored light sensor to detect phytoplankton blooms

A passive light sensor array will be moored at one or more farms. The sensor array records the intensity of the 490-µm wavelength light at different depths in the upper 15 m of the water column. The 490-µm wavelength is used since it is in the middle of the phytoplankton chlorophyll absorption range. Al-

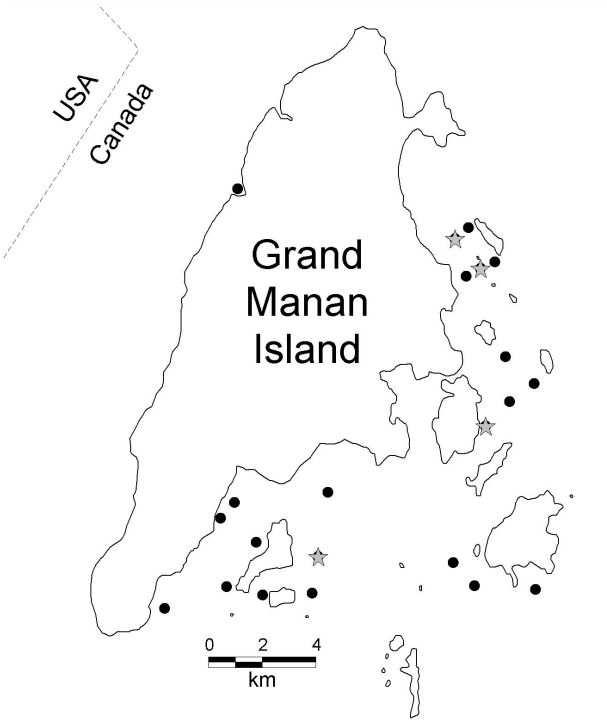

Figure 2.
Map of Grand Manan Island showing locations of enhanced phytoplankton monitoring in 2004 (stars) and other finfish aquaculture sites (black circles).

though the sensors work only during daylight, the data will be collected continuously. The data will give an indication of the temporal variability in gross phytoplankton on time scales that are not feasible to monitor using water bottle sampling approaches. The equipment will be deployed near the water sampling stations so the intensity signal can be compared to the phytoplankton species count data acquired as part of the activities described above.

It was proposed to start the initial deployment in September 2004, but this has been delayed due to difficulties in obtaining equipment.

7: Evaluate the usefulness of SeaWiFS and MODIS satellite imagery as a tool for detecting offshore phytoplankton blooms in the Bay of Fundy

Historical Sea-viewing Wide Field-of-view Sensor (SeaWiFS)[3] images (1997-2003) will be examined for evidence of being able to detect and track phytoplankton blooms within the lower Bay of Fundy. If the images appear to detect phytoplankton blooms, time series of satellite based indicators of phytoplankton will be generated for specific locations and statistical techniques for estimating the movement of blooms will be explored. The time series data can be compared with existing phytoplankton count data collected from offshore monitoring stations.

The SeaWiFS satellite images are routinely gathered and processed at the DFO Bedford Institute of Oceanography. The images can be used qualitatively as colour images or processed to give a quantitative estimate of the total amount of chlorophyll in the near surface water. The new work (currently in progress) will require reprocessing of archived satellite images and extraction of intensity data from the lower Bay of Fundy area. The SeaWiFS satellite stopped functioning in December 2004. The Moderate Resolution Imaging Spectroradiometer (MODIS)[4] satellite imagery has been operational since 2003 and will be used in the future instead of SeaWiFS. A comparison of the MODIS and SeaWiFS imagery will be made for the overlapping years.

This component will help address the situation in which blooms originate offshore of the fish farming areas of SWNB. Ideally the images will indicate the movement of a bloom toward the inshore areas and the tidal models will estimate the transport pathways of the bloom into the fish farms once the bloom reaches the inshore area.

It should be noted that the usefulness of satellite images for detecting phytoplankton blooms in the Bay of Fundy is sometimes compromised by the high turbidity of Bay of Fundy water. Furthermore, the images do not indicate the type of species that may be causing changes in water colour. Hopefully, the images may give a qualitative indication of the presence of a bloom and the species identification limitations may be overcome to some extent by examining the phytoplankton samples collected at weekly to bi-weekly intervals at existing offshore phytoplankton and hydrographic monitoring stations.

Project Status

The project has accomplished most of its proposed objectives to date. There has been considerable interest from both participating farms and other fish farms in SWNB. A workshop will be held in late 2004 or early 2005 to provide participants with an update on results to date. We will also discuss at which sites the enhanced monitoring will be conducted in 2005, as well as the possible inclusion of additional collaborating farms.

Acknowledgments

This project was funded by the Fisheries and Oceans Canada (DFO) Aquaculture Collaborative Research and Development Program (ACRDP), DFO Science, Aqua Fish Farms Ltd., Cooke Aquaculture Inc., Heritage Salmon Ltd., and Stolt Sea Farm Inc. The New Brunswick Salmon Growers' Association assisted with the project coordination and administration. We thank the following individuals who have assisted in the project: E. Gagné, R. Green, P. Green, C. Huckins, L. Kirby (Cooke Aquaculture); P. Fitzgerald, R. Lambert, V. Pedersen (Heritage Salmon); C. Davidge, T. Clinch, K. Whelan (Stolt Sea Farm); C. Saulnier, S. McGrattan, N. Bass, S. Guptill (Aqua Fish Farms); and S. Smith (NBSGA).

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Temperature Preference of Two Strains of Brook Trout (*Salvelinus fontinalis*)

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Optimal growth rates are achieved when fish are cultured at their preferred temperatures, which vary among species and strains. The objective of this experiment was to test the temperature preference of two strains of brook trout (*Salvelinus fontinalis*) raised under similar conditions. Ten replicate arenas were constructed using a central tank connected to 4 surrounding tanks (12 × 12 × 7 cm) by 2.5 cm diameter PVC pipes. The outer tanks received water at approximately 14, 15, 17, or 20°C. Individual trout (30-70 mm fork length) were placed in each arena and allowed free passage among the tanks in order to choose their preferred temperature. Fish locations were recorded at random times between 9 a.m. and 5 p.m. during a five-day period. One strain was found 78% of the time at the two higher temperatures (16.8 and 19.8°C), compared to only 38% of the time for the other strain. Such a difference in temperature preference between strains, when translated into optimal growth and the costs of heating water, can have a very dramatic impact on production costs.

On obtient des taux de croissance optimums de poissons lorsqu'ils sont élevés en milieux de température qu'ils préfèrent, qui varie selon les espèces et les souches. L'objectif de la présente expérience était de déterminer la température préférée de deux souches d'omble de fontaine (*Salvelinus fontinalis*) élevées dans des conditions semblables. Dix enceintes doubles ont été construites, composées d'un bassin central relié à quatre bassins satellites (12 cm × 12 cm × 7 cm) par des tuyaux en polychlorure de vinyle de 2,5 cm de diamètre. Les bassins satellites étaient alimentés en eau d'environ 14, 15, 17 ou 20°C. Des ombles (30-70 mm de longueur à la fourche) ont été placés dans chaque enceinte et donné la liberté d'accès à tous les bassins de sorte à ce qu'ils puissent choisir la température qu'ils préféraient. La position des ombles a été enregistrée au hasard entre 9h et 17h durant cinq jours. Pendant 78 % de cette période, une souche a fréquenté les bassins des deux températures les plus élevées (16,8 et 19,8°C), en comparaison de seulement 38 % de la période dans le cas de l'autre souche. Une telle différence dans la température préférée par les deux souches, lorsque traduite en taux de croissance optimum et coûts de chauffage de l'eau, peut avoir une très forte incidence sur les coûts de production.

Introduction

In 1981, Jobling[1] correlated optimal growth with temperature preference in a variety of fish species. When fish are cultured at their preferred temperatures, optimal growth rates are achieved. A number of abiotic factors such as light, oxygen and currents influence the temperature preference of fish[2]. Many of the chambers used to test temperature preference have not controlled for these confounding environmental factors. There are also many biotic factors that influence temperature choice by fish, including age, size, group size, and strain[3-7]. Little research has been done on the effect of strain on temperature preference of fish. Konecki et al.[8] studied the temperature preference of two strains of coho salmon (*Oncorhynchus kisutch*) and found that while the mean preferred temperature was different (9.6 and 11.6°C) between strains, there was sufficient variation within and among individuals to make this difference not statistically significant. For brook trout (*Salvelinus fontinalis*), research to date has reported temperature preferences ranging from 8.5 to 19.0°C[3-7]. This variation could be due to abiotic or biotic factors that have yet to be addressed. The objective of this experiment was to test the temperature preference of two strains of brook trout cultured under similar conditions, using a temperature arena that controls for abiotic factors.

Materials and Methods

Ten replicate arenas were constructed using a central tank connected to 4 surrounding tanks (12 × 12 × 7 cm) by 2.5 cm diameter PVC pipes. Each of the outer tanks received water at approximately 14, 15, 17, and 20°C. Fish were acclimated to 14°C and cultured under similar regimes prior to experimentation. One strain (wild) was from the Miramichi River, New Brunswick, and the other (domesticated) from Pisciculture des Alleghanys, Quebec. Each strain was test tested independently. At the start of a test, size-matched individuals (30-70 mm fork length) were placed in the 14°C tanks within each arena and allowed free passage among tanks. Only a single fish was placed in each arena. Fish locations were then recorded at random times between 9 a.m. and 5 p.m. during a five-day period, for approximately 125 observations per fish. This procedure was conducted once for each strain, using all ten arenas simultaneously. As a control, the procedure was repeated with all tanks receiving ambient water at 14°C. Trials were conducted from July 12 to August 6, 2004. Observations for each fish were totaled for four days (excluding day one as an acclimation period). Data were tested for heterogeneity and then compared to the controls using a G-test[9] with alpha level set at 0.05.

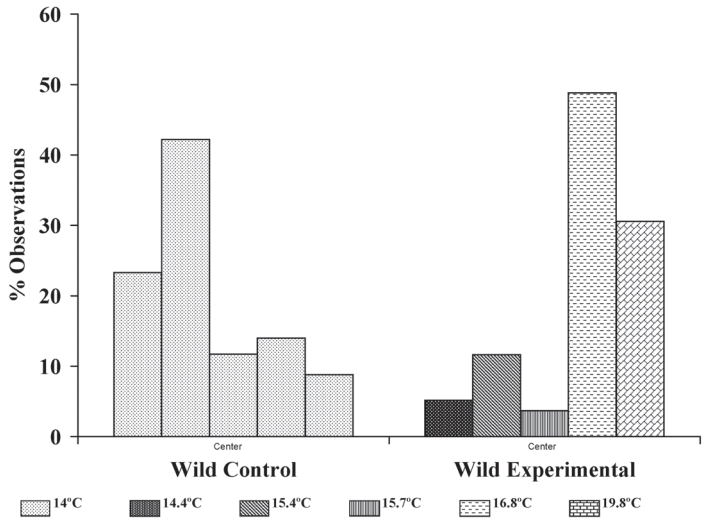

Figure 1.
Percent observations of the preferred temperature of wild brook trout with and without a temperature choice.

Results

There were significant differences in preferred temperature between control and experimental wild fish and between experimental wild and domesticated fish (Figs. 1 and 2). However, there was no significant difference between the domesticated control and experimental fish (Fig. 2). The wild fish preferred the warmer water, with 78% of the observations made at the two higher temperatures (48% at 16.8°C and 30% at 19.8°C). The domestic fish showed no temperature preference. As seen in Figures 1 and 2, both the wild and domesticated control fish showed a strong attraction to tank 2, suggesting an experimental limitation to the design.

Discussion and Conclusions

Based on the results obtained through this study, the temperature preference arenas have not met the design objectives because fish were preferentially selecting a particular tank during the control tests. The reasons for this are still under investigation. Previous researchers[3-7] have not run control trials (i.e., with no temperature gradient), so it is possible that the results reported in the literature are biased. While taking this bias into consideration, temperature preference reported here for wild and domesticated fish fell within the temperature range documented in the literature for brook trout (8.5-19.0°C)[3-7].

It appears the domesticated fish have a wider range of preferred temperatures, suggesting more individual variation within the domesticated strain[8] compared to the wild fish. When comparing the domesticated to the wild fish there is clearly a strain effect, with the wild fish demonstrating a strong preference for warmer water. This difference between the

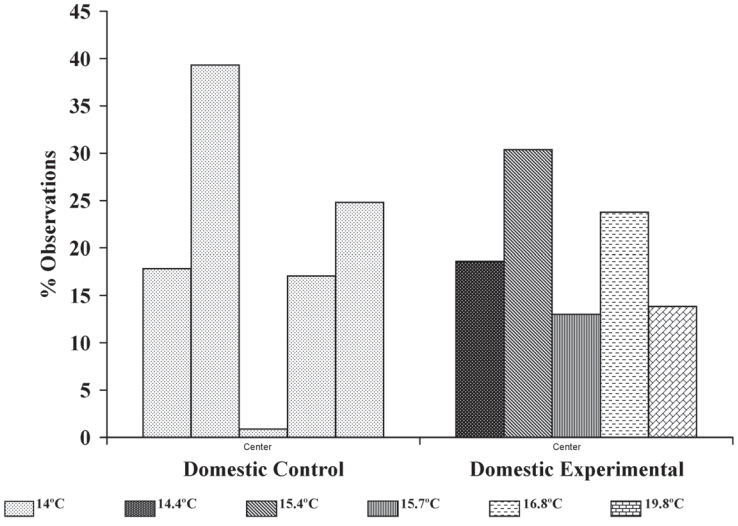

Figure 2.
Percent observations of the preferred temperature of domesticated brook trout with and without a temperature choice.

strains in temperature preference, when related back to optimal growth of the fish, may have a dramatic impact at the hatchery level. The difference in culturing temperature of one or two degrees can translate into a large difference in growth potential and/or money lost. Therefore it is important that facility managers take strain difference into consideration when optimizing culture conditions.

Acknowledgements

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Characteristics of *Alexandrium fundyense* Blooms that Affect Caged Salmon in the Bay of Fundy

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Alexandrium fundyense, a dinoflagellate that causes paralytic shellfish poisoning, has been implicated in recent years in the mortality of caged salmon in the Bay of Fundy. As part of a recently funded Department of Fisheries and Oceans Aquaculture Collaborative Research and Development Program (ACRDP) project historical data concerning the abundance of *Alexandrium* is being examined to quantify the spatial and temporal characteristics of the *Alexandrium* blooms. The abundance of *A. fundyense* has been monitored at weekly to monthly intervals since 1987 at four locations in the Bay of Fundy. The date at which *Alexandrium* has first appeared in samples has varied from day of year 105 to 179. The median date of first appearance has been day 135 in the offshore and 145 in the inshore. The null hypothesis that the date of first appearance varied randomly over the years could not be rejected (alpha=0.05) by a two-sided runs test. The median date of maximum cell abundance occurs earliest inshore (~day 200 = mid-July, station 17) and later offshore (~day 230 = mid-August, station 16). The annual maximum concentration of *A. fundyense* has varied by about three orders of magnitude and the mean has varied by about one order of magnitude. The duration of *Alexandrium* blooms has ranged from 42 to 205 days with a median duration of 112 days. The temporal character of the *Alexandrium* bloom also varied with the number of abundance pulses varying from 1 to 3 per year.

Alexandrium fundyense, un dinoflagellé causant une intoxication par phycotoxine paralysante, a été impliqué dans les dernières années dans la mortalité de saumons élevés en cage dans la baie de Fundy. Au titre d'un projet récemment financé dans le cadre du Programme coopératif de recherche-développement en aquaculture (PCRDA) du ministère des Pêches et des Océans, nous avons examiné des données historiques sur l'abondance d'*Alexandrium* en vue de quantifier les caractéristiques spatiales et temporelles des poussées de ce dinoflagellé. L'abondance d'*A. fundyense* est contrôlée à intervalles hebdomadaires ou mensuels depuis 1987 à quatre endroits dans cette baie. La date à laquelle *Alexandrium* se manifeste pour la première fois dans les échantillons varie entre les jours 105 et 179 de l'année, alors que la date médiane de la première manifestation est le jour 135 dans les eaux hauturières et le jour 145 dans les eaux côtières. L'hypothèse nulle à l'effet que la date de la première manifestation varie au hasard au fil des ans n'a pu être rejetée (alpha = 0,05), selon les résultats d'un test bilatéral. La date médiane d'abondance maximale de cellules est plus tôt dans les eaux côtières (~jour 200 = mi-juillet, station 17) et plus tard dans les eaux hauturières (~jour 230 = mi-août, station 16). La concentration annuelle maximale d'*A. fundyense* varie par environ trois ordres de grandeur, alors que la concentration moyenne varie par un ordre de grandeur environ. La durée des poussées d'*Alexandrium* varie de 42 à 205 jours, la durée médiane se situant à 112 jours. Le caractère temporel des poussées d'*Alexandrium* varie aussi, le nombre de poussées pléthoriques variant entre une à trois par année.

Introduction

Understanding the population dynamics of toxic or harmful algae species in the Bay of Fundy region has been an objective of science since well before the advent of caged salmon aquaculture in 1979. Algal toxins concentrated in the flesh of shellfish and massive kills of herring in weirs were health and economic concerns long before aquaculture was introduced into the Bay of Fundy. When blooms of some species of phytoplankton come in contact with caged Atlantic salmon the result is reduced stock performance and even substantial stock mortality. Hence, concerned fish farmers and scientists have recently teamed together to enhance our understanding of the temporal and spatial patterns in the abundance of the most potentially damaging of the algal species[1] namely, *Alexandrium fundyense*, *Chaetoceros socialis*, *Chaetoceros convolutes*, *Ditylum brightwellii*, *Eucampia zodiacus*, *Mesodinium rubrum*, *Corethron criophilum, Leptocylindrus minimus*, and *Pseudo-nitzschia pseudodelicatissima*.

A. fundyense is a dinoflagellate that produces toxins that are concentrated in shellfish and can cause Paralytic Shellfish Poisoning (PSP) in humans that eat shellfish that have accumulated the toxins. Data on PSP concentrations in shellfish have been collected from the Bay of Fundy since the 1940s and hence provide an important perspective on the interannual and seasonal pattern of *Alexandrium*[2]. The data indicate that PSP, and hence *Alexandrium*, has been present throughout much of the Bay of Fundy, particularly the lower Bay, since the early 1940s and well before, since the local natives were quite familiar with the need to avoid eating shellfish during specific months of the year. The data indicate that from day 170 (mid-June) until day 270 (end of September) the risk of PSP poisoning is the greatest and hence the concentration of *Alexandrium* cells is likely at its highest. The shellfish toxicity data also indicate both spatial and tem-

poral (interannual and decadal) variability in the intensity of toxicity. There is generally lower toxicity in shellfish from sheltered inshore areas such as Passamaquoddy Bay than in shellfish from the more exposed coastal areas of the Bay of Fundy. There was also generally higher toxicity during the 1970s and early 1980s than in the late 1980s and 1990s when the salmon aquaculture industry was rapidly expanding throughout the inshore areas of southwest New Brunswick.

The purpose of this document is to present a preliminary and brief summary of the spatial and temporal characteristics of *A. fundyense* cell concentrations as determined from a phytoplankton monitoring program. The emphasis is on describing the location, timing, duration, and magnitude of the *Alexandrium* blooms.

Data Source

A phytoplankton monitoring program has been maintained in the southwestern New Brunswick coastal zone of the outer Bay of Fundy since 1987 [3-5]. This is the area that supports the majority of the salmon cage culture in the Bay of Fundy. As part of this program water samples were collected from the near surface at weekly to monthly intervals from 4 stations (3,15, 16, and 17) throughout 1987 to 2004 and from an additional 8 stations during 1991 (12 stations in all). Phytoplankton cells have been identified and enumerated microscopically following settlement of 50 mL of the water sample using the Utermöhl technique[6]. *A. fundyense* identification has been confirmed by scanning electron microscopy.

This dataset was used to generate time series of the near surface abundance of *A. fundyense* for each of the four primary sampling stations. These time series were analysed to determine for each year in the series the first and last date of *Alexandrium* presence, the annual maximum concentration of *Alexandrium* cells, the date of the annual maximum, and the duration of the annual *Alexandrium* blooms.

Results

The information from the phytoplankton surveys reaffirm the main patterns that were previously determined from intertidal monitoring of PSP in shellfish. Figure 1 shows the annual time

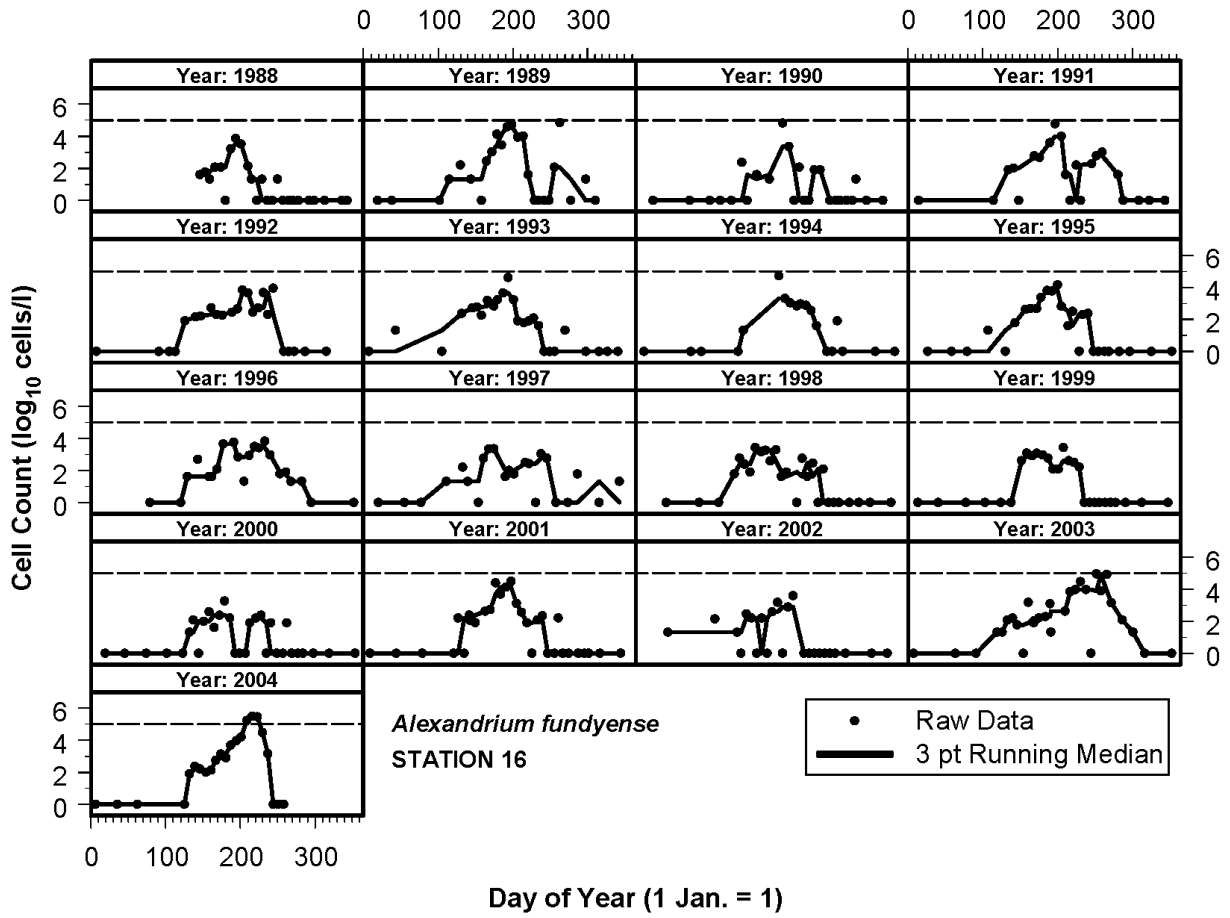

Figure 1.
Annual time series of the near surface (upper 1 m) *Alexandrium fundyense* cell concentration at a phytoplankton monitoring station (station 16) located within the offshore area of the lower Bay of Fundy. The dashed horizontal line indicates 100 000 cells per litre.

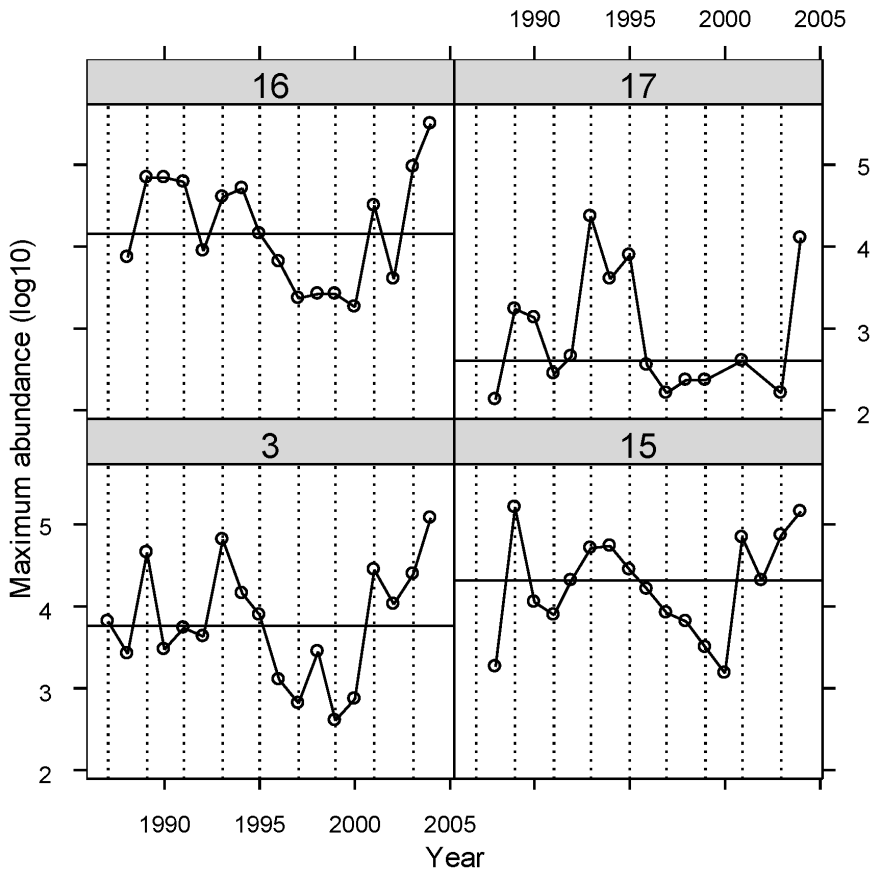

Figure 2.
Time series of the maximum observed concentration of *Alexandrium fundyense* within four phytoplankton stations routinely monitored within the lower Bay of Fundy. The numbers above each panel indicate the sampling station. Station 16 is the furthest offshore and station 17 is the furthest inshore. Stations 3 and 15 are near the mouth of inlets connected to the offshore.

series for the offshore station (station 16). From 1988 through 2004, *Alexandrium* occurred every year with the abundance in each year focused in one or more bloom events. Peak cell concentrations in surface waters exceeded 100 000 per litre offshore and were less in the inshore areas.

Close inspection of the *Alexandrium* time series indicated that each year there is a characteristic "day of first appearance" that varies inter-annually in a synchronous fashion throughout the lower Bay of Fundy area. The median "day of first appearance" occurs earlier in the offshore and coastal sites (~day 135) than in the sheltered inshore sites (~day 145). The null hypothesis that the date of first appearance varied randomly over the years could not be rejected (alpha=0.05) by a two-sided runs test.

The "date of maximum cell abundance" varies from May to October at all stations in a spatially semi-synchronous fashion with the median "date of maximum cell abundance" occurring earliest inshore (~day 200 = mid-July, station 17) and later offshore (~day 230 = mid-August, station 16).The sample with the maximum cell abundance for each year is part of a bloom, or unbroken sequence of samples with *Alexandrium* present, whose duration varies annually within the range of 10 to 160 days. There is some synchronicity in this duration across sites but the main feature is the lower variability and shorter median duration inshore (~50 days) compared to the higher variability and longer median duration in the offshore (~85 days). The "maximum cell abundance", like the date to which it is tied, is also observed to vary annually at all sites and spatially with the median ranging from 10^4 cells per litre offshore to 10^2 cells per litre inshore (Fig. 2). The total annual duration of the presence of *Alexandrium* is similar across sites (~120 days) and ranges from 50 to 200 days.

A general feature of any bloom (an unbroken sequence of two or more samples with *Alexandrium* present) is that the later in the year the bloom event occurs the less time it takes to reach its peak and the longer the bloom lasts the greater the maximum bloom concentration. The annual "maximum cell abundance" occurs in only one of these and it is usually not the first bloom event of the year.

Significance to Salmon Aquaculture Industry

The last major period of PSP and *Alexandrium* blooms was in the 1970s and early 1980s and salmon cage culture began in 1978. Since then the salmon aquaculture industry has expanded its geographic distribution throughout the inshore and nearshore coastal areas.

It is now considering expanding into the offshore Bay of Fundy where *Alexandrium* blooms are most intense and are of the longest duration. The industry did not suffer from a significant exposure to *Alexandrium* until 2003, when cell counts reached levels not seen since the late 1980s. In 2004, the cell counts were the highest seen since phytoplankton monitoring began. Losses of caged salmon that have been associated with these recent blooms have mainly been in areas adjacent to the offshore. Consequently, as this industry expands geographically it is likely to become more exposed to the higher concentrations of *Alexandrium* and PSP toxins and losses of fish may become more common.

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Rearing Opportunities of a Northern Strain of Brook Charr (*Salvelinus fontinalis*)

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Domestic brook charr reared in Quebec are characterized by a sexual maturation occurring after the second summer of growth and thereby are a small size at harvest. These constraints and other factors limit the expansion of this species for the table market. However, a northern strain of brook charr matures one year later and reaches a larger size before maturation. The Centre Écologique du Lac St-Jean (CELSJ) has investigated the strain's various characteristics (growth rate, fecundity, fillet yield, condition factor, fillet composition, and organoleptic properties). Results showed that this northern strain has a similar growth rate as domestic strains but a lower fecundity as well as a lower condition factor. Northern brook charr offer consistently higher fillet yields as well as being low in fat and highly rated by tasting panels. The strain's characteristics suggest a strong market potential. Work is continuing to confirm preliminary observations, to develop the strain and determine a successful marketing strategy.

L'omble de fontaine élevé au Québec atteint caractéristiquement la maturité sexuelle après le deuxième été de croissance, ce qui fait qu'il est de petite taille au moment de la récolte. Ces contraintes, ainsi que d'autres facteurs, limitent le développement de cette industrie aux fins de consommation humaine. Toutefois, une souche nordique atteint la maturité un an plus tard, ce qui lui permet d'atteindre une plus grande taille. Le Centre écologique du lac St-Jean (CELSJ) a étudié les diverses caractéristiques de cette souche (croissance, fécondité, rendement en filets, facteur de condition, composition du filet et propriétés organoleptiques) et les résultats révèlent qu'elle a un croissance semblable aux souches domestiques, bien qu'elle montre une fécondité moindre et un facteur de condition moins élevé. Le rendement en filets, faibles en gras et considérés très bons par des jurys de dégustation, est régulièrement plus élevé. Les caractéristiques de cette souche donnent à penser qu'elle a un potentiel de marché élevé. Les travaux se poursuivent en vue de confirmer les observations préliminaires, de développer la souche et de cerner une stratégie de marketing fructueuse.

Introduction

The domestic strain of brook charr currently raised in Québec is used for the stocking of lakes and ponds. It originates from a mix of different stocks found in Canada and the United States. However, this strain is not suitable for fish farmers who would like to sell their production for the table market. Indeed, the difficulties to produce a fillet size fish and early sexual maturation affecting flesh quality are among the major constraints for commercial production.

More than 40 years ago, Legendre(1) and Flick(2) investigated various populations of trophy brook charr (*Salvelinus fontinalis*) in the James Bay (Quebec) territory. They were interested by the longevity of these populations and their ability to reach a larger size at adult stage (usually more than 3 kg). Moreover, they noticed fast growth rates at age 3 and 4 years. Therefore, this strain represents an interesting opportunity to produce fish of sufficient size for the table market.

Considering this, the Ministry of Agriculture, Food and Fisheries of Quebec (MAPAQ) decided in 1991 to capture more than 50 specimens of brook charr from the Rupert River. They asked to the CELSJ, to keep and to spawn the fish. The fish were eventually given to the *Association des Aquaculteurs du Québec* (the Quebec fish farmers association) which transferred the spawners to Laval University, but some of their offspring remained at the CELSJ. The CELSJ kept developing this strain with the result that a fourth generation was obtained by the end of 2004.

Recently, Aquaculture Radisson, a private company established in the James Bay territory, contracted the CELSJ to maintain a breeding stock for them. It is Aquaculture Radisson's intention to produce and sell this northern strain of brook charr (Radisson brook charr) for the table market.

The objectives of this paper are to present the observations made until now on this particular strain, and to highlight some opportunities they offer commercial fish farmers. These observations have been made by the CELSJ over a period of ten years, and as of now, no independent or scientific confirmation of these results has been performed.

Observations on Various Traits of Radisson Brook Charr

Observations made on the Radisson strain of brook charr were generated at the CELSJ or its affiliate Salmotherm between 1993 and 2004. The CELSJ uses well water and raises fish in various sizes of square tanks, self-cleaning circular tanks, and ponds. Salmotherm is a site using river water, which is heated by the thermal effluents of a local pulp mill (Saint

Félicien Kraft, SFK). For more details on Salmotherm, refer to Dumas et al.[3].

Sexual Maturity and Fecundity

At the CELSJ, Radisson brook charr originating from the Rupert River normally attain sexual maturity one year later than domestic brook charr strains. This means that they have an additional year of culture before gonad development influences their growth rate and flesh quality. This behaviour is similar to that of rainbow trout.

Late sexual maturation has been observed numerous times over the last ten years at the CELSJ. As a demonstration of this, 33-month-old fish of 800 to 900 grams originating from a spawn in February 2001 were observed in November 2003 by the MAPAQ. The fish were slaughtered and their stage of sexual development and their carcass characteristics were observed. Of the 50 fish sacrificed, 26 showed no signs of sexual development while the remaining fishes gonadal development were at different stages of maturation. Of the 500 fish maintained, approximately 20 females spawned in January with about 100 males showing exterior signs of maturation. Three hundred fish that showed no signs of sexual development were selected for broodstock and spawned in the fall of 2004.

The fecundity of the Radisson stocks has been well established with three summers female fish having an egg production of 1000 to 1800 eggs per kilogram with an average of approximately 1350 eggs per kilogram. The older spawners produce from 2000 to nearly 3000 eggs per kilogram depending on their age and their size. All factors taken into consideration, since 1997, the average number of eggs per kilogram of females is 2400.

Growth Rates

Over the years numerous lots of Radisson and domestic brook charr have been raised. Excellent records have been kept of both their growth and the ambient conditions used in rearing these fish. In similar accelerated conditions (warm water), domestic brook charr barely reached 800 grams in 16 months but were then all in advanced stages of maturation[4]. The Radisson brook charr continued their growth, reaching over a kilogram in twenty months, and even then were not mature. Some Radisson brook charr were kept another three or four months and showed that they had continued growth potential under these accelerated conditions. When comparing growth of Radisson and domestic brook charr using the Growth Factor 3 (GF3) model, a modified version of the thermal unit growth coefficient perfected by the feed company Moore Clark[5], average results in growth factors for Radisson brook charr were 1.33 and 1.58 for domestic charr.

Condition Factor

The Radisson charr have consistently showed themselves to be visibly more slender than their domestic cousins. This difference can be quantified by comparing standard condition factors. Many lots of fish the same age and reared in the same conditions show that as the fish age the factor increases as well as the disparity between the strains. Young Radisson fish of just over four grams are five percent slimmer than their domestic cousins (0.882 versus 0.931). When the average weight is over 800 grams, Radisson charr are 15% slimmer than the domestic charr with condition factors of 1.24 versus 1.46, respectively.

Furthermore, carcass analysis of fish raised to over 800 grams have shown that entrails of Radisson brook charr are a smaller percentage of total weight when compared to those of domestic charr, 11.95% compared to 19%. We believe this characteristic explains the difference in condition factors as well as the fillet yields.

Fillet Yields

The CELSJ has submitted many Radisson charr as well as domestic charr and ouananiche (landlocked Atlantic salmon) for different tests concerning the quality of the fish for the table market. These tests were conducted by the Laboratoire du Centre Technologique des Produits Aquatiques of the MAPAQ. These tests measured the following items: carcass distribution (whole fish, eviscerated, headless, filleted and trimmed fillets), fillet composition (protein, lipids, etc.), and organoleptic properties (appearance, colour, odour, texture, and taste). All the tests confirmed that the Radisson brook charr offer all the characteristics of an acceptable product for the table market.

Table 1 shows comparative trimmed fillet yields between the different lines of charr as well as that of ouananiche. All these tests were done by hand except for the 2003 Radissons, which were done by a filleting machine and then hand-trimmed. This final test was conducted in November 2003 by the MAPAQ on fish maintained in coldwater conditions except for a period of four months the previous winter at Salmotherm. These fish were a year older and approximately the same size as the previous fish tested. This combined with mechanical filleting might explain why the results are slightly lower. It is expected that larger fish will result in even better fillet yields; this work is ongoing.

Table 1.
Comparative trimmed fillet yields between the different lines of charr and ouananiche (landlocked Atlantic salmon). (*Non sexually mature fish only).

Species	Radisson[6,7]	Domestic[4]	Ouananiche[4]	Radisson 2003*
Average trimmed fillet yield (%)	57.70	51.43	59.50	54.70
Max trimmed fillet yield (%)	61.02	57.78	63.82	57.20
Min. trimmed fillet yield (%)	50.27	45.61	56.31	52.00
Number of fish	40	9	18	26
Average live weight (g)	798	818	1012	862.8

Species	Live BW[a] (g)	Lipid (% FW)	Protein (% FW)	Moisture (% FW)	Ash (% FW)	Reference
BC-Radisson[c]	853	5.9	20.3	71.8	1.4	Coulombe and Blais[6]
BC-Radisson[c]	800	3.7	21.0	73.8	1.4	Blais and Coulombe[7]
BC-domestic[d]	800	9.6	17.6	71.6	1.1	Boyer et al.[4]
BC-domestic[d]	241	5.6	20.4	72.3	1.5	Rasmussen and Ostenfeld[8]
LS-ouananiche[e]	800	12.1	18.8	68.0	1.3	Boyer et al.[4]
LS-ouananiche[e]	1500	13.0	19.0	66.3	1.3	Boyer et al.[4]
RT[f]	855	10.2	19.2			Azevedo et al.[12]
AS[g]	>4000	18.2-18.9			-	Einen et al.[10]
ESB[h]	310	12.4	18.0	63.6	4.5	Lanari et al.[11]

[a]BW: body weight; [b]FW: fillet weight; [c]BC-Radisson: Radisson brook charr; [d]BC-domestic: domestic brook charr; [e]LS-ouananiche: landlocked salmon, (*Salmo salar* ouananiche); [f]RT: rainbow trout; [g]AS: Atlantic salmon; [h]ESB: European sea bass.

Table 2.
Fillet composition of various farm-raised fish.

Fillet Composition

Product quality of Radisson brook charr has to be significantly different from other salmonids available to consumers in order to allow a distinctive marketing. Consequently, the fillet composition was determined for two lots of Radisson brook charr and compared with other fish species (Table 2). All landlocked salmon and brook charr received the same feed. Protein content of Radisson brook charr is higher than all other species reported in Table 2, while the lipid content is lower. For comparison purposes, similar results for domestic brook charr, rainbow trout, Atlantic salmon, and European sea bass are included; interpretation of these results requires caution. While the proportion of protein remains constant throughout the life cycle of a fish, the lipid content may vary according to feed characteristics, feeding rate, and life stage[9,13,14]. Also, area of sampling may have influenced the results on lipid content of fillets. Indeed, studies published by Katikou et al.[15] and Einen et al.[14] clearly demonstrate that lipids are unevenly distributed in fillets. It was not possible to find out which part of the fillet the samplers analysed.

Organoleptic Characteristics

The Laboratoire du Centre Technologique des Produits Aquatiques of the Ministry of Agriculture Food and Fisheries of Quebec (MAPAQ) conducted taste tests on Radisson charr and concluded that the fillets were consistent and attractive[7]. All the panellists appreciated the taste of the fish, which was described as having a juicy and velvety texture. Colour was satisfactory and the odour of the flesh was delicate and agreeable. It was stated that the product was of excellent quality and very compatible with the market trends[6].

Opportunities Offered by the Northern Brook Charr

While most of the fish produced in Quebec is used to supply the stocking of lakes and ponds (68% in 2003, according to unpublished data from the MAPAQ), there are still some producers who are concentrating on the table market. However a decreasing production due in part by strict environmental norms and strong competition is currently forcing Quebec's fish farmers to re-think their business strategy.

A study of the table market was recently completed by Opportunité Marketing inc. for the Table filière de l'aquaculture du Québec. The focus of this study was the table market in Quebec; specifically regarding rainbow trout, brook charr, and arctic charr. In addition to the market portrait, authors of the study also proposed some preliminary strategies that could help aquaculture development in Quebec.

In Quebec, the aquaculture fish of choice for the table market has traditionally been the rainbow trout, and not surprisingly – rainbow trout is the most widely available product of the three species studied.

Quebec's trout faces strong competition from foreign production, mostly from Chile. Principal advantages of Chile's trout over Quebec's are lower prices and year-round availability of bigger fish. While low prices and better availability of trout for consumers surely contributed to the growth of the table market, it also puts a lot of pressure on Quebec's producers who are often forced to cut on their profit margin. Considering that it is very difficult to significantly reduce the production costs, a good strategy could be for Quebec's fish farmers to position their fish as a distinct, specific product[16]. An even better solution, described as ideal by the same authors, would be to create and offer a brand new distinctive product, which could have an added value over the competition.

Based on observations made so far, it is thought that the Radisson strain of brook charr could be such a product for table market development. Next steps will involve the validation of preliminary observations via a pilot project, carcass investigation and nutrient retention efficiency, and finally a demonstration of commercial feasibility.

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Growth Performance and Survival of Atlantic Halibut (*Hippoglossus hippoglossus*) Grown in Submerged Net Pens

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Atlantic halibut (*Hippoglossus hippoglossus*) were cultured in a submerged net pen located 10 km off the coast of New Hampshire, USA. Fish grew from 0.1 to about 3 kg in 31 months. Specific growth rates decreased with fish size, from 0.6 g/d for fish <500 g to 0.3 g/d for fish >500 g. Food conversion efficiency (FCR) was 1.0. Growth was slightly slower than reported by others, and was likely caused by the relatively cold temperatures experienced by the fish. Survival was 68%. Some mortality occurred when the cage was brought to the surface for repairs, and was caused by fat cell necrosis ("sunburn"). Although growth was somewhat slow, it is believed that submerged culture of halibut is preferable to surface culture, where the fish are exposed to more turbulence, bright sunlight, and seasonally high water temperatures.

Nous avons élevé du flétan atlantique (*Hippoglossus hippoglossus*) dans une cage en filet submergée à 10 km au large du New Hampshire, aux États-Unis. Les poissons ont passé de 0,1 g à environ 3 kg en 31 mois. Les taux de croissance spécifiques ont diminué en fonction de la taille du poisson, de 0,6 g/d pour les individus de < 500 g à 0,3 g/d pour ceux de > 500 g. L'indice de consommation se chiffrait à 1,0. La croissance était légèrement plus lente que cela n'était le cas lors d'autres études publiées; ceci est probablement imputable aux températures relativement froides auxquelles était soumis le poisson. Le taux de survie s'élevait à 68 %. Un certain nombre de flétans sont morts lorsque la cage a été ramenée à la surface pour être réparée; cette mortalité était imputable à la nécrose des adipocytes. Bien que la croissance était quelque peu lente, on considère que l'élevage du flétan en cages submergées est préférable à l'élevage en surface, où les poissons sont exposés à une plus grande turbulence, à la lumière intense du soleil et aux températures saisonnières élevées de l'eau.

Introduction

The declining profit margin of salmon aquaculture has stimulated the search for alternative coldwater aquaculture species in Europe and North America. One of the species that appears to have great potential is the Atlantic halibut (*Hippoglossus hippoglossus* L.). It is tolerant of cold water, has a large size, excellent taste, is relatively fast growing, and has a high market value[1].

The basic techniques for culturing halibut have been developed[2,3], and most experts agree that halibut will soon form the basis of an important aquaculture industry[4,5]. Commercial on-growing of halibut is done in both land-based tanks and in inshore net pens, and growth rates from both types of systems have been reported[6-11]. In general, fish stocked into growout systems at about 100 g reach 2 kg after 1.5 years, and 3-5 kg after approximately 2-2.5 years[4]. Halibut specific growth rates (SGRs) vary with both temperature and fish size. SGRs reported for small halibut (100-600 g) vary from 0.2-1.0 g/d[12,13], and are influenced by both diet and temperature. SGR deceases to about 0.2-0.4 g/d with increasing fish size[11-14], and under experimental conditions, is maximal at 9-10°C[14]. Variables that effect growth include diet[12,13], stocking density[10], temperature[7,14,15], and the attainment of sexual maturity[11]

Despite halibut's potential as an aquaculture species, development of the species in North America could be delayed by difficulties in siting commercial farms. Land-based sites would be expensive, and the number of suitable inshore aquaculture locations for net pens has diminished over the last several years. Indeed, salmon farms now occupy virtually all of the suitable inshore sites in New England and Atlantic Canada. For these reasons, the feasibility of offshore aquaculture is being explored by a variety of research institutions in a number of locations. Our project in New Hampshire, for example, is investigating the technical, biological, and economic feasibility of both finfish and shellfish culture at an offshore location in the western Gulf of Maine. The goal of the research reported here was to evaluate the growth performance and survival of Atlantic halibut grown in submerged net pens.

Materials and Methods

The research began in May 2001, when 2000 juvenile halibut (30 g mean weight, produced by R&R Development Ltd., Digby, Nova Scotia) were transported to the UNH Coastal Marine Lab (CML) in New Castle, New Hampshire, USA. At the CML, the fish were acclimated to temperature and salinity as needed, and then stocked into 2-m-diameter flowing seawater tanks at a density of 4.7 kg/m^2. Fish were fed 3-5% BW/day on a specially formulated halibut ration (Shur Gain™, 50% crude protein, 22% crude fat). Particle size began at 2 mm, and increased as the fish grew. Water temperature and salinity ranged from 8-16°C and 28-34 ppt, respectively. By October 2001, the

halibut had reached 100 g, and stocking density had increased to 12.7 kg/m^2. At this time they were moved to one of the offshore net pens in insulated, 1-m^3 containers.

Fish were on-grown in a submerged, 600-m^3, 15-m-diameter "Sea Station" cage (Ocean Spar Co., Bainbridge Island, WA). The bottom of the cage was approximately 20 m below the surface during most of the on-growing period. Slope of the taut bottom panels, typical of this biconical cage design, was decreased to approximately 10°C so the fish had a flatter surface on which to rest. Netting on the cage was made of 4mm nylon twine, and mesh size throughout the cage was 4.45 cm (1.75"). Because of the relatively low number of fish, initial stocking density on the 175-m^2 floor of the cage was about 1 kg/m^2. After the cage was stocked, it was submerged to 12 m below the ocean surface.

Feed (Shur Gain™ halibut diet) was pumped hydraulically through a 25 m hose from the surface to the cage. Feeding occurred as weather permitted. This was approximately 2-3 times/week during the summer and fall, and once/week during the winter and spring. Feeding rate was 2-3% body weight at each feeding. Particle size began at 2 mm, and increased to 15 mm as the fish grew. At approximately monthly intervals a sample of 25-30 fish were captured by SCUBA divers and brought to the surface for measurements. Fish were weighed (wet) to the nearest gram and measured (TL) to the nearest mm. Specific growth rate (SGR) was estimated as:

$$SGR = 100 \times \frac{(\ln W_2 - \ln W_1)}{t_2 - t_1}$$

where lnW_2 and lnW_1 are the natural logarithms of final and beginning mean weights, respectively, and t_2 and t_1 are the last and first days, respectively, of the time interval of interest. Food conversion efficiency (FCR) was estimated as the total gain in wet weight of the fish divided by the weight of feed provided (dry) during the approximately 3 years of the research.

Results

Growth in wet weight, and seawater temperature at the time of sampling, are shown in Figure 1. Temperature varied seasonally from about 3-16°C. Because temperature was only measured periodically, colder or warmer temperatures may have occurred and gone unrecorded. Fish were stocked into the cage at approximately 100 g in October 2001. At this time, they were about 17 months old. Growth was steady throughout the 3-year experiment, although there was a tendency for growth rate to slow during the coldest months of each year. After one year in the cage, mean weight had increased to about 690 g. By the end of the second year, mean weight was about 2200g, and fish were harvested at a mean weight of 2.9 kg in May 2004, after 31 months in the cage.

Specific growth rates (SGRs) from this study were 0.6 g/d for fish <500 g, and 0.3 g/d for fish >500 g. SGR for the entire period was 0.4 g/d. Food conversion efficiency was 1.0.

Survival, from stocking to harvest, was 68%. Virtually all mortality occurred between May and August 2002, when logistical problems necessitated raising the cage to the surface. Although the halibut were still approximately 4-5 m below the surface during this time, they were exposed to warm surface water temperatures (15-20°C) and bright sunlight. All fish that died exhibited lesions on the eyed-side consistent with fat cell necrosis ("sunburn")[16]. A veterinary pathologist later confirmed fat cell necrosis as the cause of death, and mortality ceased when the cage was once again submerged to 15 m.

Halibut were harvested in May 2004 at a mean wet weight of 2.9 kg.

Discussion

Because of the many variables that effect growth, comparisons of the growth performance of the Atlantic halibut raised in a submerged, offshore cage, to halibut grown in land-based tanks or inshore net pens is difficult. SGRs from this study were 0.6 g/d for fish <500 g, and 0.3 g/d for fish >500 g. These are close to the maximal values reported for similarly sized halibut[14]. These fish did not, however, grow at the rates suggested by Bromage et al.[4], who reported that cultured halibut reach 2 kg after 1.5 years, and 3-5 kg after 2-2.5 years. Instead, these fish were smaller, having an average weight of about 1 kg after 1.5 years, and 2.9 kg after about 2.5 years in the cage. Although Bromage et al.[4] did not report the culture systems or environ-

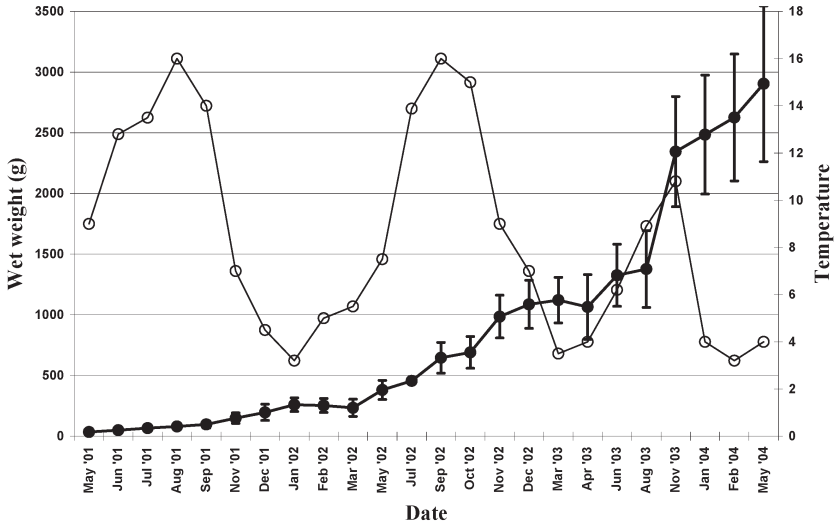

Figure 1.
Mean (± SD) wet weight (solid circles) and seawater temperature (°C) (open circles) on the sampling dates indicated.

mental parameters under which these growth rates were achieved, there may be several reasons for the slower growth we observed. The most likely is the cold ocean temperatures experienced by these fish when they were <500 g. It has been reported, based on constant temperature experiments, that the optimal temperature for 100-500 g halibut is about 11°C[14]. Temperatures experienced by these fish in this size range was <8°C. Although Bjornsson and Tryggvadottir[14] did not report optimal temperatures for halibut in the 1- to 3-kg size range, they did find that optimal temperatures decrease with increasing size, and that optimal temperature is 9-10°C for 3- to 5-kg fish. Given this, it is assumed that fish in this intermediate size range (1-3 kg) would do best at intermediate temperatures (approximately 10°C). Temperatures experienced by these fish during the months they were in this size range varied from about 3-11°C, with a mean of about 6°C. These cold temperatures may well account for the slower than expected growth observed. Growth rates of halibut raised under ambient temperatures have also been reported. In western Norway, where ambient temperatures ranged from 4.5-12.5°C, fish grew from 1.8 to 4.1 kg in one year[6], while in northern Norway, where ambient temperatures ranged from 0-13°C, fish grew from 1.6 to 3.7 kg in one year[7]. In the last year of this experiment, mean weight increased from about 1 to 2.9 kg. Although these fish had a slightly smaller starting size than those reported by others[6,7], the observed increase of 1.9 kg/yr, at temperatures ranging from 3-16°C, is comparable. Another factor that may have reduced growth was the inability to feed the fish each day. The culture site is located 10 km offshore, and 2-3-m waves are common during the winter and spring. Thus feeding only occurred on days that allowed the project vessel to safely attach to the feed hose.

As already indicated, there are a number of variables that effect halibut growth rate. It is unlikely that any of these influenced the observed growth rate as much as temperature. For example, stocking density was not a factor because these fish were stocked below the density (100% coverage of the bottom) that causes a decrease in growth rate[10]. Similarly, the attainment of sexual maturity, which slows growth in both sexes[11], did not influence the growth rate of these fish because virtually of the fish were smaller than the size at maturity, and none displayed mature gonads when the fish were harvested.

It has been suggested that halibut are capable of achieving a 1:1 FCR[4]. Experimental studies on the effect of temperature and fish size on feeding efficiencies (the inverse of FCRs) have been done[14] They showed that the highest feed efficiency for 100-500 g halibut was about 0.4, and occurred at 10.7°C, while the highest efficiency for halibut 3-5 kg was about 0.35, and was found at a lower temperature (5.5°C). Our calculated FCR (1.0) suggests that the diet we used was appropriate, and that halibut grown in submerged, offshore cages use their food very efficiently.

Results of this study indicate that halibut can be grown successfully in submerged, offshore cages. Growth performance, however, was lower than reported by others. This is attributed to suboptimal temperatures experienced by the fish in the submerged cage, and the inability to feed the fish on a daily basis. While temperatures could be increased at certain times of the year by bringing the cage to the surface, this would increase the risk of fat cell necrosis[16], and expose the fish to excessive temperatures during the summer months. Further, it would expose the fish to excessive current speeds and swells, that can cause loss of appetite, increased swimming activity, and even mortality[17]. Although there are advantages and disadvantages of both submerged and surface cage culture, it is advisable to sacrifice some growth in the colder, deeper water, in order to avoid the potentially lethal highly energetic, seasonally warm, and brightly sunlit surface waters. Automatic feeding systems, now being tested at the research site, should eliminate the problem of sporadic feeding, and thus improve halibut growth, even in offshore locations.

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Indirect Feminization of Atlantic Halibut (*Hippoglossus hippoglossus*): The Mechanism of Sex Determination and Production of Functional Sex-Reversed Females

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The production of all-female halibut stocks would provide commercial growers with an economic advantage because Atlantic halibut females grow faster and attain a larger size than males. Significant steps have been made towards achieving this goal. All gynogenetic halibut produced in 2001 were female compared to a normal sex ratio of 1:1 in control fish. The gynogenetic status of these fish was verified using microsatellite "DNA fingerprinting". Since gynogens inherit only maternal chromosomes, this result confirms that the genetic mechanism of sex determination in Atlantic halibut is female homogametic (XX). Atlantic halibut juveniles exposed to androgens (17α-methyldihydrotestosterone (MDHT)) prior to and during gonadal differentiation in 1999 have recently matured as functional males. All males were spermiating during the normal reproductive season and MDHT-treated males were crossed with normal females. The offspring from each male are being reared separately in a commercial hatchery. The sex ratio of offspring from each MDHT-treated male will determine whether that male parent is genetically male or female (i.e., sex-reversed female). Those males confirmed sex-reversed will be the first Atlantic halibut capable of producing 100% female offspring.

La disponibilité de stocks de flétan atlantique uniquement femelles avantagerait grandement les éleveurs commerciaux sur le plan économique parce que les femelles grossissent plus vite et atteignent une plus grande taille que les mâles. De grands progrès ont été faits dans la production de tels stocks. Tous les flétans gynogénotes produits en 2001 étaient femelles, en comparaison d'un sex-ratio normal de 1:1 chez les flétans témoins. L'état gynogénote de ces poissons a été confirmé par identification de l'ADN microsatellitaire. Étant donné que les individus gynogénotes n'héritent que des chromosomes maternels, cela confirme que le mécanisme génétique de détermination du sexe chez le flétan atlantique est femelle homogamétique (XX). Des juvéniles exposés à de l'androgène (17α-méthyldihydrotestostérone [MDHT]) avant et pendant la différentiation des gonades en 1999 ont récemment atteint la maturité; ce sont des mâles fonctionnels. Tous les mâles ont émis du sperme durant l'époque de reproduction normale et les mâles traités au MDHT ont été croisés avec des femelles normales. La progéniture de chaque mâle est en voie d'être élevée séparément dans une écloserie commerciale. Le sex-ratio de la progéniture de chaque mâle traité au MDHT sera établi, que le parent mâle soit génétiquement mâle ou femelle (c.-à-d., une femelle de sexe inversé). Les mâles identifiés comme étant de sexe inversé seront les premiers flétans atlantiques capables de produire une progéniture uniquement femelle.

Introduction

Aquaculturists strive to attain any acceptable biological advantage that will increase the profitability of commercial operations. This is commonly accomplished by selective breeding, improved diet formulations, or more recently, using biotechnology to produce monosex stocks. In Atlantic halibut, as with many commercially cultured species, females grow faster and attain a larger size than males. The production of all-female stocks would therefore be economically beneficial.

Our research has focused on developing techniques for indirect feminization of Atlantic halibut. This is the preferred method of producing all-female stocks since none of the marketable fish are exposed to steroids or genetic manipulations. For example, all-female salmonid populations are produced by sex-reversing genetic females into phenotypic males (neomales) capable of producing sperm that only contain X sex chromosomes[(1)]. When these neomales are crossed with regular females the resulting offspring are all females. However, to accomplish indirect feminization the mechanism of sex determination in a particular species must be determined – i.e., is the female the homogametic sex (XX) or heterogametic (WZ)? For example, among flatfish species, European plaice (*Pleuronectes platessa*)[(2)] and Dover sole (*Solea solea*)[(3)] have heterogametic

females but Japanese flounder (*Paralichthys olivaceus*) females are considered homogametic[4]. The genetic mechanism of sex determination can be determined by evaluating the sex ratio of gynogenetic offspring produced using uniparental maternal inheritance. We used protocols previously developed for producing Atlantic halibut gynogens[5] and in this study "scaled up" the techniques to mass produce gynogens for rearing until their sex could be determined morphologically.

For indirect sex reversal some fish destined to become broodstock must be exposed to hormones to alter the pathway of gonad development to produce broodstock capable of producing monosex stocks[6]. Feeding androgens for 45 days to Atlantic halibut from 30 to 38 mm fork length (FL) results in all fish developing as phenotypic males[7]. The next step in this study was to evaluate the reproductive potential of mature sex-reversed females (neomales). Since the sex-reversed fish originated from a mixed sex population some of the males exposed to androgens will be genetic males. The offspring from each potential neomale will be sexed to differentiate which are genetic females capable of producing all-female populations. Those males that produce only female offspring are then confirmed genetic females (neomales) capable of establishing the commercial production of all-female halibut stocks.

Methods

Mechanism of sex determination

On April 29, 2002, one litre of ovulated eggs from each of two wild Atlantic halibut broodstock was fertilized with UV-irradiated halibut sperm and exposed to pressure treatments to produce diploid gynogens[5]. Control fish from the same crosses were also produced. These offspring were reared under standard hatchery conditions at Maritime Mariculture Inc., St. Andrews, New Brunswick. In January 2003, 20 fish were sampled and their gynogenetic status was confirmed by determining the lack of paternal genomic contribution using microsatellite DNA genotyping[8]. Sex was also determined by histological evaluation. The remaining fish, at one year old, were transferred to the Fisheries and Oceans Biological Station and reared until February 23, 2004, at which time 121 juvenile fish were killed, sexed and fin-clipped for genotyping to confirm gynogenetic status.

Sex reversed females

The remaining Atlantic halibut juveniles exposed to androgen and estrogen treatments by Hendry et al.[7] in 1999 were reared at Fisheries and Oceans Biological Station until maturity in 2004. Sex was determined by ultrasound[9] and milt expressed by manual stripping. Milt from 9 males exposed to MDHT was used to fertilize approximately 115 liters of Atlantic halibut eggs. The offspring from each male were reared separately at two commercial hatcheries.

Results and Discussion

Of the 141 putative gynogens sampled, 120 were confirmed gynogens and all were female. The sex ratio of control fish was not significantly different from 1:1. This confirms that Atlantic halibut females are the homogametic sex. This research shows that all-female halibut stocks can be produced using similar indirect feminization techniques used to produce all-female salmonids. Direct hormonal sex reversal of genetic females into phenotypic males will result in neomales producing sperm containing only X chromosomes.

All of the retained fish exposed to MDHT treatments prior to gonadal differentiation[7] developed into functional males. Milt quality, as indicated by motility and fertilization success, did not appear different from that of regular males. There were no noticeable morphological differences between neomales and regular males.

The offspring from these 9 males were reared separately at two commercial hatcheries. It was necessary to produce large volumes of eggs fertilized with each male to allow segregated rear-

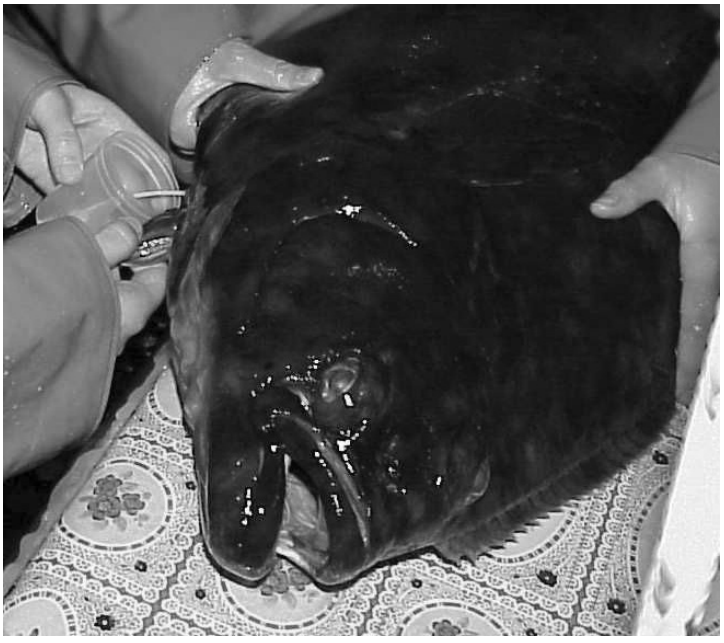

Figure 1.
Stripping milt from an Atlantic halibut male.

ing of each group of fish until they grew to about 10-12 cm FL, at which time they could be sexed morphologically. Unfortunately, one hatchery ceased operations during production and fish reared at this site were lost. The remaining offspring from 4 males are currently being reared and the sex ratios of each group of offspring will be determined in the spring of 2005 to ascertain which males are actually genotypic females. The remaining androgen-treated males (*n*=39) will be retained as broodstock and the sex ratio of their offspring determined at a later date.

In conclusion, female Atlantic halibut are homogametic. Manipulating the phenotypic sex of genetic females using androgens during early development will result in "neomales" capable of producing "feminized milt" containing only X sex chromosomes. Crossing these neomales with regular females will produce all-female stocks with higher growth potential than regular mixed sex populations.

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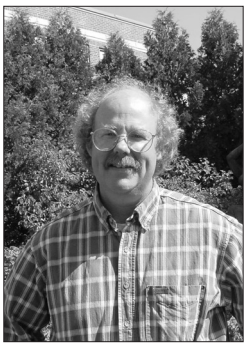
Soft-Shell Clam, *Mya arenaria*, Mariculture in Maine, USA: Opportunities and Challenges



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For each of the past five years, approximately 1100 metric tons of soft-shell clam meats have been landed by fishermen along the coast of Maine (northeastern US) with a landed value between $9.6 and $17 million USD, making clams the second most important wild fishery in the state behind lobsters. Intertidal areas, where clams are harvested exclusively, are managed by an elected stewardship committee from each coastal community. Approximately 10 of the 80 communities that manage their soft-shell clam stocks include enhancement using cultured seed from the Downeast Institute for Applied Marine Research & Education (formerly the Beals Island Regional Shellfish Hatchery), a non-profit organization whose mission is to improve the quality of life for the people of downeast and coastal Maine through applied marine research, technology transfer, and public marine resource education. Over the years, a mariculture strategy for Maine and the northeastern US has been developed based on research and community-based outreach programs originating from this organization. This paper will review the history of the Beals Island Regional Shellfish Hatchery and the results of numerous field experiments carried out over the past 15 years designed to assess the importance of stocking density, predator protection, tidal height, planting season, and site on clam growth and survival.

Au cours des cinq dernières années, 1 100 tonnes métriques de chairs de mye ont été récoltées chaque année le long de la côte du Maine (nord-est des États-Unis). D'une valeur au débarquement se situant entre 9,6 et 17 millions de dollars américains, ces prises font de la mye la deuxième plus importante ressource halieutique sauvage de l'État, le homard venant en tête. Dans chaque collectivité côtière, les zones intertidales, où la mye est récoltée exclusivement, sont gérées par un comité d'intendance élu. Environ 10 des 80 collectivités qui gèrent leurs stocks de mye les étoffent à l'aide de naissain d'élevage provenant du Downeast Institute for Applied Marine Research and Education (anciennement la Beals Island Regional Shellfish Hatchery), une organisation sans but lucratif dont la mission est d'améliorer la qualité de vie des habitants des zones côtières de l'est du Maine grâce à la recherche marine appliquée, le transfert de technologie et l'éducation du public en matière de ressources marines. Au fil des ans, une stratégie de mariculture pour le Maine et le nord-est des États-Unis a été élaborée, reposant sur des programmes de recherche et des programmes communautaires de sensibilisation du public mis sur pied par cette organisation. Nous présentons l'historique de la Beals Island Regional Shellfish Hatchery et les résultats de nombreuses expériences menées sur le terrain au cours des 15 dernières années en vue d'évaluer l'importance de la densité d'ensemencement, de la protection contre les prédateurs, de la hauteur des marées, de la saison d'ensemencement et du site en regard de la croissance et de la survie de la mye.

Introduction

In Maine, USA, commercial harvesting (clamming) of soft-shell clams, *Mya arenaria* L. from intertidal mud and sand flats has a long, rich history. Prior to the 1850s, clams were a source of food or bait, but no one bought or sold them commercially[(1)]. Shell middens containing the disarticulated valves of millions of individuals of *Mya* are prevalent along many of Maine's southern estuaries and offer testimony to the fact that Native Americans relied on this bivalve for part of their seasonal diet[(2)]. Between 1850 and 1875, clamming became a commercial industry in Maine, providing regular employment through the fall and winter for men who dug, shucked, and salted the shellfish for Grand Banks cod fishermen. In 1890, the first conservation program was enacted in Maine. It regulated the taking of clams to a period outside of a window coinciding with the spawning season[(3)] along this coast (1 June to 15 September).

By 1904, 21 factories were canning clams in Maine mostly for consumption outside the state. Shortly thereafter, stocks began to decline and the commissioner of Maine's Sea and Shore Fisheries Department, the public entity charged with the stewardship of all marine resources, initiated a seven-year investigation carried out by state wardens and private citizens to determine whether clam stocks could be enhanced using farming techniques. He developed legislation that allowed small "reservations" to be set aside for experimental purposes. Short-term ownership of these 1-2 acre intertidal clam reservations were given to most who applied with the stipulation that the recipient notify the commissioner in writing about the outcome of farming trials.

In 1911, the Maine state legislature passed a law giving selectmen of each coastal town the right to lease up to one quarter of the clam flats within its limits, the other three quarters left as common property for the public. One key problem with the law, and the

reason why few took advantage of it, was that it did not provide for exclusive ownership of the clams. State reservations continued to be used as sites of stock enhancement and rotating three-year "spawner sanctuaries" for the next few years[4], but private clam farming never became a popular or commercially important endeavor. Although it is still legally possible for Maine communities that manage their public clam stocks to set aside up to 25% of their town's intertidal flats for private clam farming, only two of the 80 coastal communities have done so in the past half century. Instead, since 1987, many of these towns – 53 – have participated in community-based shellfish management programs focusing on stock enhancement using cultured clam individuals originating from a single shellfish hatchery, the Beals Island Regional Shellfish Hatchery in the town of Beals, Maine.

This contribution examines briefly the history of that hatchery- and community-based stock enhancement effort including the field techniques and results from selected field trials designed to answer questions about how factors such as stocking density, tidal height, predator exclusion, initial planting size, and intertidal habitat affect seasonal growth and survival of both cultured and wild clams.

Beals Island Regional Shellfish Hatchery (BIRSH)

The Regional Shellfish Hatchery, located on Perio Point in the island community of Beals, Maine, opened its doors in 1987 to produce soft-shell clam seed for stock enhancement purposes. The physical plant consisted of a renovated, wooden two-story 72-m^2 building located on a commercial wharf adjacent to Moosabec Reach, the waters separating Beals Island from the town of Jonesport. The majority of funds were provided through grants from the National Oceanic and Atmospheric Administration (NOAA) and the Economic Development Administration (EDA). In 1988, a grant from the Maine Science and Technology Board enabled BIRSH to construct a 90-m^2 greenhouse that served as a clam nursery and algal mass culture building. In 1991, a 37-m^2 education center opened adjacent to the greenhouse and was named to honor Dana E. Wallace, a shellfish biologist with the Maine Department of Marine Resources for forty years. Until 1995, all grants to BIRSH were administered by the University of Maine at Machias (UMM).

Initial annual production goals were one million 8-12 mm (shell length, SL) seed clams for the six eastern coastal neighboring communities of Machiasport, Roque Bluffs, Jonesboro, Jonesport, Beals, and Addison. As the physical plant expanded, more communities from along the entire coast of Maine were invited to participate. Techniques used to spawn adults and rear soft-shell clam larvae are similar to those described in Stickney[5]. Spawning naturally conditioned wild clams was not possible before June 1. Attempts to condition animals for earlier spawning never met with consistent results. Post-set juveniles were reared in the hatchery on floating trays lined with 125µm screening and fed cultured microalgae (mostly T. *Isochrysis galbana*, *Chaetoceros calcitrans*, *C. muelleri*, *Thalassiosira weissflogii*, *Pavlova lutheri*, and *Tetraselmis maculata*). Juveniles subsequently were moved to 175-, 250-, 500-, and 1000-µm trays as they increased in size and volume.

Animals remained in the hatchery (approximately 2-3 months after settlement) until they were large enough to reside on window screening (a 1400-µm sieve was used for this purpose). At that size, animals were transferred to wooden trays lined with window screening that floated in a protected embayment near the hatchery (10 000 animals/1.1 m^2). Individuals placed into floating trays before July attained sizes >12 mm SL by the time growth ceased in November, whereas animals placed in the field nursery after July attained smaller sizes (5-9 mm SL).

From 1987 to 1991, clams were seeded onto flats under strips of protective, flexible netting (4-mm aperture) during the month of November when growth in both the nursery and the intertidal ceased. The success of these endeavors varied as a function of winter conditions. In many places, ice forming in the intertidal in early to mid January resulted in scouring and rafting of the top few centimetres of the mudflat. Since 8-12-mm *Mya* burrow very shallowly in the sediments, entire seeding efforts were lost from time to time. Other problems encountered through the fall and winter included sedimentation that tended to suffocate clams under the netting or resulted in complete loss of nets through storms or ice events. Although some seeding trials were successful (>80% survival from November through April), the inability to accurately predict icing and storm events and plant seed clams in "safe places," forced us to think differently about how to handle clams during the winter months (see Overwintering section, below).

Seed production provided BIRSH staff and university personnel with enormous opportunities to create educational and management programs with clammers, stewardship committees, local officials, educators, and school children. From 1997 to 2000, a large program was funded by the Jessie B. Cox Charitable Trust and the Dolphin Trust (Boston, Massachusetts) that allowed for all seed clams produced at BIRSH for two years to be divided evenly between three towns representing different geographic regions along the coast: southwestern, midcoastal, and downeast. This "Community Clam Culture" program successfully tested on a large scale results from small-scale research projects. Participants in two of the three communities have continued with various aspects of the seeding program.

The Downeast Institute for Applied Marine Research & Education

In 1995, a sixteen-member volunteer Board of Directors assumed administrative and financial control of BIRSH, and the following year it was granted federal non-profit 501(c)(3) status by the US federal government. The board developed a mission statement that exemplified the work to date: *To enhance Maine's soft-shell clam and other shellfish resources through aquaculture, applied research, technology transfer, and public education*. In 1999, the Board first applied for grant funds to increase the physical plant at Perio Point to accommodate a wider breadth of activities. The plan called for constructing a 440-m^2 marine laboratory with separate areas for a modest running seawater laboratory and adjacent processing rooms, classroom, office space, and expanded facilities to continue producing clams and other shellfish for stock enhancement programs. The general idea was to create a facility that could serve multiple purposes. These included: 1) a marine field station for UMM; 2) a marine laboratory with tank space for holding live organisms and processing samples; 3) a well-equipped shellfish hatchery; 4) a business incubator to serve fishermen and other entrepreneurs whose ideas about adding value to products as well as creating new products and services require space for alpha- and beta-testing; and 5) an education center for K-16 and adult learners. Although funds were not forthcoming to create the labora-

tory at Perio Point, in 2001, the Board decided to change the name of the organization from BIRSH to the Downeast Institute for Applied Marine Research & Education (DEI) to reflect these additional goals. A new mission statement was developed: *To improve the quality of life for the people of downeast and coastal Maine through applied marine research, technology transfer, and public marine resource education*.

In 2003, the DEI board moved shellfish hatchery operations 5 km from Perio Point to a 3.3-hectare coastal tract on Great Wass Island in the town of Beals, Maine. The deepwater property has approximately 0.8 km of rocky shoreline and, besides cold water, has several other features making this an ideal setting for a marine laboratory complex. These include a 600-m^2, two-story building constructed in 1998, a 12-m wharf, and two commercial tidal lobster impoundments that can be used as mesocosms during the summer months. In addition to the five goals outlined above, there are plans to construct a small (25-30-bed) dormitory and dining facility to accommodate up to 40 individuals. At present, the DEI board is renting these facilities and has raised 35% of the US$1.3 million needed to purchase it outright.

Results and Discussion

Field work conducted in eastern Maine from 1989 to 2000 has resulted in a mariculture strategy that likely has applications both north and south of the geopolitical boundaries of this State. Most intertidal experiments were well-replicated, but used small experimental units (15-cm-diameter × 15-cm-deep plastic horticultural pots) to contain clams. Many of the results, however, were tested critically on spatial scales of tens of meters in "Community Clam Culture" trials with little variation from the outcome of the small-scale tests.

Seasonal clam growth of cultured individuals

Field trials conducted at six locations over an eight-year period from 1989 to 1996 provided consistent results on seasonal growth rates. Typically, clams of varying sizes (4-15 mm SL) were planted in plastic pots at several tidal heights[6,7] and their fate followed from April to as late as December. A few studies were conducted in multiple years as Figure 1 demonstrates. The period between June and August is when 60-65% of annual growth (measured as increase in SL) occurs. This rate is independent of tidal height[7] and site, and is unaffected by stocking densities up to 2664/m^2[8]. In eastern Maine, virtually no growth occurs from late fall through early spring (Fig. 1).

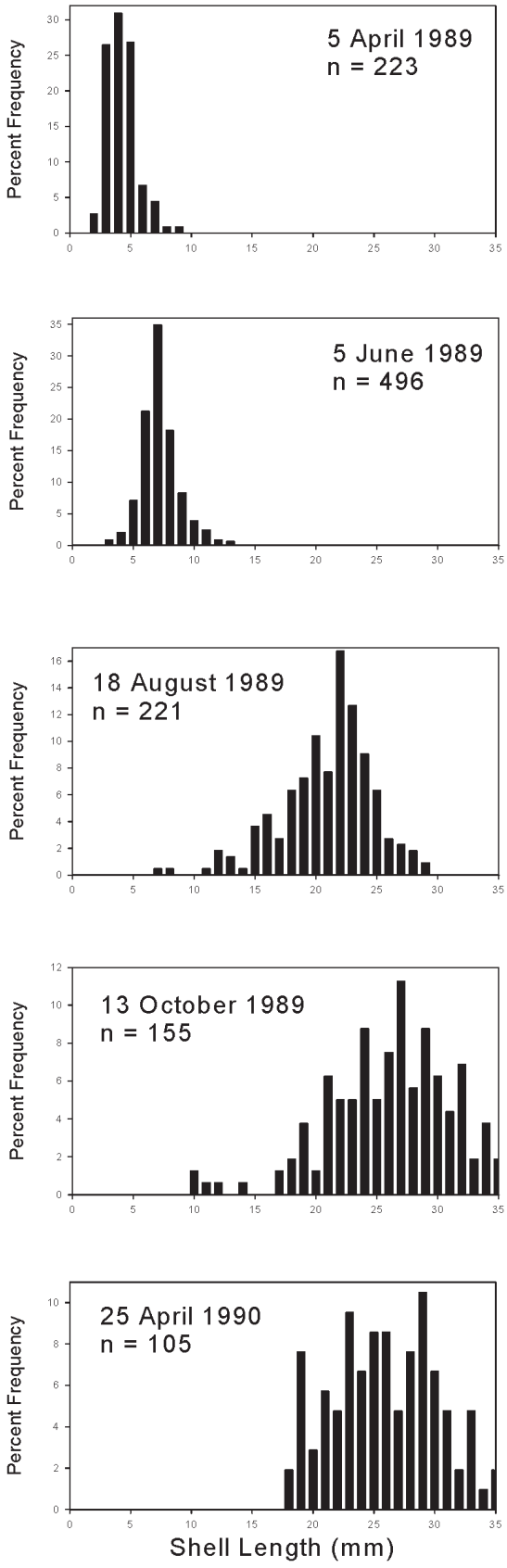

Figure 1.
Shell growth of hatchery-reared individuals of *Mya arenaria* from 5 April 1989 to 25 April 1990 at the mid-intertidal zone of a mud flat located in Cutler, Maine (44°41.00'N; 67°18.63'W). Animals were reared at the Beals Island Regional Shellfish Hatchery and were planted in 15 cm diameter × 15 cm deep plastic horticultural pots. There is no difference in the distribution of individuals between 13 October 1989 and 25 April 1990 (*G* = 8.94, *df* = 11, *P* = 0.6277).

Overwintering cultured juveniles

Because clams seeded during the fall add no appreciable shell until the following spring and intertidal survival over the winter is unpredictable, we developed a simple technique to overwinter cultured juveniles[9]. Clams (8-12 mm SL) can be held from November through April at densities up to 22 000 individuals/0.2 m^2 in bags constructed of nylon window screening (aperture 1.8 mm) that are placed in subtidal cages anchored 1-2 m off the bottom with >90% survival.

A mariculture scenario for eastern Maine and other coldwater regions

Cultured seed (8-12 mm SL) should be planted at densities between 330 and 660/m^2 in the spring near or just below the mid intertidal and protected with flexible netting (6.4-mm aperture) that is buoyed off the bottom with small floats so that netting will not interfere with clam feeding during periods of tidal inundation. In most environments, >50% of the clams will survive and attain an average size of 25-30 mm SL by November. Protective netting should be removed at that time prior to ice formation that could rip, tear, or destroy the nets. Clams reach a size refuge from most predators, including green crabs, *Carcinus maenas*, so there is no need to re-apply nets to the clams the following spring. Growth to a legal size of 51 mm will take 2-4 years.

A short-term farming strategy that takes advantage of differences (as much as 150%) in seasonal price of commercial-size clams is to impound clams >51 mm in shallowly burrowed vinyl-coated wire cages (0.6 m × 0.9 m × 0.12 m deep) with 3.8-cm aperture. Cages can hold 11-12 kg with survival rates from April to late August/early September >90%[10].

Acknowledgments

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Benthic Collection of *Mya arenaria*: A Promising Approach for Spat Supply in Îles-de-la-Madeleine (Southern Gulf of St. Lawrence)

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A reliable, abundant, and cheap seed supply is crucial for the development of soft-shell clam culture in Îles-de-la-Madeleine. Suspended collectors (scallop bags filled with Netron™) proved to be ineffective because of the huge abundance of associated mussel spat. Other approaches were tested for spat supply but the transfer of small clams (15-40 mm) from a wild population has been disappointing (very limited growth and retrieval) as well as the use of "tents" which did not increase spat settlement underneath. Hatchery production is another option but it is an expensive way to get small clams. There is an approach which is still relatively unknown: benthic collection using Astro-Turf™ mats laid down directly on the substrate. These mats collect virtually only soft-shell clams. Different trials were performed in 2003 and repeated in 2004 to gain a better knowledge on how to use mats to get optimal collection. The best results were obtained in the Havre-aux-Maisons lagoon with >1200 clams >2.5 mm/m^2 over a 3-year period. Mats could be installed throughout June (and even early July) and retrieved throughout September (and even early October) with equal success. Mats could be placed side by side with no reduction in spat collection. Despite the absence of a clear pattern, spat collection seems often higher in the intertidal zone. The mean length of the clams >2.5 mm in mid September is about 7-9 mm. Benthic collection is thus a promising avenue for spat supply in Îles-de-la-Madeleine.

Une source d'approvisionnement en naissain fiable, abondante et peu coûteuse est essentielle au développement de la culture de la mye aux Îles-de-la-Madeleine. Les collecteurs (sacs à pétoncles remplis de Netron™) suspendus dans la colonne d'eau se sont révélés inefficaces à cause de la forte abondance de naissain de moule qui s'y fixait. D'autres méthodes d'approvisionnement en naissain ont été mises à l'essai; le transfert de petites myes (15-40 mm) issues d'une population sauvage a donné des résultats décevants (croissance et récupération très limitées), tout comme l'utilisation de « tentes », qui n'a pas mené à une fixation accrue du naissain en-dessous. La production en écloserie est une autre option, quoique dispendieuse. Mais il existe une autre approche, relativement peu connue : le captage benthique de naissain à l'aide de paillassons de gazon synthétique Astro-Turf™ posés directement sur le substrat. Ces paillassons sont colonisés presque uniquement par du naissain de mye. Des essais ont été faits en 2003, puis répétés en 2004, afin de mieux comprendre comment utiliser ces paillassons pour optimiser le captage. Les meilleurs résultats ont été obtenus dans la lagune de Havre-aux-Maisons : plus de 1 200 myes de plus de 2,5 mm par m^2 ont été recueillies sur une période de trois ans. Les paillassons peuvent être installés en juin (et même au début de juillet) et récupérés en septembre (et même au début d'octobre), le niveau de succès de captage étant le même. Ils peuvent en outre être posés côte à côte sans que cela nuise au captage. Malgré l'absence d'une tendance nette, le volume de naissain capté en zone intertidale semble souvent plus élevé. La longueur moyenne des naissains de plus de 2,5 mm de longueur à la mi-septembre se situe entre environ 7 et 9 mm. Le captage benthique constitue donc une méthode d'approvisionnement en naissain prometteuse aux Îles-de-la-Madeleine.

Introduction

The first R&D activities to develop the soft-shell clam culture in Îles-de-la-Madeleine began in 1994. Since then one recurrent constraint has been to secure seed supply. During the early years, the focus of attention was on suspension collection using scallop bags filled with Netron™. However, results were disappointing. Although clam collection was possible (up to 10 000 per bag) the huge abundance of associated mussel spat in the bags made it of very limited interest for commercial operation because of sorting constraints[(1)]. As a result, the R&D program MIM (French acronym for soft-shell clam culture in Îles-de-la-Madeleine) which was put in place in 2000 focused primarily on the harvesting of small clams (15-40 mm) from a wild population as a source of seed supply. Once again, the results were disappointing because of the very poor growth and the high losses of the clams once seeded[(2)]. Nets were also deployed as "tents" to increase spat settlement (like in Cape Cod) but with no success. Since hatchery production is an expensive way to get small clams very few options were still available to secure spat supply in order to develop a profitable clam culture in Îles-de-la-Madeleine.

A new approach is through benthic collection. It was proposed by Chandler et al.[3] but very little is known about it. Benthic collection is fairly simple as it uses mats laid down directly on the substratum to increase local spat settlement and/or to retain larger numbers of settled spat between their plastic bristles. Preliminary trials were performed in 2001 and 2002 with two different mats[4]. The use of mats provided interesting results with good clam collection and no associated mussel spat. In 2001, grey doormats provided an average of 1640 clams >2.5 mm/m^2. In 2002, the Astro-Turf™ mats provided better results than grey doormats and their average collection density was 2232 clams >2.5 mm/m^2. The clams >2.5 mm reached a mean length of 7-9 mm in mid September. These results prompted a larger experiment in 2003 and 2004 to confirm the potential of benthic collection and to gain a better knowledge on how to use the Astro-Turf™ mats to get optimal collection.

Materials and Methods

Most experiments were performed in the Havre-aux-Maisons lagoon on a clam culture lease. The Astro-Turf™ mats (45 cm × 61 cm) were deployed at mid-intertidal level in mid June and retrieved in mid September, otherwise stated. They were fixed to the medium-sand substratum with metal hooks planted at each corner. Individual mats are separated from each other by ~1 m. There were usually 10 replicates and 10 controls per treatment. A control was a 0.02-m^2 sample of substratum (~10-cm deep) taken nearby the mats. Control results are not presented here as the corresponding mats have always provided much higher spat densities (7-40×). Therefore, mats considerably increase spat settlement/retention compared to the adjacent natural sandy substratum.

At retrieval, mats were placed in individual plastic bags and brought back to laboratory. They were cleaned with pressurized tap water and the dislodged material was sorted through 2.5-mm and 1-mm mesh sieves. Only results about clams >2.5 mm are presented here as these are of primary interest for commercial clam culture.

Several parameters were studied in 2003 and in 2004 to assess the inter-annual repeatability of the results.

Period of installation

This experiment was designed to define the optimal installation period of the mats. Three periods were examined in 2003 (June 1, 15, and 30) and four in 2004 (June 1, 15, 30, and July 7). All mats were retrieved in mid September.

Figure 1.
Experimental Astro-Turf™ mats placed individually or side by side.

Period of retrieval

This experiment was designed to define the optimal retrieval period of the mats. On one side, the later the mats could be retrieved, the larger size the clams will reach. On the other side, there are possibly higher risks to loose spat during fall when the higher frequency and intensity of the storms increase bedload transport. Further, the increase in spat size with time could possibly result in higher numbers being trapped between the mat bristles. All mats were installed in mid June. Three periods of retrieval were examined in 2003 (September 1, 15, and 30) and four in 2004 (September 1, 15, 30, and October 7).

Spatial variation

Almost all the preliminary trials with mats were performed in the Havre-aux-Maisons lagoon and results were promising. But could it be possible to find better or other interesting collection sites in Îles-de-la-Madeleine? Overall, 8 different sites were examined all around the Îles-de-la-Madeleine in 2003 and 2004. These sites were chosen based on the presence of wild clam populations nearby.

Tidal level

Chandler et al.[3] only looked at different intertidal levels. However, it is important to know whether benthic spat collection is restricted to the intertidal zone or whether it could also be successful in the low-depth subtidal zone. These information could be useful to optimize management of a clam culture lease. A preliminary trial in 2001 suggested the clam collection was better in intertidal than in subtidal zone[4]. In 2003 and 2004, spat collection at intertidal and subtidal levels was compared at 3 different sites.

Large-scale collection

A large number of mats would be needed to provide seed at a commercial scale. Mats will probably have to be placed side by side to minimize the needed surface for spat collection. However, preliminary observations in 2003 were somewhat worrying as clam density was much lower on mats placed side-by-side (mean = 828 clams >2.5 mm/m^2) than on individual mats (mean = 1402 (±137 SE) clams >2.5 mm/m^2). Since this comparison was unplanned, the mats were not placed in the same area initially. So, it was difficult to reach firm conclusion about this anecdotal observation. Was this difference simply a coincidence or was it real ? In 2004, side by side mats were compared to individual mats in three replicates (Fig. 1). For each replicate, the mean of 6 individual mats was compared to the mean of 6 mats taken from a group of 28 mats placed side by side.

Results

Period of installation

There were no significant differences in spat abundance in mid September from mats installed throughout June 2003. However, the clam density tended to decrease with time from 1660 (±212 SE) clams/m^2 in early June to 1175 (±114 SE) clams/m^2 in late June. In 2004, there were no significant differences in clam densities on mats installed between early June and early July. In contrast to 2003, the clam density was quite constant through time with values ranging between 1026 (±212 SE) clams/m^2 and 1384 (±318 SE) clams/m^2.

Period of retrieval

There were no significant differences in spat abundance on mats installed in mid June and retrieved all along September 2003 (Fig. 2). However, clam density tended to decrease with time from 1633 (±267 SE) clams/m^2 in early September to 1288 (±165 SE) clams/m^2 in late September. In 2004, there were no significant differences between periods of retrieval from early September to early October. In contrast to 2003, the density tended to increase through time with values ranging from 1127 (±191 SE) clams/m^2 in early September to 2136 (±318 SE) clams/m^2 in early October (Fig. 2)

Spatial variation

Of the 8 sites examined in 2003 and 2004, only the Havre-aux-Maisons lagoon provided abundant and stable spat collection with 1402 (±137 SE) clams/m^2 in 2003 and 1384 (±318 SE) clams/m^2 in 2004. At all other sites, spat collection was ≤802 (±42 SE) clams/m^2 with one exception. The Pointe-à-Marichite site provided 2895 (±371 SE) clams/m^2 in 2003 but only 421 (±38 SE) clams/m^2 in 2004.

Tidal level

Three of the previous sites were used to compare collection in subtidal and intertidal levels in 2003 and 2004. There were significant site × zone interactions during both years. There was no clear pattern but clam collection often seemed to be better in the intertidal zone.

Large scale collection

In 2004, clam density was similar for side by side mats and spaced mats when both were installed in the same area, with 738 (±48 SE) and 721 (±152 SE) clams/m^2 respectively.

Discussion

Astro-Turf™ mats could be installed throughout June and even early July with good collection success. They could also be retrieved throughout September and even in early October with comparable collection success. However, it seems that spat collection could probably have better results if mats are installed in early June and retrieved in late September-early October. Interestingly, it seems the mats could be retrieved in late fall with no important losses due to storms. Further, the quantity of clams >2.5 mm increased with time (at least in 2004). This was probably due to the growth of the smaller clams and their subsequent shift to the larger size class (>2.5 mm). These information are highly valuable in a commercial context as they suggest that clam growers could have a certain flexibility in their management of spat collection.

There were huge spatial variations in spat collection in Îles-de-la-Madeleine. However, it seems that spat collection in the intertidal zone of the the Havre-aux-Maisons lagoon provides the most reliable results. Spat collection in the Havre-aux-Maisons lagoon has been quite stable over 3 years with yearly average 1495 (±86 SE) clams/m^2 with a mean size of 8 mm. These are promising results as the main clam culture ac-

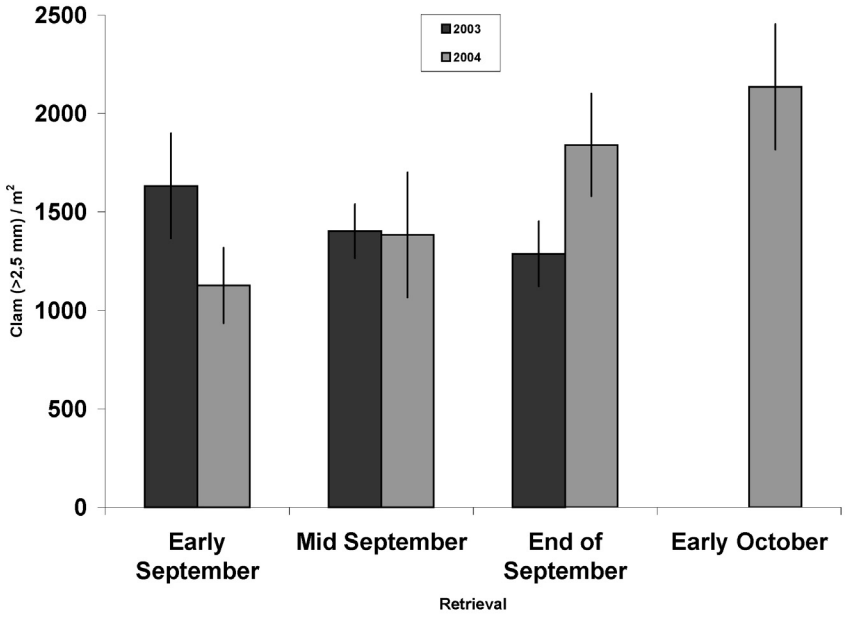

Figure 2.
Spat density (mean ± SE) on Astro-Turf™ mats installed in mid June and retrieved at different periods in fall 2003 and 2004.

tivities have been concentrated in this lagoon. Large-scale spat collection should be possible even though the mats are placed side by side to minimize the needed collection area in the Havre-aux-Maisons lagoon.

Although there is no mussel spat on the mats, clam sorting is time consuming. Young clams must be sorted from broken clam shells and other debris. Further work is needed to ease sorting. Nevertheless, benthic collection could be profitable despite this constraint since the estimated costs to get 6 000 000 spat (mean size of 8 mm) is about $23 000.

This approach seems promising, at least in Îles-de-la-Madeleine. Similar experiments were performed elsewhere in Quebec but provided disappointing results probably due to substratum composition and sites exposure to waves[5]. Over the past years we have acquired a better knowledge on how to use mats but further work is still needed.

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Influence de Variables Physiques et Biologiques sur le Temps d'Enfouissement de la Mye Commune (*Mya arenaria*)

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Peu de choses sont connues sur la façon optimale d'élever les myes. Parmi les éléments importants à considérer figure la vitesse d'enfouissement. Le présent projet a pour objectif principal d'évaluer des facteurs physiques et biologiques sur la vitesse d'enfouissement. Plus spécifiquement, les différents facteurs examinés étaient: la taille des myes, la densité d'ensemencement, l'ameublissement du substrat, la durée de la période d'émersion précédant l'ensemencement et la période de l'année. La variabilité saisonnière de la vitesse d'enfouissement a, également, été étudiée. Les résultats ont démontré que (1) la vitesse d'enfouissement diminue avec l'augmentation de la taille, (2) des densités d'ensemencement jusqu'à 350 myes (25-30 mm) · m^{-2} n'ont pas entraînées d'impacts négatifs, (3) il n'est pas nécessaire d'ameublir un substrat constitué de sable moyen, (4) les myes peuvent subir une émersion de 4 heures sans effets négatifs, (5) une température de l'eau atteignant 20-23°C ne cause pas de ralentissement de l'enfouissement, (6) une diminution substantielle de la condition des myes, associée ou non à la ponte, semble avoir un impact négatif et (7) les ensemencements d'automne (à partir de septembre) ne sont pas recommandés. Ainsi, les informations obtenues ont permis de favoriser le maintien des organismes sur place et leur survie.

Little is known about the optimal way to grow soft-shell clams. One important aspect to consider is burrowing time. The main objective of this project is to evaluate the physical and biological factors influencing burrowing time. More specifically, the various factors examined were: clam size, seeding density, substrate consolidation, duration of emersion preceding seeding and time of year. Seasonal variability of burrowing time was also studied. The results demonstrated that (1) burrowing time decreases with increased size, (2) seeding densities of up to 350 clams (25-30 mm) per m^2 had no negative impact, (3) it is unnecessary to loosen a substrate consisting of medium sand, (4) clams can tolerate emersion for four hours with no negative effects, (5) water temperatures as high as 20 – 23°C cause no reduction in burrowing time, (6) a substantial decline in the condition of clams, whether or not associated with spawning, appears to have a negative impact, and (7) fall seedings (beginning in September) are not recommended. The information obtained were used to promote the maintenance on site and survival of clams.

Introduction

Peu d'études écologiques ont été faites sur l'enfouissement des bivalves, et plus particulièrement sur la mye commune. De plus, aucune n'a été conduite dans une perspective d'ensemencement. Cette étude a été conduite pour mieux documenter un aspect spécifique lié à l'ensemencement des myes, soit l'influence de différents facteurs biologiques et environnementaux sur la vitesse d'enfouissement. Les différents facteurs examinés étaient: la taille des myes ensemencées, la densité d'ensemencement, l'ameublissement du substrat avant ensemencement, la durée de la période d'émersion précédant l'ensemencement et la période de l'année. L'objectif général de cette étude était d'évaluer l'influence de ces facteurs sur la vitesse d'enfouissement dans des conditions se rapprochant le plus possible de celles rencontrées lors des activités d'élevage.

Matériels et Méthodes

Les travaux ont été réalisés aux Îles-de-la-Madeleine sur la zone aquacole de la Dune du Nord dans la lagune du Havre-aux-Maisons. Pour la présente étude, les myes ont été prélevées sur un gisement naturel dans le secteur sud-ouest de la lagune du Havre-aux-Basque. Par la suite, les individus ont été transférés au site expérimental de la lagune du Havre-aux-Maisons pour les essais d'enfouissement.

Les expérimentations on eu lieu en milieu subtidal à marée montante en zone peu profonde sur une aire limitée. Les myes ont été placées individuellement au centre d'un cylindre de PVC de 10 cm de diamètre et elles étaient réparties aléatoirement. Pour ce qui est de la période de l'année, la vitesse d'enfouissement a été examinée dans des bassins pour limiter l'influence des variables marées, courants et vents. Pour tous les volets il y a

20 myes de chaque traitement qui ont été étudiés et il y a 9 réplicats qui ont été fait par volet sauf pour le densité où il y en a eu 6.

La vitesse d'enfouissement a été mesurée à partir de la proportion enfouie de l'individu (sortie du pied, 1/4, 1/3, 1/2, 2/3, 3/4, tout enfoui) et notée à différentes périodes de temps (aux 15 min pour les 2 premières heure et, par la suite, à toutes les 30 min jusqu'à 3 ou 4 h après l'ensemencement).

Chaque semaine, différents paramètres environnementaux étaient mesurés au site de prélèvement de la lagune du Havre-aux-Basques afin de relier leur évolution avec les changements temporels observés au niveau de la vitesse d'enfouissement des myes. Des mesures ponctuelles de température et de salinité ont été réalisés et des échantillons d'eau ont été obtenues pour la mesure des concentrations de seston total, organique et inorganique.

Chaque semaine, 25 myes de 25-30 mm étaient aussi récoltées au site de prélèvement et congelées jusqu'au moment de la détermination de la masse sèche de leurs tissus. Ceci a permis de caractériser l'évolution de l'état général des myes.

Une expérience complémentaire fut menée dans les bassins de l'Institut Maurice-Lamontagne (Pêches et Océans Canada, Mont-Joli) afin d'obtenir des informations sur l'influence du niveau d'enfouissement des myes sur leur capacité de résister aux déplacements passifs créés par l'action des courants.

Résultats

En laboratoire, les myes qui sont enfouies à 50 % et plus résistent à des courants atteignant 30 cm/sec. Un enfouissement de 50 % de la coquille réduit donc de façon substantielle les problèmes de déplacement passif car la mye peut alors résister à des courants importants. Dans cette optique, la période nécessaire pour obtenir un niveau d'enfouissement de 50 % fournira une information utile et pratique.

Il y a différentes classes de tailles qui ont été étudiées car ce facteur à une répercussion sur l'enfouissement des organismes benthiques (15-20 mm, 25-30 mm, 35-40 mm).Les résultats ont démontrés que les myes les plus petites s'enfouissaient plus rapidement que les plus grosses (Fig. 1).

La densité d'ensemencement pourrait avoir une influence sur l'enfouissement des myes car une densité trop importante pourrait entraîner des interactions physiques entre les individus. Donc le temps d'enfouissement a été examiné pour une faible (100 myes/m^2), moyenne (225 myes/m^2) et une forte (350 mye/m^2) densités. Les résultats ont démontrés que les différentes densités n'avait pas d'effets négatifs sur l'enfouissement.

Actuellement, pour les ensemencements, la parcelle est traitée avec une récolteuse hydraulique ce qui a pour effet d'ameublir le substrat. L'enfouissement à donc été examiné en fonction du temps écoulé entre l'ameublissement et l'ensemencement (pas d'ameublissement, 1 jour, 7 jours et 14 jours après) pour pouvoir déterminer si cette étape était nécessaire. Les résultats ont démontrés que l'ameublissement du substrat ne facilitait pas l'enfouissement.

L'émersion est un facteur important parce qu'il soumet les myes à la dessication ainsi qu'à des écarts importants de température. La vitesse d'enfouissement a été examinée suite à différents temps d'émersion (immersion continue, 1 heure, 2 heures et 4 heures). Les résultats ont démontrés qu'une durée d'émersion jusqu'à 4 heures n'avait pas d'effets négatifs sur l'ensemencement (Fig. 2).

Le myiculteur est dépendant des conditions ambiantes au moment des ensemencements et ces conditions varient au cours de l'année puisque plusieurs facteurs interviennent en conjonction : la salinité, la température, la disponibilité de nourriture et la période de reproduction, par exemple. C'est pourquoi la vitesse d'enfouissement a été examinée toutes les 3 semaines entre mai et octobre 2002. Les résultats ont démontrés que la vitesse d'enfouissement était variable au cours de la période estivale mais maximale vers la mi-août.

Les paramètres environnementaux et biologiques ont varié au cours de la période estivale dans la lagune du Havre-aux-Basques, là où étaient prélevées les myes expérimentales. Aucun de ceux-ci ne peut individuellement expliquer sans ambiguïté les fluctuations observées au niveau de la vitesse d'enfouissement des myes. L'effet combiné des différents paramètres environnementaux et biologiques sur la vitesse d'enfouissement (% d'enfouissement observé après 4 heures) a été évalué à l'aide d'une régression multiple. Les deux facteurs dont la contribution est la plus importante pour expliquer la variation observée au niveau de la vitesse d'enfouissement étaient la température et la masse sèche des myes.

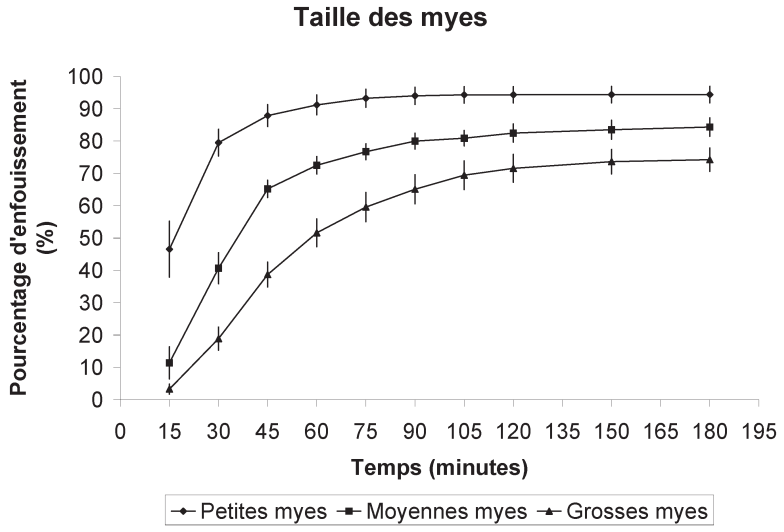

Figure 1.
Évolution temporelle de la vitesse d'enfouissement des myes (*Mya arenaria*) en fonction de trois classes de taille (15-20, 25-30 et 35-40 mm), en milieu naturel, aux Îles-de-la-Madeleine.

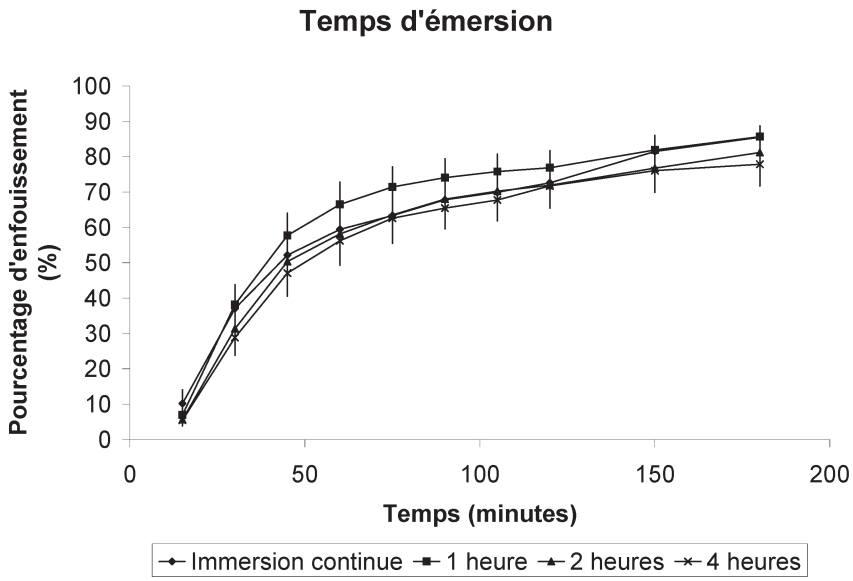

Figure 2.
Évolution temporelle de la vitesse d'enfouissement des myes (*Mya arenaria*) en fonction de quatre durées d'émersion (immersion continue, 1 heure, 2 heures et 4 heures), en milieu naturel, aux Îles-de-la-Madeleine.

Discussion

L'ensemble des résultats obtenus pour les différents volets montre que la taille des myes et la période de l'année ont eu une influence significative sur la vitesse d'enfouissement. L'évolution de l'enfouissement a suivi une courbe asymptotique pour tous les volets sauf pour la « densité » où les observations ont été faites lorsque la température de l'eau avait chuté. Baptist[1] et Pfitzenmeyer et Drobeck[2] ont aussi rapporté des courbes asymptotiques pour décrire l'évolution temporelle du pourcentage d'enfouissement des myes.

La vitesse d'enfouissement était corrélée négativement avec la taille des individus. Les myes les plus petites (15-20 mm) ont atteint un niveau d'enfouissement de 50 % après un peu plus de 15 minutes tandis qu'il a fallu 1 heure pour les plus grosses (35-40 mm). D'autres études ont aussi rapporté des relations inverses entre la taille et la vitesse d'enfouissement[1-3]. De leur côté, Beal et Vencile[4] rapportent que des myes d'écloserie de 12 mm s'enfouissent habituellement en moins de 10 minutes dans des sédiments vaseux en mai et que la plupart des individus ne sont plus visibles après seulement 5 minutes.

Le processus d'enfouissement n'a pas été ralenti par des densités d'ensemencement variant entre 100 et 350 myes de 25-30 mm/m^2. Pourtant, une densité élevée pourrait éventuellement avoir un impact négatif sur l'activité d'enfouissement des myes en raison des interactions physiques possibles entre les individus. Ces contacts physiques pourraient créer des obstacles ou générer un stress qui entraveraient ou ralentiraient l'enfouissement. Ceci pourrait s'expliquer par le fait que, même à cette densité, l'ensemble des myes ne couvraient qu'environ 16,3 % de la surface expérimentale si l'on considère qu'un individu de 27,5 mm de longueur occupe une surface d'environ 465 mm^2 (~ longueur × largeur, où largeur = 0,615 longueur; obs. pers.) lorsqu'il repose à plat sur le sédiment. Il faudrait donc que les myes soient ensemencées à des densités plus élevées pour entraîner des contacts physiques suffisamment nombreux pour éventuellement voir apparaître un impact négatif sur l'enfouissement.

Ameublir le substrat avant l'ensemencement à l'aide d'un râteau hydraulique n'a pas facilité l'enfouissement des myes. Il a été suggéré qu'un ameublissement pouvait avoir un effet favorable sur l'enfouissement[5,6]. Dans la présente étude, les myes ensemencées sur des surfaces qui avaient été ameublies 1, 7 ou 14 jours plus tôt ne se sont pas enfouies plus rapidement que celles ensemencées sur des parcelles non traitées. Ceci pourrait être en accord avec les observations de Trueman et al.[7] selon lesquelles le délai de retour à un degré de compaction naturelle est de 24 heures pour un sédiment sablonneux perturbé. Nous avons également observé une compaction rapide du sédiment perturbé au cours de l'étude.

Une émersion pouvant durer jusqu'à 4 heures sous le soleil matinal de juin n'a pas eu d'effets négatifs sur l'enfouissement de la mye commune. En émersion, la mye doit passer d'un métabolisme aérobique à un métabolisme anaérobique. Or, ce changement des voies métaboliques entraîne une diminution très importante des dépenses énergétiques de façon à ne maintenir que les fonctions essentielles à la survie[8,9]. Lors de la réimmersion, les produits de la respiration anaérobique qui ont été accumulés pendant la période d'émersion sont expulsés grâce à une activité exacerbée de pompage[9]. Il était possible que ces changements ralentissent la capacité de réaction de la mye en terme d'enfouissement lors de l'ensemencement (réimmmersion).

La vitesse d'enfouissement fut très variable au cours de la période estivale avec un maximum vers la mi-août (20-22 août) et une diminution rapide par la suite. La température de l'eau et la masse sèche des tissus des myes expérimentales ont été les deux facteurs expliquant dans des proportions similaires la plus grande partie de la variation observée entre mai et octobre au niveau du pourcentage d'enfouissement atteint après 4 heures d'observation. Ces observations ont été faites pendant une seule saison (mai à octobre 2002) et elles ne peuvent donc être extrapolées directement. Il suffirait que l'année 2002 ait été une année différente des autres pour limiter l'étendue des conclusions. Les résultats obtenus fournissent néanmoins des indications générales très utiles pour la planification des périodes d'ensemencement.

Les myes de 25-30 mm qui sont à demi enfouies peuvent résister à des courants de l'ordre de 30 cm/sec tandis que celles déposées à plat sur le sédiment étaient emportées passivement par des courants de l'ordre de 12 cm/sec. Ceci dépend de la position de

la coquille face aux courants. Plus la surface offerte par la coquille est grande, plus la force du courant peut agir pour déloger la mye . Un enfouissement de 50 % de la coquille réduit donc de façon substantielle les problèmes de déplacement passif car la mye peut alors résister à des courants importants.

Conclusion

Cette étude a donc permis de mieux définir les conditions les plus propices pour faciliter l'enfouissement des myes lors des ensemencements. Certaines balises utiles ont pu être établies:

- La vitesse d'enfouissement diminue avec l'augmentation de la taille
- Des densités d'ensemencement jusqu'à 350 myes (25-30 mm)·m^{-2} n'ont pas d'impacts négatifs sur la vitesse d'enfouissement
- Il n'est pas nécessaire d'ameublir un substrat constitué de sable moyen pour améliorer la vitesse d'enfouissement
- Les myes peuvent subir une émersion de 4 heures sans effets négatifs sur l'enfouissement
- Une température de l'eau atteignant 20-23°C ne cause pas de ralentissement de l'enfouissement
- Une diminution substantielle de la condition des myes, associée ou non à la ponte, semble avoir un impact négatif sur la vitesse d'enfouissement
- Les ensemencements d'automne (à partir de septembre) ne sont pas recommandés en raison de la diminution marquée de la vitesse d'enfouissement
- Un enfouissement de 50 % de la coquille réduit substantiellement les possibilités de déplacements passifs causés par les courants

Remerciements

Merci à l'équipe technique du Programme MIM et l'équipe de la Station Technologique Maricole des Îles-de-la-Madeleine pour leur aide précieuse sur le terrain et en laboratoire. Merci à Pêche et Océans Canada, l'Institut des Sciences de la mer, Québec-Océan et le Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec pour leur support financier.

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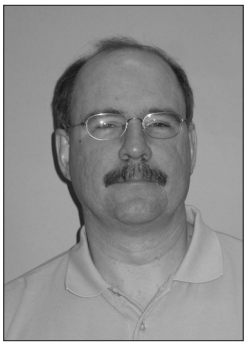
Mesures de la Dispersion de Myes Communes (*Mya arenaria*) Ensemencées dans le Barachois de La Malbaie, Québec



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En 2003 et 2004, des travaux ont été réalisés sur des myes communes dans le barachois de La Malbaie afin de mesurer l'étendue, l'orientation de leur déplacement et estimer leur survie ainsi que le taux de retour à la suite de leur ensemencement. Les 480 myes (35-40 mm de longueur), ont été mesurées, numérotées et marquées avec une petite tige métallique. Ces myes ont été séparées en 10 lots puis répartis sur 10 parcelles espacées de 20-30 m sur des substrats sablonneux à vaseux. Les parcelles avaient de 10 à 30 cm d'eau à marée basse et ont été nettoyées de tous débris métalliques avant de procéder aux ensemencements. Sur chaque parcelle, 48 myes ont été installées par groupe de six myes, à intervalle de 20 cm, sur chacun des huit axes d'un gabarit orientées selon les points cardinaux. Lors des suivis subséquents toutes les myes ont été localisées le long des axes du gabarit à l'aide d'un détecteur de métal. En octobre 2003, toutes les myes sur l'axe ouest de six des parcelles ont été récupérées, afin d'évaluer la rétention des marques métalliques et la mortalité des myes. En juillet 2004, toutes les myes restantes ont été retirées de cinq parcelles et cinq nouvelles parcelles avec 240 spécimens (25-30 mm) ont été ajoutées. Les premiers résultats indiquent que la distance moyenne parcourue par les myes est faible, autour de 10 cm, et cela peut importe l'orientation du déplacement. Les déplacements vers le nord-est, l'est et le sud-est, soit ceux correspondant à la direction générale du courant de marée descendante, sont toutefois sensiblement plus nombreux. Le taux de récupération a été de près de 90 % des myes ensemencées et le taux de survie de plus de 80 % après 3 mois et respectivement de 66 % et 70 % après 1 an. Le déplacement des plus petites myes des parcelles de 2004 était plus faible, près de 5 cm, et elles avaient pris une autre direction de celles de 2003. Les mesures de déplacement effectuées sur les myes retirées des cinq parcelles de 2003, indiquent une plus grande distance moyenne de 17 cm. Ces distances sont cependant faibles dans le contexte d'ensemencements commerciaux. Une grande majorité des myes ne démontrent aucun mouvement et certaines se perdre hors de la zone des suivis. Lorsque des mouvements sont mesurés, d'autres facteurs environnementaux et la direction du courant doivent avoir une grande influence et devront être considérés.

This soft-shell clam study was performed during 2003 and 2004 in the barachois of La Malbaie in order to measure the displacement, orientation of movement, and the survival and retrieval rates following seeding. Each of the 480 (35-40 mm size) soft-shell clams was measured, numbered, and tagged with a metal pin. These clams were separated into 10 groups individually placed on 10 parcels separated by 20-30 m and located on sandy to muddy substrates. Parcels had 10-30 cm of water at low tide and were cleaned of any metal debris before enhancement. On each parcel, 48 clams were installed by groups of six at 20-cm intervals on each of eight axes on a cardinal point template. In subsequent visits, a metal detector was used to find all clams. In October 2003, all clams on the western axis of six parcels were dug to evaluate metal tag retention and mortality. In July 2004, all clams were retrieved from five parcels and five new ones containing 240 (25-30 mm) clams were added. The first results indicate that the mean distance travelled by clams inside our parcels was around 10 cm, and didn't follow any particular orientation. More clams of the 2003 parcels moved towards the northeast, east, and southeast, following the general direction of the low tide current. Recuperation rate of seeded clams was close to 90% and the survival rate more than 80% after three months and 66% and 70%, respectively, after one year. Displacement of 2004 parcels smaller size class clams was lower, with nearly 5 cm and was oriented in a different direction than the 2003 parcels. Clams retrieved on the axis of 2003 parcels show a more important mean distance travelled of 17 cm. This may indicate that commercially seeded clams should have very limited movement out of their seeding site. The majority of clams did not show any movement. Where movement was calculated, it may have been influenced by other environmental conditions and direction of currents at the time of seeding.

Introduction

Différentes études biologiques[1,2] évaluant la biomasse, la répartition spatiale, et la distribution de taille des myes ont été réalisées dans le sud de la péninsule gaspésienne afin d'évaluer la productivité de certains bancs naturels, les quantités exploitables commercialement pour établir une activité économique durable basée sur cette ressource renouvelable. Les résultats de ces travaux ont été utilisés pour évaluer la faisabilité bioéconomique[3] de la myiculture en Gaspésie. La précision de cette

étude économique a toutefois été limitée par l'absence de données précises pour les secteurs à l'étude. La présente étude, qui vise à combler certaines de ces lacunes, a pour objectif principal d'évaluer le taux de récupération de myes ensemencées. Afin d'y parvenir, il est prévu évaluer la mortalité naturelle et d'estimer la distance et l'orientation des déplacements des myes de deux classes de taille sur le site de La Malbaie.

Matériaux et Méthodes

En 2003, 480 myes de 35-40 mm, ont été mesurées, numérotées avec un marqueur permanent et marquées avec une petite tige métallique collée sur la valve droite. Ces myes ont été séparées en 10 lots puis réparties sur 10 parcelles espacées de 20-30 m sur des substrats sablonneux à vaseux dans le barachois de La Malbaie. Les parcelles, qui avaient de 10 à 30 cm d'eau à marée basse, ont été nettoyées de tous débris métalliques avant de procéder aux ensemencements. Sur chaque parcelle, 48 myes ont été installées par groupe de six myes, à intervalle de 20 cm, sur chacun des huit axes d'un gabarit orienté selon les points cardinaux (nord, nord-est, est, sud-est, sud, sud-ouest, ouest et nord-ouest). Le suivi de ces parcelles se faisait après une semaine, un mois et en octobre en 2003; puis en juillet, août et octobre 2004. Lors des suivis les myes ont été localisées à l'aide d'un détecteur de métal et les déplacements mesurés le long et perpendiculairement aux axes du gabarit. Les distances parcourues étaient calculées par rapport aux positions initiales de chacune des myes sur les axes. Les distances moyennes et le nombre moyen de déplacements sont calculés à partir des myes ayant effectué un déplacement et ne tient pas compte des myes n'ayant pas bougées lors du suivi et de celles qui sont sorties des parcelles. En octobre 2003, toutes les myes sur l'axe ouest de six des parcelles ont été récupérées afin d'évaluer la rétention des marques métalliques, le taux de récupération et le taux de mortalité des myes. Toutes les myes restantes ont été retirées de cinq parcelles sur les dix parcelles initiales de juillet 2004 afin d'évaluer les mêmes facteurs sur un an. À la même période, cinq nouvelles parcelles avec 240 spécimens de 25-30 mm ont été installées selon la même procédure et les suivis réalisés avec la même fréquence des suivis qu'en 2003.

Résultats et Discussion

La composition des sédiments des 10 parcelles de 2003 n'était pas identique. Il est intéressant de noter que sur les trois parcelles composées d'un substrat de sable (8 à 18 déplacements/parcelle), il semble y avoir eu moins de déplacements par rapport aux myes localisées sur des parcelles situées sur des substrats comportant plus de vase, de sable-vase et de zostères (25 à 30 déplacements/parcelle). Les suivis réalisés en 2003 ont permis d'estimer la distance moyenne parcourue par les myes retrouvées sur les sites expérimentaux à 9,7 ± 5,0 cm. Il semble que ces déplacements soient orientés en direction vers le nord-est, l'est et le sud-est. Le nombre moyen de déplacement a été de 21,5 ± 12,6 déplacements/point cardinal. En ajoutant les suivis 2004, la distance moyenne reste comparable (11,1 ± 6,3 cm) et l'orientation générale de ces déplacements reste semblable (Fig. 1a). Près de 15 % des déplacements/point cardinal s'orientent vers le nord, le nord-est et le sud-est, contre près de 10 % dans les directions sud-ouest, ouest et nord-ouest.

En 2004, les lots de myes de la classe de taille de 25-30 mm ont parcouru une distance moyenne de 5,2 ± 3,6 cm. L'orientation générale de ces déplacements diffère sensiblement de l'orientation mesurée sur le lot ensemencé en 2003 (Fig. 1b). Les directions ouest, nord-ouest et nord comptaient chacune près de 18 % des déplacements totaux comparativement à 5 à 11 % vers le nord-est, l'est et le sud-est.

La récupération des myes le long d'un seul axe, parmi les huit possibles, à six des dix parcelles dès le premier automne, en octobre 2003, a permis de calculer un taux de récupération de près de 89 % et un taux de survie de 81 % après quatre mois. Ces taux ont été respectivement de 66 et 70 % après 12 mois d'ensemencement sur les parcelles expérimentales échantillonnées à la fin juin 2004.

La récolte complète de quelques sites a permis de vérifier le déplacement individuel réel des myes par rapport à leur lieu d'ensemencement initial. À la différence des suivis avec le détecteur de métal, où il y avait des incertitudes quant à l'identité de la mye localisée, la récolte complète des sites permettait de calculer la distance réelle parcourue par chacune des myes retrouvées. Les résultats finals ont démontré que les myes de 35-40 mm s'étaient déplacées d'environ 17 cm comparativement aux 11 cm estimés avec la méthode utilisant le détecteur de métal. Comme les suivis n'ont été faits qu'à l'intérieur du périmètre de chacune des parcelles, ceci a pour conséquence que la distance moyenne des déplacements est sous-estimée puisque cette moyenne ne tient pas compte des myes qui n'ont pas été retrouvées au cours des suivis.

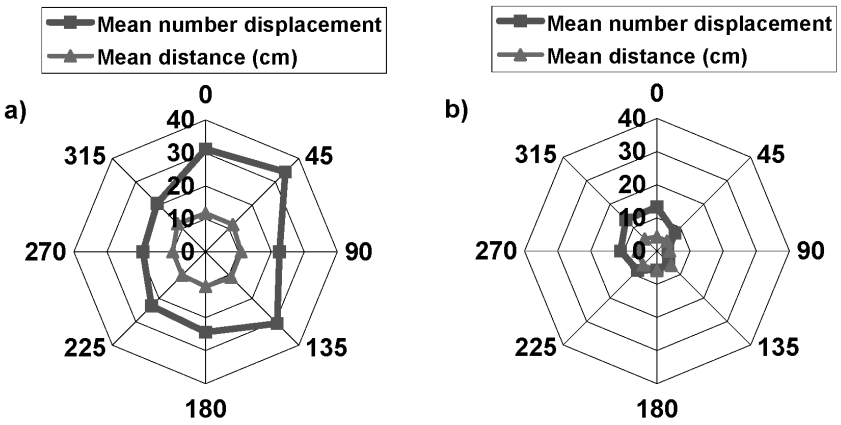

Figure 1.
Nombre moyen, distance moyenne et orientation des déplacements parcourus par les myes a) de 35-40 mm en 2003-2004 et b) de 25-30 mm en 2004.

Conclusion

Les myes détectées parcourent une faible distance si on ne tient pas compte dans le calcul des 10 à 34 % qui ne sont pas retrouvées après 4 et 12 mois d'expérimentation. L'orientation dominante des déplacements a varié en 2003 et 2004 et en conséquence d'une classe de taille à l'autre. L'orientation observée des déplacements est probablement le résultat des conditions environnementales entourant l'ensemencement, la marée, le vent et autres facteurs environnementaux ont probablement plus d'impact que la taille des myes ou l'année de l'expérience. Ces résultats devront être pris en compte lors d'ensemencements afin de minimiser les déplacements, les mortalités et maximiser les taux de survie et de récupération.

Remerciements

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Open-sea Culture of Mussels (*Mytilus edulis*) in Îles-de-la-Madeleine: A Promising Avenue

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Mussel culture in Îles-de-la-Madeleine is concentrated in lagoons, but the available space is limited. Future development depends on farming in open-sea areas where the conditions are different from those in lagoons – barely sheltered sites situated far from the coasts and subjected to drifting ice. A project has been ongoing since 2002 to determine the parameters of production in this new environment. The site is 20 minutes by boat from the nearest harbour, at a depth of 19 meters. Usually free of ice, it is accessible by boat nearly year-round, allowing a winter harvest. The longlines with their buried anchors have remained stable since their installation. The growth of the mussels in the sea is comparable to those in the lagoon. The use of spat from Bassin in Havre-Aubert helps the mussels reach commercial size only one year after sleeving. Spat can also be collected onsite throughout the entire water column. The yield in cooked meat remains very high (~50%) during a good part of the summer. This site thereby provides mussels of superior quality at a time when lagoon mussels have spawned and thus have a low meat yield. The results are promising.

La production mytilicole des Îles-de-la-Madeleine est concentrée dans les lagunes mais l'espace disponible est limité. Le développement futur devra passer par l'élevage en milieu ouvert, où les conditions sont bien différentes des lagunes: sites peu abrités situés à distance des côtes et soumis aux glaces dérivantes. Un projet est en cours depuis 2002 pour déterminer les paramètres de production de ce nouvel environnement. Le site est à 20 minutes de bateau du port le plus proche, à une profondeur de 19 m. Habituellement libre de glace, il est accessible par bateau presqu'en tout temps permettant ainsi la récolte hivernale. Les lignes avec leurs ancrages enfouis sont demeurées stables depuis leur installation. La croissance des moules en mer est comparable à celle en lagune. L'utilisation du naissain du Bassin de Havre-Aubert permet d'obtenir des moules commerciales un an seulement après leur mise en boudin. On peut aussi s'approvisionner en naissain directement sur le site, sur toute la colonne d'eau. Le rendement en chair cuite demeure très élevé (~ 50%) durant une bonne partie de l'été. Ce site fournit donc des moules de qualité supérieure au moment où les moules en lagune ont pondu et sont presque vides. Les résultats sont prometteurs.

Introduction

Mussel culture in Îles-de-la-Madeleine has been going on in lagoons for close to 20 years. These sheltered and favourable areas for mussel culture are restricted however, and there is no available space for new leases. Certain indicators lead us to believe that an interesting potential lies off the coasts in open-sea areas. First, this avenue could fulfill or resolve in part the problem of access to the commercial mussels on a regular basis with which the industry is faced. The important losses (fall-offs) associated with the summer lagoon harvest could possibly be reduced. Moreover, the meat yield observed in the sea during the summer could eventually be higher to that observed in lagoons, due to the different environmental conditions. It was with the goal of examining these premises that the Station technologique maricole undertook this exploratory project in 2002. Since then, other partners have joined the project: Société de développement de l'industrie maricole du Québec (SODIM) and Canada Economic Development.

Methodology

Choice of site

Several elements came into play when choosing the site. First, it was desirable to avoid any user conflicts. In this way, the chosen site does not conflict with commercial fishing and is considered an adequate distance from any maritime navigation (Fig. 1).

The site, which covers an area of 25 hectares (500 m × 500 m) at a depth of 19 meters, is deep enough to install longlines at a sufficient depth to avoid the drifting ice. The site is situated at the limit of the Baie de Plaisance, therefore not far from a fishing harbour.

Deployment of longlines

In June 2002, three submerged longlines were installed, anchored with buried Japanese anchors. The longlines are 100-m long and are installed in parallel at 75 m from one another.

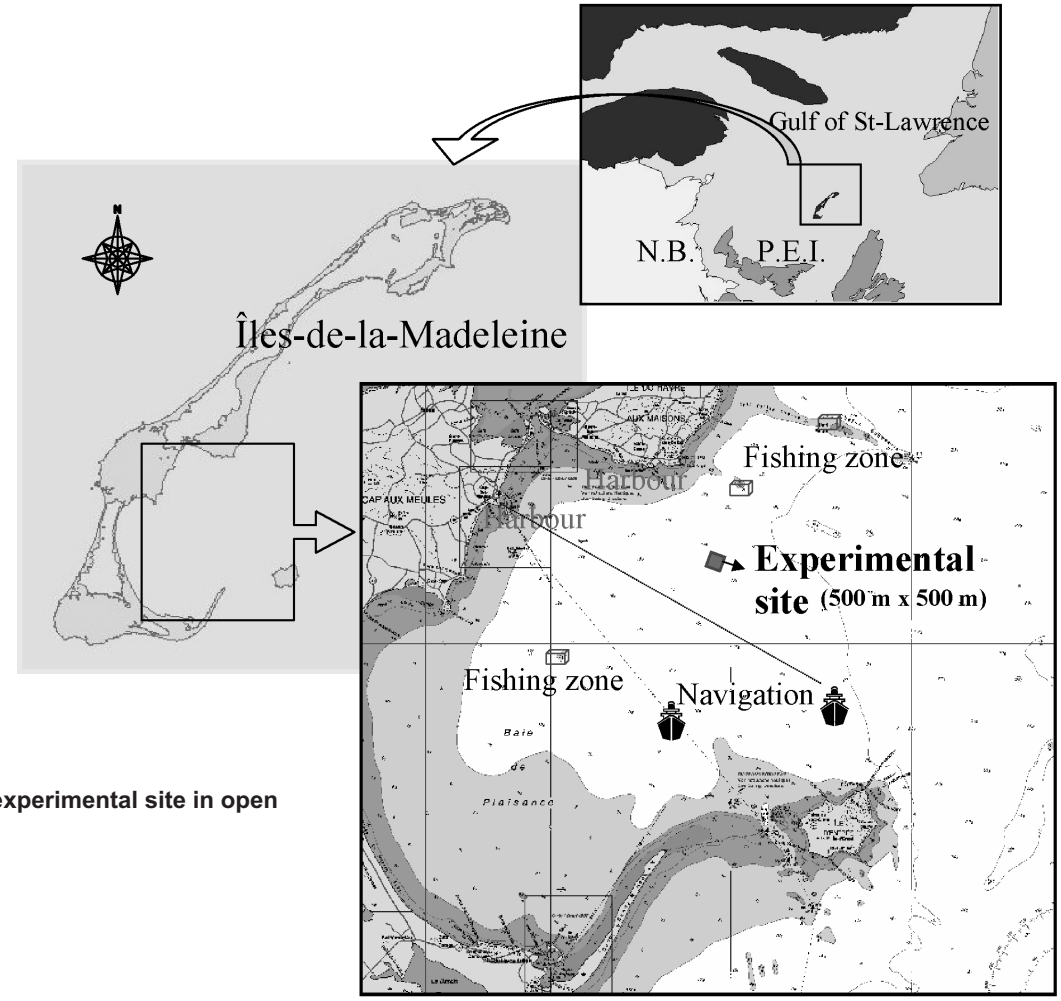

Figure 1.
Localisation of experimental site in open sea.

Environmental assessment

Prior to the deployment of the experimental longlines, Tita et al.[(1)] carried out an environmental assessment of the study site. Sediment characteristics and benthic communities were sampled between May and July 2002. Water currents were also monitored from June through October 2002.

Evaluation of the performance of different stocks

In order to evaluate the possibility to use one or the other of the different stocks of mussel spat available in Îles-de-la-Madeleine, growth from spring until autumn 2003 was compared. Four stocks were followed: those from the Bassin in Havre-Aubert, the lagoon in Havre-aux-Maisons, the lagoon in Grande-Entrée, and the Baie de Plaisance. Mussel spat from the four groups which had been collected in 2002 were placed in Vexar® cages (five cages per stock) in May 2003. Measurements (to the closest mm) were taken in May and then in November.

Spat collection

In 2002, the potential for spat collection and whether second set could be a problem on commercial sleeves throughout the water column were evaluated. In June, 15-m long polyrope collectors were installed on a longline left at 3 m from the surface for three months. Additional collectors were set up on an entire line in June 2003 and taken out a year later to get an idea of the potential of a "commercial" spat collection.

Growth and production

In anticipation of obtaining the lease, and in order to quickly get first growth results, mussel spat taken from the Bassin of Havre-Aubert was sleeved in November 2001. These sleeves were overwintered on a longline in the lagoon of Havre-aux-Maisons. In June 2002, the sleeves were transferred to the open-sea site and followed until summer 2003.

To evaluate a complete production cycle in open sea, mussels were sleeved in October 2002 and installed on the site and in the Havre-aux-Maisons lagoon in order to draw comparisons. The experiment was repeated in November 2003.

Evolution of the meat yield

The evolution of the summer meat yield of 1^+ mussels was observed in 2003 and 2004. In the beginning of May, Vexar® cages containing medium-sized mussels (~60 mm) were placed in the open sea and in the lagoon. Three cages were retrieved bimonthly in 2003 and weekly in 2004 from each of which 10 mus-

sels were used to evaluate the cooked meat yield (n = 30). The cooked meat yield was assessed as: (weight of meat / (weight of meat + weight of shell)) ×100.

Results

Site access

The site is easily and rapidly accessible. At a speed of 10 knots, it is about 30 minutes from the harbour of Cap-aux-Meules and 20 minutes from the harbour of Pointe-Basse. In the winters of 2002-2003 and 2003-2004, the presence of ice was limited in the Baie de Plaisance and that would have allowed to access the site at almost any time.

Behaviour of longlines

In the autumn of 2004, the three longlines were still in place. The anchorage resisted well and the chosen depth for the longlines – about 10 m from the surface – allowed for avoiding the drifting ice.

Environmental assessment

The fine, well-sorted sediment (median = 96 ± 4.5 µm) had 0.38% of organic carbon and 0.17% total nitrogen. Polychaetes were the dominant macrofaunal group (84%), while nematodes dominated meiofauna (87%). As commonly observed in this type of sediment habitat, polychaetes displayed low species diversity (n = 13; Shannon-Wiener index = 1.17 ± 0.30; equitability = 0.43 ± 0.08), compared to nematodes (n = 62; Shannon-Wiener index = 4.33 ± 0.38; equitability = 0.88 ± 0.04)

Water currents had an average velocity of 6.9 ± 3.72 cm/s and predominantly flowed in an East-North-East direction. Tita et al.[1] suggested that such a current system might mitigate the sediment organic enrichment associated to farming activities. Dispersion of organic matter conveyed by mussels in the form of feces and pseudofeces may indeed result from local water currents. Reduced local effects on benthic communities may then be expected.

Performance of stocks

All stocks showed similar growth. Starting at a mean length of 22-26 mm, the mussels reached a mean size of 46-51 mm six months later. There does not seem to be an important stock effect on this experimental site in open sea.

Spat collection

The maximum density of spat (~15 000 mussels/m) collected in September 2002 was observed between 13 and 15 meters from the surface . At the depth targeted for mussel culture (10 m from the surface), the collection was still relatively important with ~ 5000 ind./m. That could possibly lead to problems with second set on sleeves in the future.

Spat collection occurred later in open sea than in a lagoon and consequently, the mussel spat reached a smaller size in the autumn. As a result, the 2003 spat was sleeved in early summer 2004. Several cohorts of mussels were observed on the collectors as well as some associate species which were present in great abundance: hydroids and *Caprella*. Moreover, a heavy presence of small starfishes (*Asterias vulgaris*) was noticed on the collectors.

Growth and production

The young mussels sleeved in November 2001 and transferred in June 2002 responded well to the culture conditions in open sea. Their modal size reached 60 mm less than a year later (May 2003) whether in open sea or in the lagoon (control group). Consequently the sleeves in open sea provided up to 11 kg/m of commercial mussels (>50 mm).

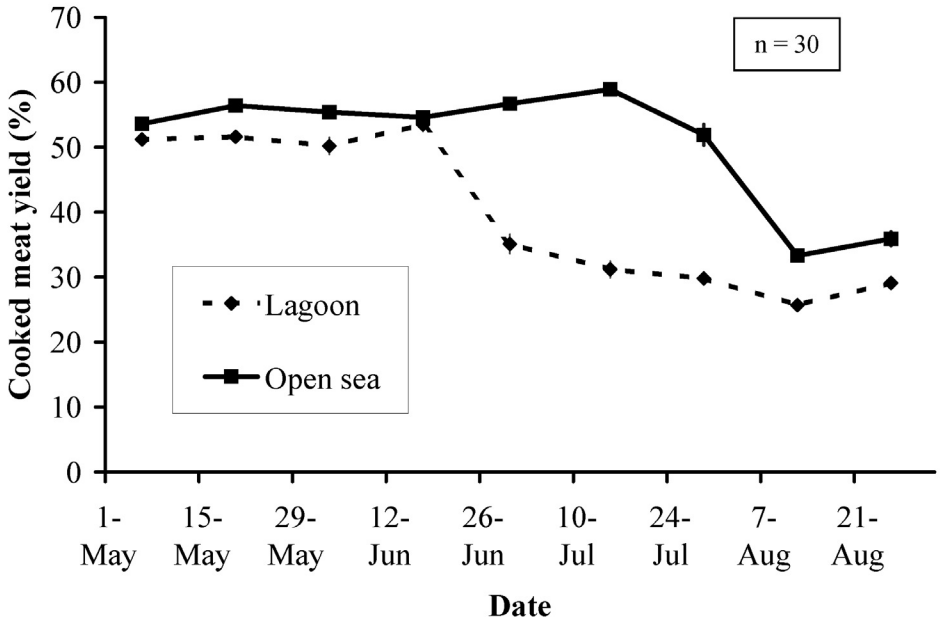

Figure 2. Evolution of commercial mussel meat yield (mean ± SE) in 2003.

The sleeves placed in open sea in October 2002 experienced heavy losses due to fall-offs in September 2003 when flotation on the longlines was adjusted. At this time a heavy second set of young mussels had added weight to the sleeves. However, the remaining 1^+ mussels had reached commercial size in November 2003, one year after sleeving.

Another sleeving operation was undertaken in November 2003. The modal size of the sleeved mussels was 23 mm and they reached a modal size of 53 mm in less than a year (end of September 2004).

Evolution of the meat yield

In 2003, the spawning period differed greatly at the two sites (open sea vs lagoon). From the early May until mid-June, the cooked meat yield remained >50% in both the lagoon (Havre-aux-Maisons) and the open-sea site. On June 30, a sharp drop (from 53% to 35%) was observed in the lagoon, thus indicating a massive spawning. (Fig. 2). As for the yield in the open-sea, it remained high for 6 additional weeks, the drop having been noted August 12.

In 2004, the meat yield pattern differed from the previous year as no synchronized massive spawning was observed at neither sites. Nevertheless, the mussels in open sea always had higher meat yield values than those kept in the lagoon. The open-sea mussels went progressively from a cooked meat yield of 59% in May to 35% at the end of August. During the same period, the mussels in the lagoon went from 46% to 25%.

It should be noted that, though of equal size, the weight of the mussels' shell in 2004 was always much higher in the lagoon than in open sea. From the beginning of May to the end of August, the mean weight of shells (± SE) in the lagoon went from 7.5±0.1 g to 10.0±0.2 g. compared to 4.7±0.2 g to 6.3±0.2 g in open sea.

Discussion and Conclusion

The first findings regarding the accessibility to the site are positive. No conflict of use has been noted since setting up the longlines in the sea. Technologically speaking, the methods used have so far responded well to our expectations.

Abundant spat collection seems to be possible at the open-sea site. Doing so will require appropriate management which will take into account competitors and predators on the collectors. Also, sleeving this spat will have to wait the following spring to get mussels of required size. However, spat from Havre-Aubert have repeatedly reached commercial size in only about one year after sleeving at this site.

Second set could be abundant even if longlines are submerged as low as 10 m below the surface. In 2002, the setting of the sleeves took place on October 10 when mussel larvae were still present in the water column. That probably explains the intense second set observed on the sleeves during the following year and the subsequent fall-offs. Losses due to fall-offs were less important in 2004 on mussels sleeved in November 2003. There were probably very few or no larvae at this time, limiting the problem. The problem of fall-offs caused by excess weight due to the second set will certainly have to be taken into consideration.

The high meat yield of mussels in culture in open sea is very advantageous when targeting summer and fresh markets. The consumers would certainly come out a winner. Thus, the high summer meat yields observed in 2003 and 2004 provide great marketing potential for mussels cultured in open sea in Îles-de-la-Madeleine. However, the low weight of the shells could lead to a higher fragility and thus could possibly undermine the mussel processing because of higher proportions of broken shells. That should be examined closely.

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The Size Refuge against Rock Crab (*Cancer irroratus*) Predation Is Not Totally Reached by Commercial-Sized Mussels (*Mytilus edulis*)

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Do the rock crab, *Cancer irroratus*, prey on cultured mussels of commercial size? Despite their large size (≥50 mm), the shell of the cultured mussels is thinner (lighter) and more flattened than wild mussels of similar length. Thus, the cultured mussels are possibly vulnerable to crabs, particularly the larger ones found on the sleeves. This question is of great importance for mussel growers who sink longlines to control the abundance of second set (mussels <25 mm) on commercial sleeves. Mussels from different size-classes (10-15, 20-25, 40-45, 50-55, and 60-65 mm) were provided to two groups of rock crabs: medium (70-89 mm) and large (100-119 mm). With no choice of prey, both groups of crabs could eat mussels ≥50 mm but only large crabs (≥100 mm) could eat the largest mussels (60-65 mm). No crabs attacked mussels ≥60 mm when they had choice of prey, and only the largest crabs were successful with 50- to 55-mm mussels. Both groups of crabs selected second-set mussels (≤25 mm) rather than commercial-sized (≥50 mm) mussels. This is good news for mussel growers who sink longlines to control second set abundance on commercial sleeves. However, one should be sure that (1) rock crabs are the main benthic predators around and (2) large crabs do not predominate.

Le crabe commun, *Cancer irroratus*, fait-il des moules cultivées de taille commerciale sa proie? Malgré sa grande taille (~50 mm), la coquille des moules cultivées est plus mince (plus légère) et plus aplatie que celle des moules sauvages de même taille. Les moules cultivées sont donc peut-être vulnérables à la prédation exercée par le crabe commun, en particulier les gros individus présents sur les boudins. La réponse à cette question importe beaucoup aux mytiliculteurs qui calent des filières pour limiter l'abondance du naissain de moule de fixation secondaire (< 25 mm) sur les boudins de culture commerciale. Des moules de différentes classes de taille (10-15, 20-25, 40-45, 50-55 et 60-65 mm) ont été offertes à deux groupes de crabe commun : moyens (70-89 mm) et gros (100-119 mm). Sans choix de proies, les deux groupes ont mangé des moules > 50 mm, mais seuls les gros crabes (> 100 mm) ont mangé de grosses moules (60-65 mm). Aucun crabe n'a attaqué des moules > 60 mm lorsqu'ils avaient un choix de proies, et seuls les crabes les plus gros se sont attaqués aux moules de 50-55 mm. Les deux groupes de crabes ont préféré les moules de fixation secondaire (> 25 mm) aux moules de taille commerciale (> 50 mm). C'est là une bonne nouvelle pour les mytiliculteurs qui calent des filières pour limiter l'abondance du naissain de deuxième fixation sur les boudins. Ils doivent toutefois s'assurer que 1) le crabe commun est le principal prédateur benthique au site de culture et 2) les gros crabes ne prédominent pas.

Introduction

The second set is made of all the young mussels that had settled on and among the larger mussels in the sleeves. It usually occurs during the summers following sleeving when young post-larvae settle on all available and adequate substrata. When abundant, second set may cause major impacts on commercial production by adding weight on the lines, creating intra-specific competition for food and space, and increasing fall-offs of commercial mussels[(1)]. In PEI, some mussel growers sink longlines to control the abundance of the second set on their commercial sleeves. However, little information is available for its possible use in the Iles-de-la-Madeleine. For example, there is a large variation in the sinking time of the longlines among growers. This experiment was performed as part of a larger study to investigate the potential of sinking longlines in Iles-de-la-Madeleine[(2)].

The concept of sinking longlines is fairly simple in theory. The longlines are sunk voluntarily for a certain period of time in fall so that the lower part of the commercial sleeves lies on the bottom. As a consequence, the benthic predators – mainly rock crabs – are attracted by this abundant food and climb onto the sleeves to feed. Crabs would attack the second set (mussels <25 mm) and ignore the larger mussels including the commercial-sized mussels (≥50 mm) based on the assumption that they have reached a size refuge against predation. Thus, this procedure is based on the selective feeding of the predators and/or the size refuge possibly attained by the commercial-sized mussels. But what is really happening on the sleeves once they have been sunk? Are the commercial-sized mussels at risk? Are we sure the crabs will ignore the large commercial-sized mussels when encountering them? This should be validated while evaluating the real potential of sinking longlines.

No adequate information was available. Almost no studies report on the predation of cultured mussels[3] and only few on the predation of mussels by rock crabs. Most studies use relatively small crabs (<80 mm carapace width) from different species – blue crabs (*Callinectes sapidus*) and green crabs (*Carcinus maenas*) – fed with relatively small (<40 mm) wild mussels. Further, the mussels are usually offered as individuals and not in clumps as on the sleeves. The experimental conditions in these studies were quite different from those of interest in the present study. Further, suspension-cultured mussels have a thinner (lighter) and more flattened shell than wild mussels of similar size; two characteristics easing crab predation which is largely based on shell crushing[4]. Therefore, one may hypothesize that a crab could attack larger cultured mussels than wild mussels due to these morphological differences of the protective shell. Data obtained from wild mussels are thus difficult to use for cultured mussels. Further, large crabs (>100 mm carapace width) are often found on the sleeves. Given the correlation between crab size and prey size[5], it is possible that rock crabs may prey on large mussels.

Considering the lack of adequate information, a series of experiments was designed to answer two questions: (1) do the commercial-sized mussels have reached a size refuge against rock crab predation and (2) if not, do the crabs select the smaller mussels when given a choice (as on the commercial sleeves) ?

Materials and Methods

The experiments were performed in October-November 2003 in a flow-through tank whose water temperature was maintained at 13-15°C. The tank contained 18 cages (33 cm L × 28 cm W × 13 cm H) distributed in 6 rows of 3 cages with one crab in each. The rows of cages were permutated each day to minimize any possible tank effect.

Crabs from two size classes were used: medium (70-89 mm carapace width) and large (100-119 mm carapace width). Crabs belonging to these size-classes are found frequently on the sleeves. The crabs from the two classes were placed in alternate positions within the tank. Before the experiments, crabs were fed with mussels to be sure they have experienced feeding on this prey. Then they were starved for 3 days just prior to the beginning of each series of experiment to standardize their hunger level.

Mussels from five size classes were used: 10-15, 20-25, 40-45, 50-55, and 60-65 mm. The first two size classes are considered as second set while the last two classes are commercial-sized mussels. The intermediate size class (40-45 mm) was used as a transition between the second set and the commercial-sized mussels. However, few mussels from this intermediate size-class are found on 1-year-old commercial sleeves in fall.

Different quantities of prey were provided to the crabs according to mussel size with higher numbers of smaller mussels: 20× 10-15 mm, 15× 20-25 mm, 7× 40-45mm, 5× 50-55 mm, and 3× 60-65 mm. Crabs should not be limited by the available food but rather must have the opportunity to feed ad libitum. Further, crabs are mostly tactile and visual predators[6], so encounter probability is related to prey size. Each mussel was offered only once to the crabs.

Short-term experiments

The only way to study selection is to compare what the predator can potentially eat (no choice) to what it actually eats (with choice)[7]. Thus, a series of short-term (15-h) experiments was performed to characterize the predation potential of the crabs as well as their food selection. During the first part of the experiment, crabs had no choice. Each day a single size-class was randomly provided to the crabs from 17h00 to 8h00 the following day. On the sixth day, crabs were given choice when mussels from all the 5 size-classes were provided together in the same proportions as previously done, for a total of 50 individuals. On the seventh day, the 50 mussels from all size classes were pro-

Table 1. Proportions in numbers and volume of mussels from different size-classes provided in the diet and eaten during short-term (15 h) experiments.

Mussels	Presentation	70-89-mm crabs (%)	100-119-mm crabs (%)
	Numbers		
10-25 mm	50 individual mussels	97.8	90.7
(70% number)	Clumps of 50 mussels	100	86.5
40-45 mm	50 individual mussels	2.2	4.6
(14% number)	Clumps of 50 mussels	0	10.8
50-65 mm	50 individual mussels	0	4.7
(16% number)	Clumps of 50 mussels	0	2.7
	Volume		
10-25 mm	50 individual mussels	81.2	48.8
(12% volume)	Clumps of 50 mussels	100	32.1
40-45 mm	50 individual mussels	18.8	18.5
(26% volume)	Clumps of 50 mussels	0	47.0
50-65 mm	50 individual mussels	0	32.7
(62% volume)	Clumps of 50 mussels	0	20.9

vided but in clumps to add obstacles to selection through constraints in searching and handling of preys.

Long-term experiments

A series of long-term experiments was also performed to characterize the possible shift in selection when preferred prey decline or disappear. This situation is more similar to what occurs on sleeves when crabs likely select their preferred prey first and then their second choice.

Fifty mussels from the 5 size classes were provided together to the crabs for 11 days. Each morning, eaten mussels were counted and mussels which had attached together to form groups were separated to keep the experimental mussels as individual prey. Then, clumps of 50 mussels from the 5 size classes were provided to the crabs for 11 additional days. Eaten mussels were not counted on a daily basis to avoid any change of the clumps structure. Three medium-sized crabs moulted during this experiment and were discarded from data analyses.

Results

Short-term experiments

As expected, crabs faced no shortage of mussels from any size class during the short-term experiments. In absence of choice, both medium (70-89 mm) and large (100-119 mm) crabs preyed on commercial-sized mussels ≥50 mm but only the large crabs were successful with mussels ≥60 mm. There were no significant differences in mussel consumption for both groups of crabs when they had no choice; i.e. for any given mussel size class, medium- and large-sized crabs ate the same quantities of prey.

When given a choice, only the large crabs selected mussels ≥50 mm provided either individually or in clumps. However they did not select 60-65 mm mussels. Medium crabs preyed only on ≤45-mm mussels when provided individually and only on ≤25-mm mussels (second set) when offered in clumps.

Selection can clearly be seen when the proportion of eaten mussels from each size class is compared to their initial proportion in the provided diet. Mussels from second set (10-15 and 20-25 mm) which represented 70% (35/50) of the total number of mussels provided to the crabs were eaten in larger proportions (>85%) by both groups of crabs (Table 1). Medium crabs selected no commercial-sized mussels (50-55 and 60-65 mm) while <5% of the prey eaten by the large crabs belonged to this category. Yet, mussels ≥50 mm represented 16% (8/50) of the total number of individuals in the diet.

The number of eaten mussels is possibly not the best way to examine selection as it neglects the huge variation of size for the experimental mussels. For example, the volume of a 65-mm mussel is about 170 times larger than a 10-mm mussel. This difference in size certainly influences the probability of prey detection and handling. Further, satiation is probably greater after the consumption of a large mussel and that may reduce searching for other prey. Selection may be examined through the comparison of the total volume of the provided and eaten prey since this parameter integrates their size and numbers. Selection of small mussels is still clear even though the prey are characterized by their volume rather than their numbers only (Table 1).

Long-term experiments

When a variety of mussels were provided over a longer period of time (11 days), both groups of crabs had the same feeding pattern; i.e. both consumed the same relative proportion of mussels of the different size classes. That was true for either individualized and clumped mussels. Further, there were no significant differences in the feeding patterns of the crabs when mussels

Mussels	Presentation	70-89-mm crabs (%)	100-119-mm crabs (%)
	Numbers		
10-25 mm	50 individual mussels	87.4	87.8
(70% number)	Clumps of 50 mussels	89.8	80.5
40-45 mm	50 individual mussels	11.4	9.9
(14% number)	Clumps of 50 mussels	8.3	15.3
50-65 mm	50 individual mussels	1.2	2.3
(16% number)	Clumps of 50 mussels	1.9	4.2
	Volume		
10-25 mm	50 individual mussels	41.0	41.5
(12% volume)	Clumps of 50 mussels	45.5	25.9
40-45 mm	50 individual mussels	49.7	42.4
(26% volume)	Clumps of 50 mussels	39.3	46.0
50-65 mm	50 individual mussels	9.3	16.1
(62% volume)	Clumps of 50 mussels	15.2	28.1

Table 2. Proportions in numbers and volume of mussels from different size-classes provided in the diet and eaten during long-term (11 d) experiments.

were provided individually or in clumps. Therefore, mussel clumping did not constrain prey selection.

In contrast to the short-term experiment, both groups of crabs preyed on commercial-sized mussels (>50 mm) over the 11-day experiments. However, only the large crabs preyed successfully on the 60-65 mm mussels. Thus, medium and large crabs can prey on commercial-sized mussels even though some small mussels are still available. They just have to be in contact with commercial-sized mussels for a while.

As for the short-term (15-h) experiment, both groups of crabs preferred the smallest prey over a 11-day period (Table 2). That was true for the number as well as the volume of eaten prey relative to their proportion in the provided diet. There was an important shift towards the intermediate-sized (40-45 mm) mussels by the medium crabs compared to the short-term experiment (Table 1). For both groups of crabs, this size class was chosen roughly according to its initial proportion in numbers (8.3-15.3% vs. 16% provided) but in larger proportion in terms of prey volume (39.3-40.7% vs. 26% provided). However, both groups of crabs preyed on second set in smaller proportion and on commercial-sized mussels in larger proportion than in the short-term experiment.

Discussion

Size refuge against rock crab predation is not totally reached by commercial-sized (≥50-mm) mussels. Crabs ≥70 mm can prey on ≥50-mm mussels but only the large (100- to 119-mm) crabs can successfully prey on mussels ≥60 mm. However, crabs prey selectively on the second set mussels (<25 mm) when they have a choice. This situation is comparable to what is observed on the sleeves. With time (11 d) medium crabs will also prey on intermediate-sized (40- to 45-mm) mussels in larger proportion than during the short-term experiment. Although they primarily select the smallest mussels (10-25 mm), the medium crabs will prey on the 40- to 45-mm mussels secondarily.

Crabs could be used efficiently to clean the commercial sleeves from heavy second set in Iles-de-la-Madeleine by sinking lines . This operation would be done during the fall following sleeving in Iles-de-la-Madeleine. At this period, the modal length of the commercial mussels is ~60 mm. At this size, they can be preyed on only by the largest crabs (>100 mm). That should greatly minimize the losses of commercial mussels resulting from the intentional face to face with their predators. However, one should be sure that (1) rock crabs are the main benthic predators around and (2) large crabs do not prevail.

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La Dépuration en Vrac et l'Entreposage Humide de la Moule Bleue (*Mytilus* spp.) en Gaspésie, QC, Canada – de la Démonstration Expérimentale à l'Implantation Commerciale, 2002-2003

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La mytiliculture en Gaspésie rencontre plusieurs défis, dont le développement de centre(s) de traitement et d'un procédé de purification des moules. Un projet a donc été entrepris afin de 1) établir la faisabilité technico-économique de la dépuration en vrac et de l'entreposage humide des moules et 2) implanter un centre de traitement intégré. Le système de dépuration en vrac développé en Grande-Bretagne a été testé. Les résultats démontrent que les moules reprennent rapidement leur activité physiologique normale après leur mise en vrac dans le système. Les résultats obtenus en circuit ouvert selon deux entassements (225 kg et 365 kg) de moules et deux débits (40 et 90 L/min) respectent les exigences du PCCSM concernant la qualité de l'eau, et un débit de 10,6 $L \cdot h^{-1} \cdot kg^{-1}$ de moules permet le maintien des niveaux en oxygène pour des températures variant entre 3°C et 15°C. Lors d'un projet pilote, les coûts du procédé de dépuration en vrac et d'entreposage humide ont été estimés à environ 0,69 $ / kg net de moule, soulignant l'importance d'intégrer ce procédé dans un contexte de traitement post-récolte, pour permettre sa faisabilité économique. L'implantation commerciale d'un centre de traitement est en cours depuis l'automne 2003.

In Gaspé peninsula, the mussel culture industry is facing several challenges. Among others, there is the implementation of a brand new processing plant including a mussel depuration system. Thus, a multistage project has been undertaken which aims (1) to assess the technical and economical feasibility of a depuration system linked to a standard live holding system and (2) to set a processing facility. A standardized bulk-bin depuration flow-through system, based on United Kingdom technologies, has been tested. It has been demonstrated that mussels resume their normal physiological activity a few hours following their transfer in deep layers. Results obtained with 2 mussel crowding (225 and 365 kg) at 2 flow rates (40 and 90 $L \cdot min^{-1}$) met Canadian Shellfish Sanitation Program requirements, the most important being to maintain 50% oxygen concentration at the tank outlet for a temperature ranging from 3 to 15°C. During the pilot scale project, it has been estimated that depuration and live holding costs should reach a high of $\$0.69 \cdot kg^{-1}$. This result underlined the importance to view the depuration as a necessity into the marketing process of Gaspé mussels. A commercial processing plant has been established in Rivière-au-Renard since 2003.

Introduction

Afin d'assurer la commercialisation efficace de la moule en Gaspésie (Québec, Canada), le besoin d'implanter un centre intégré de transformation permettant de regrouper l'offre, de faciliter l'entreposage et le traitement, s'est rapidement fait sentir. La problématique particulière de la production de la moule dans la baie de Gaspé entraîne quant à elle la nécessité de mettre en place une unité de purification de la moule, et d'en déterminer la faisabilité technico-économique. Une équipe formée par la Direction de l'innovation et des technologies (DIT) et la Société de développement de l'industrie maricole (SODIM) s'est donc penchée sur cette problématique, en adoptant une approche menant d'une démonstration expérimentale à une implantation commerciale, en utilisant le procédé de dépuration des moules développé au Royaume-Uni, le « Bulk bin system for mussels[(1)] ». L'objectif général de la phase de démonstration expérimentale était de démontrer la faisabilité technique du procédé de dépuration en vrac de la moule bleue, en regard des exigences du Programme canadien de contrôle de la salubrité des mollusques (PCCSM)[(2)]. Il ne s'agissait pas d'une démonstration de la dépuration au sens strict, étant donné la faible contamination de départ des moules de la baie de Gaspé[(3)]. Les objectifs spécifiques étaient de déterminer si les moules, soumises à un entassement important, possèdent la capacité de s'ouvrir et de montrer une activité de filtration « normale », et de déterminer les débits d'eau minimaux à fournir dans les bacs pour répondre aux seuils critiques de qualité des eaux de traitement exigés par le PCCSM[(2)], en fonction de l'entassement des moules. La phase de projet pilote avait comme objectif général d'établir les bases

économiques d'un procédé de dépuration et d'entreposage humide de la moule. Plus spécifiquement, il s'agissait de valider à une échelle commerciale la faisabilité technico-économique du procédé de dépuration et d'entreposage humide. Quant à la phase d'implantation commerciale, son objectif était d'implanter et d'opérer un centre intégré de traitement de la moule, incluant des capacités de dépuration et d'entreposage humide.

Matériels et Méthodes

Démonstration expérimentale

Afin de déterminer l'impact de l'entassement sur leur activité, une évaluation de l'activité de filtration des moules à partir d'indicateurs visuels tel que la sortie des cils et des siphons[4, 5], la sortie du pied et la formation de byssus fut réalisée. Des moules furent entassées sur 60 cm d'épaisseur dans trois colonnes d'observation en acrylique[6] . De l'eau de mer à 13-16°C fut ajoutée aux colonnes à raison d'un débit de 16,7 L/min. Des observations des moules furent effectuées à trois hauteurs à toutes les six heures pendant 48 heures, de façon à mimer un cycle de dépuration. Afin de déterminer les débits d'eau minimaux à fournir dans les bacs de dépuration pour répondre aux seuils critiques de qualité des eaux de traitement exigés par le PCCSM[2] en fonction de l'entassement des moules, des cycles de dépuration ont été effectués dans l'unité de dépuration expérimentale érigée au Centre aquacole marin de Grande-Rivière[6], entre juin et novembre 2002. Des lots de moules provenant de la baie de Gaspé furent soumis à des cycles de dépuration en fonction de deux entassements et deux débits, soit 35-40 cm de hauteur (225 kg de moules) pour un débit de 40 L/min et 55-60 cm de hauteur (365 kg de moules) pour un débit de 90 L/min. Trois cycles par traitement furent effectués, en circuit ouvert, avec trois réplicats. Un suivi des paramètres de la qualité de l'eau prescrit par le PCCSM[2] fut effectué à toutes les six heures pendant les cycles de dépuration. Un suivi de la teneur en coliformes fécaux rencontrés dans les moules à dépurer a aussi été effectué, selon la méthode NPP/BLT, confirmation EC. À cette fin, des échantillons ont été prélevés à T_0, T_{24}, et T_{48}.

Projet pilote

Le projet pilote s'est déroulé du début novembre à la mi décembre 2002. Pour répondre aux objectifs de ce volet, une unité commerciale de dépuration et d'entreposage humide des moules a été installée dans les locaux de l'entreprise « Les pêcheries Marinard Ltée. ». L'unité de dépuration était dotée d'une capacité de 18 bacs isothermes disposés en neuf colonnes, alors que l'unité d'entreposage humide possédait une capacité de 48 bacs disposés en 12 colonnes. Chaque bac était équipé de sa propre arrivée d'eau salée. Les moules étaient récoltées dans la baie de Gaspé par les mytiliculteurs selon les méthodes habituelles. Les moules étaient généralement disposées dans les bacs de dépuration à raison de 225-275 kg/bac. Le débit d'eau salée a été fixé à 50 L/min. Les paramètres de la qualité de l'eau de traitement était suivis conformément au PCCSM[2] durant les cycles de dépuration. Un suivi de la teneur en coliformes fécaux rencontrés dans les moules a été effectué, tel que décrit au paragraphe précédent. Après la dépuration, les moules étaient transférées en entreposage humide, selon les mêmes quantités/bac, à un débit de 10-15 L/min. Une analyse des points critiques, une évaluation des coûts et une analyse de temps et mouvements ont été effectuées.

Implantation commerciale

L'implantation et les opérations commerciales du procédé de dépuration et d'entreposage humide dans un centre intégré de traitement de la moule se sont effectuées du 16 octobre à la mi décembre 2003, à des températures de l'eau de mer variant de 8°C à 1°C. Une unité commerciale de dépuration et d'entreposage humide des moules a été installée dans les locaux de l'entreprise « Les pêcheries Rivière-au-Renard Inc. ». Les moules provenant de la baie de Gaspé étaient disposées dans les bacs de dépuration et d'entreposage humide à raison de 275 kg/bac. Le débit d'eau salée a été fixé à 45-50 L/min par bac pour la dépuration. Les paramètres de la qualité de l'eau de traitement étaient suivis conformément au PCCSM[2] durant les cycles de dépuration. Un suivi de la teneur en coliformes fécaux rencontrés dans les moules a été effectué tel que décrit précédemment. Après la dépuration, les moules étaient transférées en entreposage humide, selon les mêmes quantités/bac. Les bacs d'entreposage humide étaient alimentées en eau selon un système en cascade, c'est-à-dire que le bac supérieur d'une colonne recevait de l'eau neuve, et se drainait dans le bassin du milieu, qui se drainait à son tour dans le bassin inférieur. Chaque bac d'entreposage humide était aussi muni d'une entrée d'air et d'un bulleur. Une étude a été entreprise afin de déterminer la combinaison de débit d'eau et d'air permettant d'obtenir une teneur en oxygène dissous supérieur à 60% à la sortie du bassin inférieur. En cours d'opération, une analyse des points critiques et des coûts a été effectuée.

Résultats

Démonstration expérimentale

Les observations visuelles effectuées montrent que les moules reprennent rapidement leurs activités après avoir été entassées dans les colonnes d'observation en acrylique. Six heures après la mise en eau, les valves s'écartent, les siphons et les cils sortent, et ce aux trois positions observées. De plus, les moules ont commencé à secréter du byssus. Les résultats des essais de détermination des débit ont montré que les combinaisons entassement*débits testées permettent le respect des critères de qualité de l'eau de traitement du PCCSM[2], pour des températures d'eau de mer entre 3°C et 15°C, en circuit ouvert. La déplétion en oxygène dissous entre l'entrée et la sortie des bacs de dépuration varie de façon exponentielle avec la température de l'eau. La figure 1 présente les teneurs moyennes de l'eau de traitement lors des 3 cycles de dépuration effectués avec un entassement de moules de 35-40 cm et un débit de 40 L/min.

Projet pilote

Huit cycles de dépuration ont été effectués durant le projet pilote, pour un total de 30 000 kg brut de moules, soit 16 200 kg net. La plus haute teneur en coliformes fécaux rencontrés dans les moules à T_0 fut de 130 cf/100 g. La teneur moyenne en coliformes des moules à T_{48} fut de <20 cf/100 g. Une diminution de la teneur en coliformes fécaux des moules a été observée. Les critères de qualité d'eau de traitement du PCCSM[2] ont été respectés. Après la dépuration, tous les lots ont reçu un verdict d'acceptabilité. Le procédé de dépu-

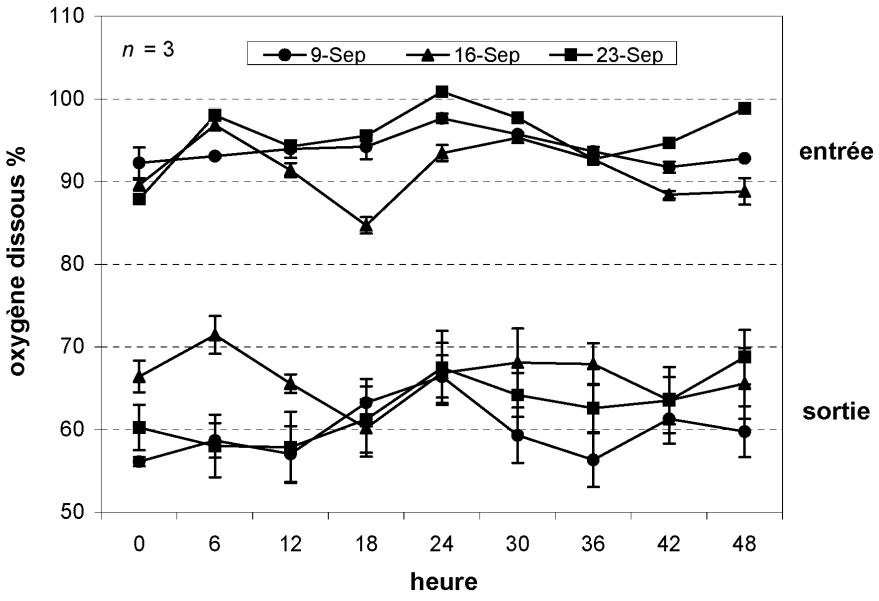

Figure 1.
Teneurs moyennes de l'eau en oxygène dissous (%) à l'entrée et à la sortie des bacs de dépuration lors des 3 cycles de dépuration effectués avec un entassement de moules de 35-40 cm (225 kg) et un débit de 40 L/min.

ration et d'entreposage humide ne semble pas affecté les qualités organoleptiques des moules, pour une durée d'entreposage < 7 jours. Les coûts du procédé ont été évalués à 0,69 $ /kg net de moules. Le tableau 1 présente la ventilation des coûts du procédé.

Implantation commerciale

L'unité de dépuration était dotée d'une capacité de 24 bacs isothermes disposés en 12 colonnes, alors que l'unité d'entreposage humide possédait une capacité de 63 bacs disposés en 21 colonnes. Six cycles de dépuration ont été effectués sur des moules de la baie de Gaspé, pour un total de 36 300 kg brut de moules, soit 20 500 kg net. La plus haute teneur en coliformes fécaux observée dans les moules à T_0 était de 130 cf/100 g. La teneur moyenne en coliformes des moules à T_{48} fut de <20 cf/100g. Une diminution de la teneur en coliformes fécaux des moules a été observée. Les critères de qualité d'eau de traitement du PCCSM[(2)] ont été respectés. Après la dépuration, tous les lots ont reçu un verdict d'acceptabilité. L'entreposage humide s'est effectué sur 70 350 kg brut de moules, soit 41 700 kg net, incluant les moules dépurées de la baie de Gaspé et des moules non dépurées provenant de la baie des Chaleurs. Le débit d'eau par colonne a été fixé à 20-30 L/min, avec une adduction d'air de 5-7 cfm/bac. Des fluctuations dans le système d'approvisionnement en eau de mer ont causé des problèmes d'ajustement des débits. Selon les résultats obtenus sur un lot de moules, l'entreposage humide de plus de deux semaines pourrait avoir entraîné une diminution de la qualité organoleptique des moules.

Discussion et Conclusion

La démonstration expérimentale a permis de démontrer la faisabilité technique de la dépuration en vrac de la moule bleue, en regard des exigences du PCCSM ayant trait à l'activité des moules et de la qualité des eaux de traitement. Le projet pilote a permis de valider la faisabilité à l'échelle commerciale d'un procédé de dépuration et d'entreposage humide, mais à un coût élevé, soit 0,69 $ le kg net, ce qui limite la rentabilité de ce procédé. La qualité organoleptique des moules ne semble pas affectée par le procédé lorsque l'entreposage humide est de courte durée, soit < 7 jours. Le projet pilote a également permis la commercialisation de moules dépurées pour la première fois au Canada. La phase d'implantation commerciale a permis d'ériger un centre intégré de traitement de la moule et une commercialisation à partir d'une base régionale. Des travaux supplémentaires sont nécessaires pour améliorer les paramètres d'opération de l'entreposage humide et pour déterminer la possibilité de diminuer les débits d'eau lors de la dépuration, tout en respectant les critères de la qualité de l'eau de traitement. Les problèmes de fluctuations dans l'approvisionnement en eau devront aussi être solutionnés.

Tableau 1.
Ventilation des coûts associés au procédé de dépuration en vrac et d'entreposage humide lors du projet pilote, par bac de 225 kg et par kg de moule (*54% du poids brut).

	$ / bassin	$ / kg moule		%
		brut	net*	
Opérations	29.19	0.13	0.24	35
Matériels / équipement	54.61	0.24	0.45	65
Eau de dépuration 50 L/min	21.6			26
Analyses bactériologiques	27			31
TOTAL	83.80	0.37	0.69	100

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Comparative Rates of Byssal Thread Production and Crawling Speed in Hatchery-Produced *Mytilus trossulus* and *Mytilus edulis* under Varying Salinity, Temperature, and Feeding Conditions

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Mussels, *Mytilus edulis* and *M. trossulus*, occur sympatrically in culture sites throughout eastern Canada. The proportions of each species vary according to site and year class. In spite of this, there has been little attempt to ascertain species performance under various environments. In the present study, individually numbered hatchery-produced juveniles were exposed to a variety of treatment combinations in multifactorial trials representing natural conditions: fed or unfed; salinities – 15, 20, 25, or 30 ppt; temperatures 3°C or 11°C. Mussels were assessed on a daily basis for two weeks for the following: byssal threads/day, crawling rate in mm/min, and proportion attached. Survival was 100% in all treatments. *M. trossulus* juveniles produced significantly more byssal threads, were more active (mm/min), and showed a greater proportion of attached individuals under all experimental treatments. The rate of byssal thread production and attachment was highest in *M. trossulus* at 20 ppt while the highest rates in *M. edulis* were at salinities of 30 ppt. The findings suggest that *M. trossulus* juveniles may tolerate a wider range of conditions than *M. edulis* in culture and that conditions of reduced food and salinities may favor *M. trossulus*.

Les moules *Mytilus edulis* et *M. trossulus* partagent le même habitat (espèces sympatriques) dans les sites de culture à l'échelon de l'est du Canada. Bien que la proportion d'individus de chaque espèce varie selon le site et la classe d'âge, on a rarement tenté d'établir avec précision la performance de chacune dans diverses conditions. Dans le cadre de la présente étude, des moules juvéniles de culture numérotées ont été exposées à une gamme de combinaisons de traitements lors d'essais multifactoriels représentant des conditions naturelles : apport de nourriture ou non; salinités de 15, 20, 25 ou 30 ppm et températures de 3 °C ou 11°C. Les éléments suivants ont ensuite été évalués quotidiennement pendant deux semaines : quantités de fils de byssus produites par jour, vitesse de déplacement en mm/min et proportion d'individus fixés. Toutes les moules ont survécu aux traitements. Les juvéniles de *M. trossulus* ont produit significativement plus de fils de byssus, étaient plus actifs (vitesse de déplacement en mm/min) et se fixaient dans une plus grande proportion dans toutes les conditions expérimentales. Chez cette espèce, les taux de production d'un byssus et de fixation étaient plus élevés à une salinité de 20 ppm, tandis que chez *M. edulis*, ils étaient plus élevés à une salinité de 30 ppm. Ces résultats suggèrent que les juvéniles de culture de *M. trossulus* tolèrent peut-être mieux une plus vaste gamme de conditions que leurs congénères de *M. edulis* et que, lorsque la nourriture est moins abondante et que la salinité est moins élevée, *M. trossulus* performe mieux.

Introduction

Mytilus edulis and *M. trossulus* cohabit similar and widely diverging environments in Atlantic Canada. They occur sympatrically in natural populations and in culture throughout eastern Canada[1-3]. *M. trossulus* is a high-value species cultured for the past 20 years on rafts in Washington State[4] . Only two or three studies have examined the culture performance of both species under similar environments in eastern North America, and those that have suggest *M. trossulus* may be less desirable in terms of production characteristics (e.g., thinner shell, slower growth, higher mortality)[5-7]. However, these same researchers acknowledge that culture performance is influenced greatly by the environment and genetics and there are certainly examples of *M. trossulus* outperforming *M. edulis* under rope culture conditions in Atlantic Canada.

Byssal thread production in *Mytilus* spp. is influenced by a variety of biological and environmental factors, including size, salinity, temperature, turbulence, and food levels[8]. Both *M. trossulus* and *M. edulis* display shifts in salinity tolerance with age, with *M. trossulus* showing a tolerance for reduced salinities at all ages[9]. In addition, *M. trossulus* demonstrated significantly higher rates of food consumption than *M. edulis* under varying food conditions[10]. Anecdotal evidence suggests that *M. trossulus* is more active at low temperatures than *M. edulis* (C. Couturier, unpublished observations). These recent findings all suggest that different environmental conditions may favor one species over the other in Atlantic Canada, and that environmental conditions will influence culture performance of both species.

The objectives of the present study were to compare the rates of byssal thread production and general behavioural patterns of hatchery produced *M. trossulus* and *M. edulis* juveniles in the laboratory when held under varying conditions of food, salinity, and temperature. It was hoped that the information would further contribute to the understanding of the culture performance of the

two species in Atlantic Canada and by so doing provide useful information for use by the mussel culture industry.

Materials and Methods

Source and maintenance of juveniles

Hatchery produced juvenile mussels of known genetic background were obtained for the present study. They originated from broodstock mussels which were typed by species using DNA markers[4], subsequently bred in the laboratory and reared on a mixed algal diets until use. Approximately 140 individuals of each species, comprising a mixture of several families for each species, were made available for our purposes.

Upon receipt, all mussels were individually identified with numbered labels affixed to the shell with cyanoacrylate glue. The shell length of each mussel was measured to the nearest 0.1 mm. The mussels were held in recirculating seawater raceway systems (aerated, 3ºC, 32 ppt salinity, unfed) until required for experiments, generally within a few days of transfer.

Byssal thread experiments

Ten mussels of each species were randomly assigned to plastic containers with one of the following 16 treatment combinations: (Temperatures: 3ºC and 11ºC) × (Salinities: 15 ppt, 20 ppt, 25 ppt, 30 ppt) × (Food level: Fed (2% per day), unfed).

Mussels were acclimated to temperature increases for 7 days prior to experimentation.

Water was changed every second day, byssal threads gently cut on all mussels, and counted the following day for each mussel[8]. This was repeated over a two week period so that 6-7 estimates of byssal thread production were obtained for each mussel.

Crawling rate experiments

Immediately following the byssal rate trials, mussels were placed in the same conditions in the center of a clear tray with 1-cm^2 gradations marked on the bottom. Each mussel's position on the grid was marked every 15 minutes for a total of 60 minutes observation time (4 measurements per mussel). The average distance traveled under the various treatments was computed for each mussel.

Results and Discussion

Juveniles of both species were similar in size at the onset of the experiment. Mean size of *M. trossulus* was 17.8 ± 1.3 mm (SD) and *M. edulis* was 19.0 ± 2.2 mm (SD). Survival was 100% throughout all experiments.

Byssal thread production

Figure 1 shows the daily rate of byssal thread production between the two species held under four salinity and two feeding conditions. *M. trossulus* produced significantly more threads under all feeding and salinity regimes than *M. edulis* (ANOVA, $P<0.05$). *M. edulis* produced fewer threads as salinity decreased whereas *M. trossulus* maintained similar thread production rates at all salinities. When nutrionally deprived, *M. edulis* produced significantly fewer threads than fed *M. edulis* under all salinity combinations (Fig. 1); the absence of food only slightly reduce byssal thread daily production in *M. trossulus* juveniles.

Byssal thread production rates were similar at 3ºC and 11ºC for each species, however *M. trossulus* produced 2 to 3 times the number of threads at each temperature compared with *M. edulis* (ANOVA, $P<0.05$). This effect was observed in both fed and unfed mussels but more pronounced in the unfed groups.

The above results on hatchery produced juveniles are in general agreement with the few previous studies on wild specimens of both species whereby *M. trossulus* tends to be more tolerant of reduced salinities and poorer environmental conditions[7,10,11]. The implications are that under unfavorable or rapidly changing environmental conditions in culture, *M. trossulus* may be favored.

Crawling rate

Figure 2 shows the crawling rate (mm/min) for juveniles of the two species. *M. trossulus* is much more mobile than *M. edulis* of comparable size (ANOVA, $P<0.05$), and both species appear to be more mobile at the lower temperature.

When exposed to varying salinity levels, again *M. trossulus* exhibited greater levels of activity (as measured by crawling rate) than *M. edulis* juveniles exposed to the same conditions. The absence of food increased the crawling rate in both species, suggesting an adaptive response to low food levels. The implications for culture here are that under low food or unfavourable food conditions, mussel juveniles are likely to be more active, perhaps searching for more favourable conditions. This in-

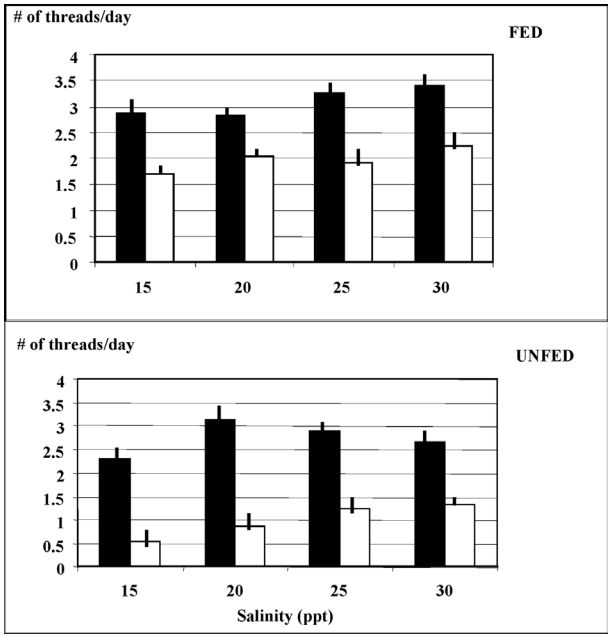

Figure 1.
Daily rate of byssal thread production under various salinity and feeding regimes. Shaded histograms are *M. trossulus*, open histograms are *M. edulis*. Bars represent means ± SE, *n* = 10 mussels.

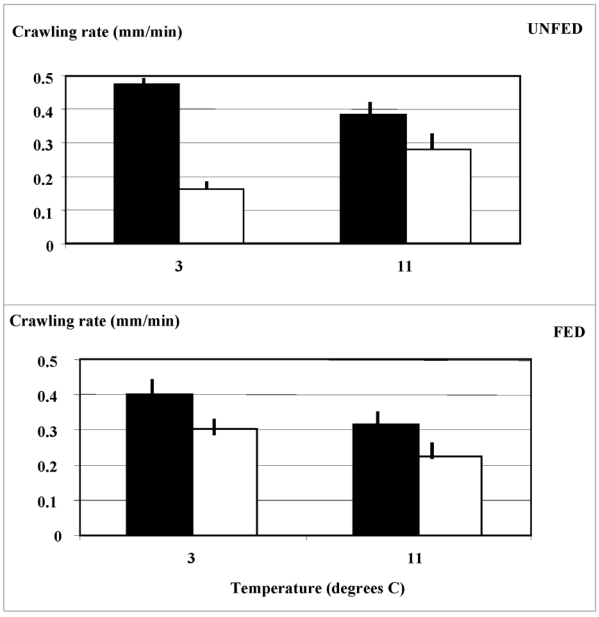

Figure 2.
Crawling rate (mm/min) for two temperatures and feeding conditions. Shaded histograms are *M. trossulus*, open histograms are *M. edulis.* Bars represent means ± SE, *n* = 10 mussels.

creased movement could result in a greater incidence of drop-off from culture socks, and might serve to explain changes in frequencies towards *M. edulis* in rope cultures observed by several researchers (D. Innes, unpublished observations).

The higher crawling rate of *M. trossulus* at all temperatures, salinities, and food combinations is interesting, and may confer an advantage in terms of escape from predators compared with *M. edulis.* This is speculative, however it would be interesting to investigate further.

Conclusions

Reduced rates of byssal thread production at lower temperatures might result in poorer retention rates of juvenile mussels when socked at lower temperatures.

Environments with reduced food and/or salinity levels may provide more favorable conditions for *M. trossulus* compared to *M. edulis.*

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Improving the Quality of Sea Scallop (*Placopecten magellanicus*) Spat Collection by a More Accurate Determination of the Immersion Period of Collectors in Îles-De-La-Madeleine, Québec, Canada

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Overabundant settlement of undesirable organisms on scallop collectors can i) negatively affect survival rate and growth of young scallops, ii) increase time to handle and clean collectors, iii) slow sorting operations in the plan, and (iv) increase production costs. For spat collection on a commercial scale, it is very important to know if it is possible to reduce abundance of undesirable species without affecting scallop spat collection. A study done in 2003-2004 near a commercial site of spat collection, located east of Îles-de-la-Madeleine (Québec, Canada), aimed to evaluate if it was possible to target with more accuracy the immersion time of collectors to reduce abundance of undesirable species and maintain high collection of scallop spat. After spawning season determined by GSI monitoring, evolution of larval abundance of scallop and undesirable species was followed by weekly sampling, and characterization of spat collection by weekly immersion of spat collectors for a week. These two tools were evaluated to know if they could help to target the best immersion time of collectors. Other experimental collectors were immersed weekly between the third and eighth week following spawning and retrieved in December to evaluate cumulative collection for six different immersion periods. Very few larvae (>200 µm shell height) of scallop and *Hiatella* were observed during the sampling period. Maximum densities were respectively of 0.054 ± 0.008 larvae/L and 1.524 ± 0.871 larvae/L. Numbers of mussel larvae were higher with a maximum of 15.957 ± 1.068 larvae of mussels/L on September 2. Even if it is possible to detect the presence of larvae >200 µm into the sea column, it does not seem possible to establish any relation between larval abundance and the collecting rates observed. The weekly spat collection monitoring appears more effective to identify peak settlement of scallop and undesirable species and could be a good tool to help producers choose the optimal time to immerse their collectors. Results of 2004 will help clarify the interest of this tool.

La fixation en surabondance d'organismes indésirables sur les collecteurs de pétoncles peut i) avoir une incidence négative sur le taux de survie et de croissance des pétoncles juvéniles, ii) nécessiter que plus de temps soit consacré à la manutention et au nettoyage des collecteurs, iii) ralentir les opérations de tri à l'usine et iv) accroître les coûts de production. Pour capter du naissain à l'échelle commerciale, il est très important de savoir s'il est possible de réduire l'abondance des espèces indésirables sans nuire au captage du naissain de pétoncle. Une étude réalisée en 2003-2004 près d'un site de captage commercial de naissain, situé à l'est des Île-de-la-Madeleine (Québec, Canada) visait à évaluer s'il était possible de cibler avec plus de précision le moment de mise à l'eau des collecteurs afin de réduire l'abondance des espèces indésirables tout en maintenant une bonne collecte de naissain de pétoncles. Après la ponte du pétoncle géant, déterminée par un suivi de l'indice gonadosomatique (IGS), l'évolution de l'abondance larvaire des pétoncles et des espèces indésirables a été suivie par un échantillonnage hebdomadaire alors que le captage de naissain a été caractérisé par l'immersion hebdomadaire de collecteurs laissés à l'eau pendant une semaine. Ces deux outils ont été évalués afin d'établir s'ils pouvaient servir à cibler la meilleure période d'immersion des collecteurs. D'autres collecteurs expérimentaux ont été mouillés de façon hebdomadaire entre la troisième et la huitième semaine suivant la ponte et récupérés en décembre afin d'évaluer le captage cumulatif pour six différentes périodes d'immersion. Très peu de larves de pétoncles et de hiatelles de taille > 200 µm ont été observées durant la période d'échantillonnage. Les densités maximales de larves se chiffraient respectivement à 0.054 ± 0.008/L et 1.524 ± 0.871/L. Les larves de moules étaient en nombre plus élevé. Le maximum, soit de 15.957 ± 1.068 larves/litre, a été observé le 2 septembre. Même s'il est possible de détecter la présence de larves de taille > 200 µm dans la colonne d'eau, il ne semble pas possible d'établir une relation entre l'abondance larvaire et les taux de collecte observés. Le suivi hebdomadaire de la collecte de naissain semble plus efficace pour ce qui est d'identifier le pic de fixation du pétoncle et des espèces indésirables et pourrait alors être un bon outil pour aider les producteurs à établir le moment optimal pour immerger leurs collecteurs. Les résultats de 2004 aideront à confirmer si cet outil est vraiment utile.

Introduction

Each year, undesirable organisms settle on sea scallop spat collectors. The excess of bio-fouling on collectors can result in reducing buoyancy of long lines (gear damage), in slowing down scallop growth (competition for food)[1], in increasing scallop mortality (predation and packing) and in slowing handling and cleaning operations, with the result that labour costs are increased and profitability reduced[2,3]. In 1998, predation by starfish (*Asterias vulgaris)* inside collectors resulted in spat's high mortality[4]. In 2001, overabundance of bivalves *Mytilus edulis* and *Hiatella arctica* in collectors caused high mortalities. Many collectors dragged the sea bottom because the weight of long lines was too high[5].

The main objective of this study was to look at different potential tools to target the best immersion period of collectors to maximize sea scallop spat collection while minimizing the presence of undesirable organisms.

Specific objectives were i) to determine the spawning period of sea scallops, ii) to evaluate if larval monitoring and/or post larval monitoring for peak settlement can be used as tools to determine an optimal immersion period of spat collectors and iii) to estimate growth and survival rates of sea scallop (*Placopecten magellanicus*) and main undesirable species (*Hiatella arctica*, *Mytilus edulis*, *Anomia* sp., *Asterias vulgaris*) in collectors during the year following settlement.

Materials and Methods

Study area

The sampling sites were located in open waters in the Îles-de-la-Madeleine in the southern Gulf of St-Lawrence (Québec, Canada). The monitoring was conducted near the spat collection commercial site of Pearl Reef (Fig. 1).

Scallop reproductive development

The reproductive cycle of the sea scallop (length >90 mm) was investigated in 2003 and 2004 by monitoring the gonado-somatic index (GSI). This index was calculated as the percent ratio between the wet weight of gonad and the one of remaining soft parts. Weekly samples of twenty adult scallops (for a ratio of 50% female and 50% male) were collected by dredging in the Chaîne-de-la-Passe fishing area and in the Fond du Sud-Ouest, a natural bed closed to fishing since 1991 (Fig. 1). A Digby drag was used for sampling wild sea scallops at the depth of 28-30 m, between the end of July and the middle of September. Scallops were dissected to determine the GSI.

Larval monitoring

To determine larval densities, a sample of known volume of seawater was pumped between two and eight meters off the bottom at three stations of the Pearl Reef. Commercial collectors were habitually immerged at these depths. In 2003, the larval monitoring was conducted between July 4 and October 29 on the spat collection site of the Pearl Reef (Figure 1). In 2004, the larval monitoring was conducted between August 2 and November 8. Larvae of *Placopecten magellanicus*, *Hiatella arctica*, *Mytilus edulis*, *Anomia* sp. and *Asterias vulgaris* were identified and counted. A random sub-sample of 30 larvae of each species was also measured under a Leica Bioquant IV stereomicroscope analyzer (100×).

Spat collection monitoring

Recruitment of newly settled sea scallop spat was estimated using artificial collectors immersed for seven days intervals during the scallop larval settlement period. The scallop spat collectors deployed throughout this study were made of four Netron™ sections inside a 2-mm-mesh spat collecting bag. Collectors were suspended at 2 m off the bottom. Collectors were immersed weekly at three sites on the Pearl Reef (water depth from 30-32 m), for eleven consecutive weeks in 2003, starting on August 19 and for eight consecutive weeks in 2004, starting on September 9. A week after deployment, collectors were retrieved from each site and cleaned over a 250-µm mesh sieve. The content of each collector was preserved in 95% ethanol. The number of sea scallops (*Placopecten magellanicus*) spat and four undesirable species (molluscs: *Mytilus edulis*, *Hiatella arctica*, *Anomia* sp. and echinoderm: *Asterias vulgaris*) found in the collectors was counted. A random sub-sample of 30 specimens of each species was also measured. In 2004, to verify loss of spat during collection, sleeves of 250-µm meshes were gently placed over the collectors underwater before their retrieval.

Six immersion periods of scallop spat collectors

Other spat collectors done as described above, were tagged and placed in the spat collection site of the Pearl Reef at two meters off the bottom. Three series of five collectors were deployed weekly on the Pearl Reef (water depth from 30-32 m), for six consecutive weeks, starting September 15, 2003. On December 6, thirty collectors (5 for each immersion period) were retrieved and cleaned. The content of each collector was preserved in 95% ethanol and analyzed as described above. In the same way thirty collectors were retrieved in May 2004 and the thirty remaining, in October 2004.

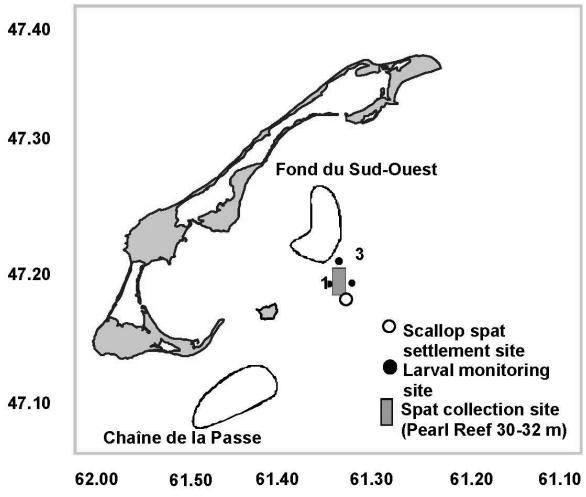

Figure 1.
Location of site for sea scallop spat collection, study sites of scallop spat settlement and abundance of larvae and fishing grounds in the Îles-de-la-Madeleine in the Gulf of St-Lawrence, eastern Canada.

Results

Scallop reproductive development

The GSI monitoring showed that adult scallops initiated spawning by August 26, 2003. Maximum GSI of 47.5% on the Fond du Sud-Ouest and 40.4% on the Chaîne-de-la-Passe was found on August 19. Spawning was completed on September 15.

Larval monitoring (1st tool)

Figure 2 A shows the density of larvae pumped between 2 and 8 meters off the bottom from August 26 to October 29 2003. Larvae of bivalve *Mytilus edulis* were most abundant. Highest larval density of 15.96 larvae/liter (>200 µm shell height) was recorded on September 2. Other species had densities significantly lower than mussels. During the monitoring, we observed a peak of larvae of *Anomia* on October 20 (2.71 larvae/L), of *Hiatella arctica* on October 1 (0.45 larvae/L) and of sea scallop on October 29 (0.10 larvae/L).

Spat collection monitoring (2nd tool)

Figure 2B shows spat numbers found in collectors immersed for one week at 2 meters off the sea bottom. Of the undesirable organisms, the bivalve *Mytilus edulis* was the most abundant. The highest density (1148 spats/collector) was observed on August 26. Other mollusks in order of decreasing abundance included: *Hiatella arctica* (354/collector on October 26) and *Anomia* sp. (47/collector on September 8). Starfish (*Asterias vulgaris*), a major predator of scallop spat, was only present in low numbers (between 0.33 and 6/collector) except on September 22 with 26/collector (diameter between 0.8 and 1 mm). The highest collecting rate of scallop was obtained on October 20 (465/collector). For spat collection monitoring in 2004, use of divers to recuperate a sub sample of collectors inside bags with mesh of 250 µm showed that there was no loss of organisms when collectors were recovered directly from the boat.

Six immersion periods of scallop spat collectors

Figure 2C shows the spat numbers of sea scallop and main undesirable species in collectors immersed at 6 different periods. These collectors were retrieved on December 6. We can observe a peak of scallops in collectors immerged on October 7 (6147/collector). The highest densities of mussel, arctic saxicave (*Hiatella arctica*), and jingle shell (*Anomia* sp.) were found on collectors immersed on September 15; 2727/collector, 5154/collector, and 3760/collector, respectively. Collectors immersed eight weeks after the beginning of the spawning of sea scallop (October 21) contained 3281 scallops, 532 mussels, 683 hiatellas, 165 anomias. Starfishes were found in low number on collectors in 2003 (only 1 on September 15 and October 13)

Discussion

Comparison of the first tool with six immersion periods of scallop spat collectors

Larval monitoring is not a good tool to predict the best immersion period of collectors because it is difficult to link densities of larvae in seawater and spat settlement in collectors. In fact, scallop larvae are always the less abundant (Fig. 2A) but this species is always the one most abundant on collectors (Fig. 2C). On collectors immersed on October 6 and emerged on December 6, the numbers of scallops, mussels, arctic saxicaves and jingle shells were respectively 6147, 1138, 2227, and 1143 per collector (Fig. 2C). Larval densities observed on October 6 and one and two weeks before (Fig. 2A) cannot be associated with the collecting rates observed. In fact the best concordance between larval densities and spat collection rates is obtained with mussels. In Figures 2A and 2C, the curves for this species present the same tendency to decrease with time. Monitoring of larval abundances permit to identify peak of larvae >200 µ m but except for mussel, it does not seem to have a relation between larval abundance and spat collecting rate. In 2003, with the results of mussel larval monitoring (Figs. 2A and 2C), it would have been possible to recommend to scallop growers to start immersion of their collectors on September 23. The number of mussels would have been reduced by half with still a good scallop collection rate.

Comparison of second tool with six immersion periods of scallop spat collectors

The weekly monitoring of spat collection, is a good tool to identify peak of settlement. Figure 2B shows that high collecting rates of mussels and hiatellas were observed at the end of August and at the beginning of September. Abundance of these two species would have been probably very high on scallop collectors immersed at that time. Immersion of commercial collectors usually starts at the end of September. In 2003, weekly monitoring of spat collection would not have permitted to reduce the number of mussels and hiatellas on collectors. However, producers have observed that the abundance of these two species on collectors is variable from one year to the other. In 1998, hiatellas were so abundant that important losses of scallop spat were associated with that problem. We can suppose that in certain years, the first peak of the collection of mussel and hiatellas may be later in September. When it happens, weekly spat monitoring could be a useful tool to identify the problem and to advice producers to slightly delay the immersion of scallop spat collectors. Another useful utilization of these results is the possibility to know if there is still interest for producers in immersing collectors. Meteorological conditions are particularly rough in fall and it is not always possible for producers to immerse their collectors within a short period of time. So if they have to immerse collectors late in the season, they have to know if these collectors will still collect scallops. As we see in Figures 2B and 2C, weekly monitoring indicates that scallop collection was still good at the end of October (October 21, 296 scallops/collector) and the number of scallops found on collectors immersed at that time and retrieved in December was still high (3281 scallops/collector).

For the period from September 16 to October 28, cumulative numbers per collector obtained with weekly monitoring were 1331 scallops, 1731 mussels, 1128 hiatellas and 52 anomias. In comparison, numbers per collector found on experimental collectors immersed at the same time and retrieved in December were 5391 scallops, 2727 mussels, 5154 hiatellas, and 3760 anomias. Aside from for mussels, weekly monitoring seems to underestimate the collecting success of scallops, hiatellas, and anomias. In 2004, recuperation of collectors by divers showed that there was no loss of organisms when collectors are retrieved from the surface as in 2003. Possible explanation of the low col-

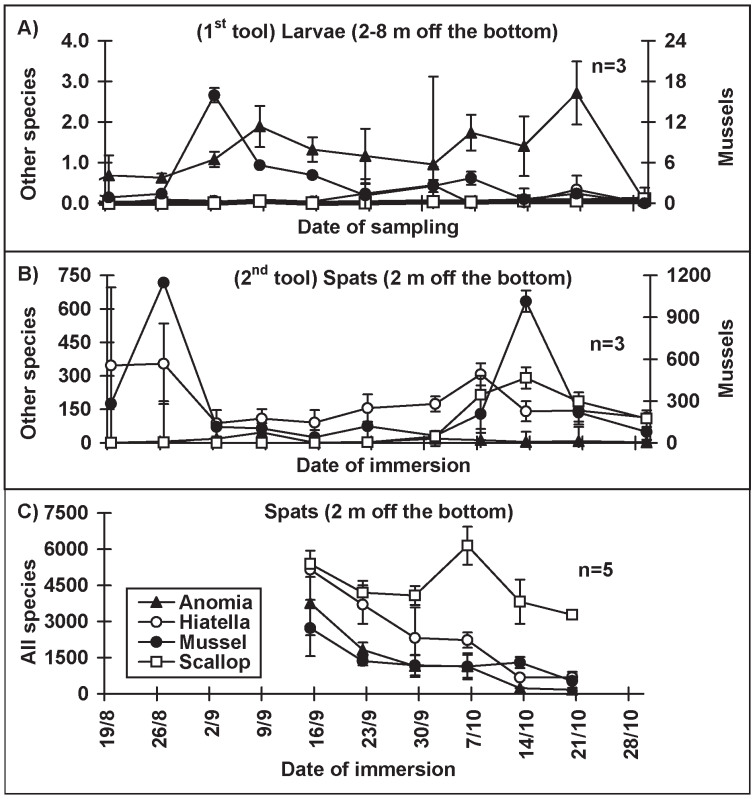

Figure 2.
A) Number of larvae > 200 µm per liter pumped ±SE (scale for mussels at right); B) number of spats per collector immersed one week ±SE (scale for mussels at right); and C) number of spats per collector immersed at different periods ±SE. Collectors were retrieved on December 6.

lecting success of certain species is that collectors immersed for one week are not fully effective for certain species possibly because conditioning of collecting substrate (bacterial biofilm) is not optimal[6,7]. Other experimental works will be necessary to validate this hypothesis.

Conclusion

The first tool, the larval monitoring, is a good tool to track the presence of larvae >200 µm into the sea column but difficult to link with spat settlement. The second tool, the weekly spat collection monitoring, is the best tool to identify peak settlement. With this tool, two kinds of advice could possibly be given to the producers: first to delay the immersion of their collectors if the abundance of hiatellas and mussels are high at the end of September and secondly to immerse collectors even late in season if collection is still good on weekly collectors. It would be very interesting to evaluate if collectors conditioned for one or two weeks in sea water before being immersed would be more effective to collect scallops, hiatellas and anomias. Other experimental work will be necessary.

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Identifying Commercially Viable Sea Scallop (*Placopecten magellanicus*) Spat Sites

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Sea scallop (*Placopecten magellanicus*) spat collection was investigated from 1995 to 2003 throughout the southern Gulf of St. Lawrence in the Department of Fisheries and Oceans (DFO) Gulf Region and along the Nova Scotia coast in the DFO Maritimes Region. In many areas, the collectors were deployed annually and consistent interannual spatial patterns of scallop spat settlement were observed. Commercially viable numbers of scallop spat were documented at various study sites. Based on results it is recommended to deploy collectors offshore at a site depth of 21 to 35 meters (70 to 115 feet) except if a scallop culture site is nearby, where collectors can be placed in shallower water of 12-20 meters (40-64 feet).

Les possibilités de captage de naissain de pétoncle géant (*Placopecten magellanicus*) ont été évaluées de 1995 à 2003 à l'échelon du sud du golfe du Saint-Laurent, dans la Région du Golfe du ministère des Pêches et des Océans (MPO), et le long du littoral de la Nouvelle-Écosse, dans la Région des Maritimes du MPO. Dans de nombreux secteurs, des collecteurs ont été mouillés tous les ans et des tendances spatiales persistantes de fixation du naissain ont été observées d'une année à l'autre. Des concentrations commercialement viables de naissain de pétoncle ont été documentées à divers sites d'étude. D'après les résultats obtenus, nous recommandons que les collecteurs soient mouillés au large à une profondeur se situant entre 21 et 35 m (70 à 115 pi), sauf si un site de pectiniculture est situé à proximité, dans quel cas des collecteurs peuvent être mouillés en eau moins profonde, soit de 12 à 20 m (40 à 64 pi) de profondeur.

Introduction

Securing scallop spat is the first step in ensuring successful scallop aquaculture or enhancement activities. Scallop spat can be obtained by collecting them from the wild or by producing them in hatcheries. In this study, the potential of wild scallop spat collection was investigated. Successful sea scallop collection depends on deployment date and site location. Ideal deployment date can be predicted based on the gonadosomatic index (GSI) of the adult scallops; however, the best collection location depends on hydrographic and geographic conditions (e.g. currents, depth, and presence of land mass)[(1)]. Wild spat collection can be cost effective if spat are collected at high enough densities. The Australians have demonstrated that as little as 500 scallop spat per collector bag can be commercially viable[(2)]. In Japan, they target a collection of more than 1000 spat per bag[(3)]. However, sometimes they collect only 200 spat per bag. We launched a sea scallop (*Placopecten magellanicus*) spat collection study from 1995 to 2003 throughout the southern Gulf of St. Lawrence in the Department of Fisheries and Oceans (DFO) Gulf Region and along the Nova Scotia coast in the DFO Maritime Region. The purpose of our investigation was to seek and identify sites that had an average collection rate between 1000 to 2000 scallop spat per bag. However, sites where over 2000 scallop per bag were collected were still considered to be potential commercial sites.

Materials and Methods

Collector bags consisted of a 3-mm mesh bag filled with 3 pieces of Netron® or monofilament that provided similar surface area. Three collector bags were secured to a mooring system and were deployed at each study sites. Each mooring had an anchor and buoys that was set-up to secure the three bags in suspension, three to six meters off the bottom. The gonadosomatic index (GSI) of the adult scallop (over 80 mm) from wild populations was monitored in several areas throughout the study region. Four weeks after the adult scallops initiated spawning, the fisher or aquaculturist participating in this project deployed the collectors with moorings. Deployment occurred at the end of September or beginning of October. If possible, the fisher and aquaculturist were requested to select sites with a depth of 25-30 m. This was based on results from prior successful undocumented scallop spat collection investigations.

In the Gulf Region, collection sites were located in the Baie des Chaleurs, along the northeastern coast of New Brunswick, throughout the Northumberland Strait, along the coast of Prince Edward Island and eastern Cape Breton. At one site in the Baie of Chaleurs, the fisher was requested to select four sites at different depths in the same area. In the Maritimes Region, collector sites were in St. Ann Bay, Mira Bay, Chedabucto Bay, Whitehead Harbour, and near Goose Island. At all sites, the collectors and moorings were retrieved one to four months following deployment. After retrieval, collectors were placed in large plastic bags and frozen until the contents of the bag could be analysed. Analysis consisted of emptying and washing the thawed contents of the collector bag onto a 150-µm sieve. The contents were then fixed in 5% buffered formaldehyde for a minimum of three days. The contents were later subdivided using a Folsom plankton splitter if more than 200 scallops were estimated to be in the sample contents. The scallops in the sub-sample that contained about 200

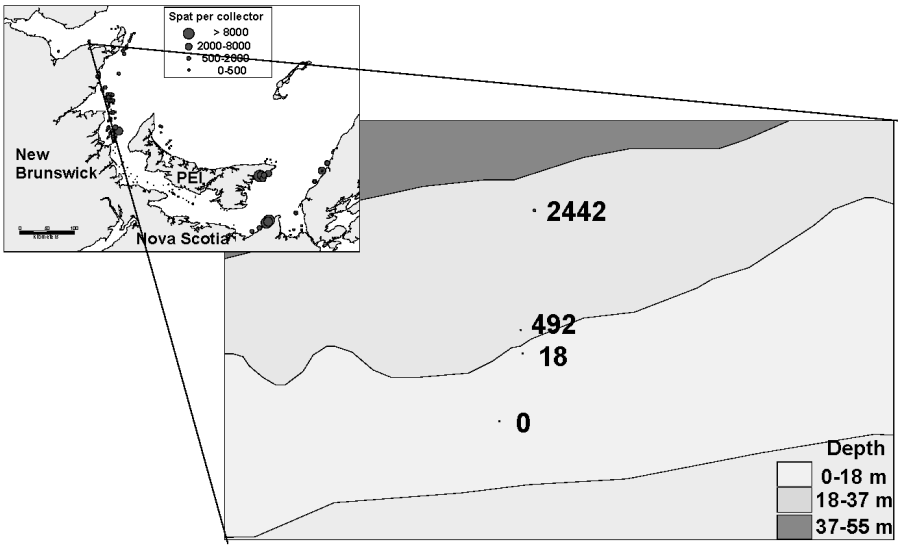

Figure 1.
The average number of scallop spat per bag at study sites in the Gulf Region and at four sites with various depths in one area of Baie des Chaleurs.

scallops, were measured and counted. The sample was then preserved in 70% alcohol.

Results

In the Gulf Region, an average of 1000 scallop per bag or more were found in most bags that were deployed at all the collection sites except in the Northumberland Strait (Fig. 1). The bags deployed in the Northumberland Strait were heavily fouled with silt. Results of the scallop spat count found at various depths at the site in the Baie des Chaleurs, are presented in Figure 1.

In the Maritimes Region, an average of 1000 scallop per bag or more were found at all the collection sites where water depth was between 22 and 35 m. Poor collection was observed at a very deep site of 38 m. Most of the collectors deployed at shallow sites with a depth of 9 to 20 m collected poorly except for those in Tor Bay and Whitehead (Fig. 2). The collection sites in Tor Bay were 12 m to 18 m deep and those in Whitehead Harbour were 18 to 20 m; however scallop farms are located there: Jamieson's Giant Scallops and Foggy Farms. In Figure 2, the low counts of 186 to 258 scallop spat per bag were collected at sites where the water depth was 9 to 18 m while the high count of 1104 scallop spat per bag was collected at a 21-m-deep site.

In St. Ann Bay, especially around Bird Islands, in Chedabucto Bay and at one site in Tor Bay the collectors had an exceptional collection where most of the contents consisted of scallop spat with very little fouling of other species or silt.

Throughout the course of this study, the following very important anecdotal observations were recorded:

- If spat collector bags were allowed to touch the bottom, their content were quickly lost (due to predation or other causes).
- Entire spat collector lines can easily be lost during a storm if they not properly anchored and anchors made from old railroad ties were very efficient.
- A large number of unwanted predators and competitors were often collected along with the scallops at many sites.

Discussion

Scallops beds are located throughout the Northumberland Strait and preliminary results of a scallop larval study indicate that the larvae are also found throughout the Strait (unpublished

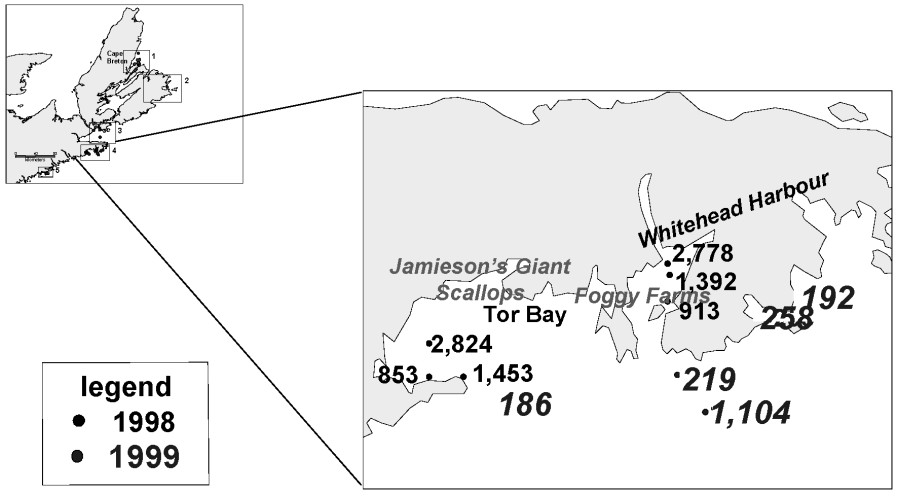

Figure 2.
Average number of scallop spat per bag at study sites in the Maritimes Region including the numbers found in Tor Bay and Whitehead Harbour where scallop farming was practiced.

data). The poor collection in central part of the Northumberland Strait was mainly attributed to the silt and other fouling that accumulated inside the collectors. The silt in the bag may have prevented the scallop spat from settling in the collectors or may have been responsible for its mortality. High silt levels are often found in the water column in areas located between two land masses where high water flow resuspends bottom sediments. When collecting scallop spat, it is recommended to avoid sites that often contain high silt levels in the water column.

Scallop farming is not yet practiced in the Gulf Region, therefore the source of spat are from the wild scallop population. In the Maritimes Region, there are several scallop farming operations. Scallop farmers have observed that when they began their operation the initial spat collection at their site was not very plentiful but improved over the years as the scallop farm matured (see Parsons et al.[4]). At one farm in Nova Scotia, the wild scallop bed near the farm site had not being fished for many years when the farm first started; however after they had been culturing scallops for several years one or two boats had started dragging scallops again[5]. When collecting wild scallop spat in these regions, it is recommended to deploy collectors at sites with a water depth of 25 to 35 m. However, high counts can also be obtained in shallower (12-20 m deep) water near scallop farms.

Summary

- Best spat collection was obtained when collectors were deployed offshore at a site depth of 21 to 35 m (70 to 115 feet) **except** if a scallop culture site is nearby where collectors can be placed in shallower water of 12 to 20 m (40 to 64 feet).
- Poor spat collection occurred when collectors were deployed in heavily silted water (between two land masses).
- High spat loss occurred when collectors touched the bottom.
- To prevent collector loss, collectors must be well anchored.
- Large numbers of unwanted predators and competitors were often collected with scallops.

Future Studies

Two key areas for future study are to determine approaches to collecting scallops without fouling of other species and silt and approaches to optimize scallop survival and growth rate while they are in the collectors.

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A Comparison between Different Scallop Spat Collector Designs

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Pecten UPM-MFU Inc. manages a sea scallop (*Placopecten magellanicus*) enhancement project for three local corporations. Each corporation collects their own spat using similar commercial collectors and is always interested in improving spat collection efficiency. A study was launched to investigate the efficiency of collectors of six different designs. In the fall of 2003, these six collector designs were deployed at two of the three Pecten spat collection sites (Detroit and Miramichi) off the eastern coast of New Brunswick. By June, 2004, spat collection varied from 2900 to over 7600 scallop spat/collector. In terms of scallop spat, preliminary results indicate that the commercial collectors presently employed by Pecten rated as the 3rd and 4th most efficient collector design at the Detroit and Miramichi sites, respectively. Furthermore, commercial collectors rated second as far as the lowest numbers of *Hiatella* at both sites. *Hiatella* was by far the most abundant species found in the collectors in numbers ranging from 2 to 8 fold that of scallops. Fuzzy rope collectors collected significantly less scallop and *Hiatella* spat than all other collector designs evaluated. Further studies are needed to better understand the variations which occur in spat collection.

Pecten UPM-MFU Inc. gère un projet d'ensemencement du pétoncle (*Placopecten magellanicus*) pour trois corporations locales. Chaque corporation possède son propre site de collecte et est toujours à la recherche de collecteurs plus efficaces. Une étude a été réalisée afin de comparer l'efficacité de six constructions différentes de collecteur. En automne 2003, ces six designs de collecteur ont été deployés à deux des trois sites de Pecten (Detroit et Miramichi) sur la côte est du Nouveau-Brunswick. En juin, 2004, le taux de collecte variait de 2900 à 7600 pétoncles par collecteur. En nombre de pétoncles, les résultats préliminaires indiquent que les collecteurs commerciaux présentement utilisés par Pecten se sont classés au troisième et quatrième rang par rapport aux autres designs de collecteur dans les sites de Detroit et Miramichi, respectivement. En plus, ces collecteurs se sont classées au deuxième rang pour avoir collecter le moins de *Hiatella*. Le *Hiatella* était l'espèce retrouvée en plus grand nombre dans les collecteurs atteignant de deux à huit fois le nombre de pétoncles. Les collecteurs de corde ont capté significativement moins de pétoncles et de *Hiatella* que les autres designs de collecteurs. D'autres études sont nécessaires afin de mieux expliquer les variations qui se manifestent lors de la collecte de naissains de pétoncles.

Introduction

Pecten UPM-MFU inc. has been involved in scallop enhancement in New Brunswick since 1997 and relies on wild spat collection as their source of seed. In 2003, commercial spat collectors were deployed on October 2 (collection site) according to the gonosomatic index. Peak spat settlement typically occurs 35 to 40 days after the initiation of spawning[(1)] and in 2003, occurred during the week of September 29.

Industry continuously seeks to improve collection methods while reducing costs. The ideal is to obtain between 1000 and 2000 scallop spat per collector while collecting few or none of the associated species. These compete with scallops for space and food and therefore can greatly decrease growth. Predatory species are also undesirable in collectors for obvious reasons. Various substrate types have been studied for scallop spat collection including nylon netting, burlap, fibreglass, polyethylene film[(2)] plastic mesh (Netron®), and multi-cord[(3)]. In the mid 1980s, the standard collection unit consisted of monofilament gillnetting enclosed in a small mesh (<3 mm) onion bags[(4)]. However, Netron® is replacing gillnetting and becoming the substrate of choice in terms of cost and handling[(5)]. Over the years, Pecten has consistently used onion bags filled with Netron® but with little consideration to the mesh size of the bags or the amount of Netron®. The objective of this study is to compare different variations of the scallop spat collector and to advise Pecten according to results.

Materials and Methods

In this study, collectors were constructed with various mesh size onion bags filled with different substrate material (Table 1). On October 2, 2003, nine collectors of each design were deployed on one long line at each the Detroit and Miramichi commercial collection sites. Three collectors of each design were retrieved on November 3, 2003, and another three on June 22, 2004. Collectors were individually analysed for animal count by species and scallop spat shell height.

Results

For collectors retrieved in November, 2003, one month after deployment, average scallop spat counts varied from 2501

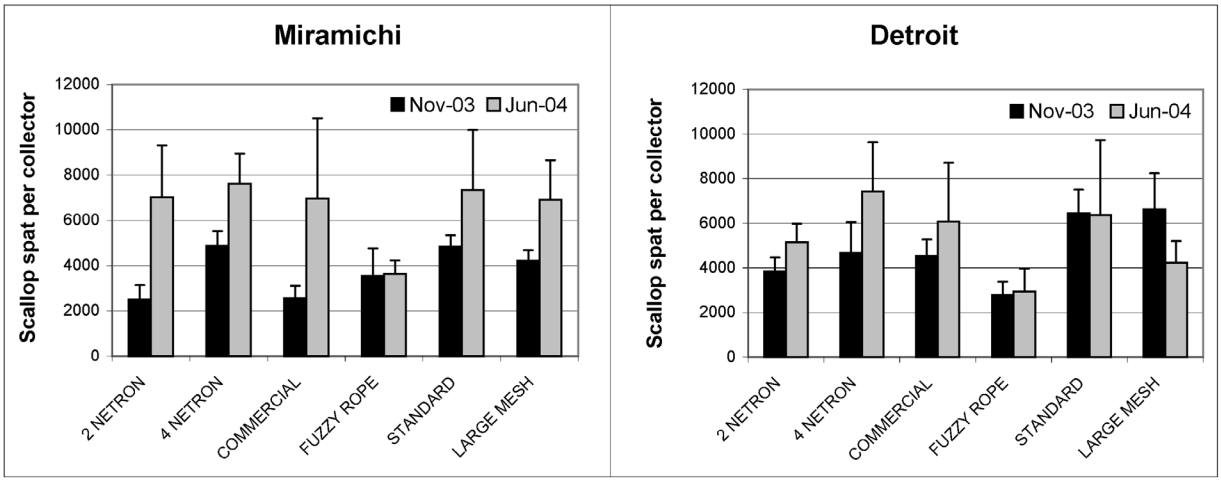
Name	Bag colour	Mesh size of bags	Substrate material
2 Netron®	Green	2×2 mm + 2×1 mm	2, 100×40 cm pieces of blue Netron®
4 Netron®	Green	2×2 mm + 2×1 mm	4, 100×40 cm pieces of blue Netron®
Commercial	Green	2×4 mm + 2×1 mm	2, 100×40 cm pieces of blue Netron®
Fuzzy Rope	Green	2×2 mm + 2×1 mm	300 cm of roughened rope (13mm)
Standard	Green	2×2 mm + 2×1 mm	3, 100×40 cm pieces of blue Netron®
Large Mesh	Blue	3×3 mm + 3×1 mm	3, 100×40 cm pieces of blue Netron®

Table 1.
Design description of scallop spat collectors deployed in October 2003.

spat/collector with the 2 Netron® collectors to 4875 spat/collector with the 4 Netron® collectors at the Miramichi site (Fig. 1). Meanwhile, it varied from 2789 spat/collector with the fuzzy rope collector to 6624 spat/collector with the large mesh collector at the Detroit site. When collectors were retrieved in June 2004, scallop spat/collector ranged between 6900 and 7600 in all Miramichi collector types except for fuzzy rope collectors which had an average of 3627 spat/collector. Spat collection varied greatly between the different collector types at the Detroit site with average spat counts of above 6000 spat/collector in the commercial, standard and 4 Netron® collectors while yielding counts of 5141, 4224, and 2941 spat/collector for the 2 Netron®, large mesh and fuzzy rope collectors, respectively. The average size of scallops from all collector designs combined was 3±1.4 mm, in June 2004.

Other species found in the collectors included *Hiatella*, mussels, brittle stars, seastars, cockles, macoma, various worms, jingle shells, foraminifers, and *Mitrella*. *Hiatella* was by far the most abundant associated species. In November 2003, *Hiatella* counts were 624 animals/collector in the commercial collectors of Miramichi but climbed to 16064 animals/collector in June 2004. Only the fuzzy rope collector had lower counts of 10 960 animals/collector in June 2004 at this site. Other collector types had average *Hiatella* counts which varied from 18 784 animals/collector with 2 Netron® collectors to 20 309 animals/collector for the 4 Netron® collector. A similar trend occurred at the Detroit site. Seastars were only found in the Detroit samples at counts of 5 seastars/collector with fuzzy rope collectors to 43 seastars/collector for 2 Netron® collectors. However, seastars were not found in the 4 Netron® and commercial collectors.

Figure 1.
Scallop spat collection rate for different collector designs deployed at the Miramichi (left) and Detroit (right) sites in the fall of 2003 and retrieved in November 2003 and June 2004.



Discussion

In scallop spat collection, the industry seeks a collection rate of between 1000 and 2000 scallops/collector for economic viability while avoiding associated species such as *Hiatella* and mussels and predators such as seastars. The values found at the two sites are well above these figures but also had a great amount of associated species, especially *Hiatella*. Therefore it is important to understand if the collector design can play a role in managing spat collection.

In general, higher scallop counts were observed when collectors were retrieved in June 2004, eight months after deployment compared to those retrieved in November 2003. Also, the trends for different collector designs did not follow through from November 2003 to June 2004. This indicates that caution should be exercised when predicting spat collection and tendencies from early fall samples.

If we focus on the June sample, which is closest to the enhancement period, we find that the commercial collectors ranked fourth and third for scallop spat collection in Miramichi and Detroit, respectively, and had the second least amount of *Hiatella*. Commercial collectors gave the best overall ranking (considering both good

scallop spat counts and low *Hiatella* counts) and had the largest mesh size and only 2 pieces of Netron®. Nevertheless, fuzzy rope yielded the least scallop spat while remaining above economic viability, and had the least *Hiatella* and the least mussels. Fuzzy rope is much less expensive than Netron which makes it an interesting option for Pecten. As expected, the 4 Netron® collector, consisting of more substrate, collected the most scallop spat but also the most *Hiatella* than all other designs. However, Pecten could be advised that 2 Netron® collectors also collected economically viable amounts of scallop spat, but at half the cost of 4 Netron® collectors.At both sites, *Hiatella* counts were lower than scallop counts in November 2003 but were two to nine times the amount of scallop counts in June 2004. Another sample will be taken at both sites in the fall of 2004, just prior to the seeding activities, and will shed more light on what to expect as final collection results from the different designs. It would be interesting to conduct trials with larger mesh sizes than were used here.

Conclusion

- Commercial collectors (largest mesh size) gave good results in terms of scallop spat settlement and least *Hiatella*.
- Black fuzzy rope collected lower numbers of scallop and *Hiatella*.
- *Hiatella*, mussels, brittle stars, sea stars, cockles, macoma, worms, jingle shells, foraminifers, and *Mitrella* were also found in collectors.
- Mesh size of onion bags used to construct collectors may be important to reduce *Hiatella* collection.

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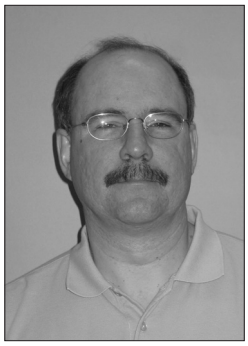
Bilan des Activités de Captage du Pétoncle Géant (*Placopecten magellanicus*) Réalisées entre 1999 et 2004 en Gaspésie, Québec



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Les premières études sur le captage du pétoncle géant en Gaspésie furent effectuées par des promoteurs privés au milieu des années 1980 et par des équipes universitaires au début des années 1990 dans un nombre restreint de sites. Plus récemment, de 1999 à 2001, une relance des activités de recherche et développement sur le captage a été réalisée tout le long de la côte sud de la Péninsule de la Gaspésie, entre Miguasha et Gaspé, et de façon plus ciblée depuis 2002 dans deux secteurs offrant de bons succès de captage. Au cours de ces années, la répartition spatiale et temporelle des deux espèces de pétoncle (*Placopecten magellanicus* et *Chlamys islandica*) et des espèces associées ont été regardées. Selon les années, les suivis des capteurs ont été réalisés après trois mois ou après dix mois et plus d'immersion. Des différences notables entre le nombre de pétoncles ont été observées après ces deux périodes de suivi. Seuls les capteurs immergés au moins dix mois ont permis de distinguer le pétoncle géant du pétoncle d'Islande. Les meilleurs succès de captage du pétoncle géant, soit une moyenne de 1200 à 3400 pétoncles géants/capteur, ont été obtenus dans les baies Tracadigache et de Gaspé. Le nombre moyen de naissains de pétoncle/capteur diminue en fonction de la durée de la période d'immersion. Selon le secteur, ces diminutions peuvent représenter jusqu'à 80 % des naissains captés initialement. Le patron de distribution verticale du captage du pétoncle géant entre 2 et 8 du fond, change d'une année à l'autre. Des résultats contradictoires sont observés entre les deux années des essais de détermination de la période du pic de captage et des essais supplémentaires seront nécessaires.

The first studies regarding Giant scallop spat collecting activities in Gaspésie were done by the private sector in the mid 1980s and by universities in early 1990s in a small part of the peninsula. Between 1999 and 2001, new activities were realized along the coast from Miguasha to Gaspé. Since 2002, more precise factors were examined in the two most successful sectors. Within the last two years, the vertical and temporal distributions of the two scallop species (*Placopecten magellanicus* and *Chlamys islandica*) and other associated organisms were examined. In some years, the collecting success was evaluated after three and ten months of immersion. Some differences were noticed between these two periods. Only the collectors immersed for ten months allowed easy distinction of the two scallop species. In the two sectors of interest, Bays of Tracadigache and Gaspé, a mean of 1200-3400 giant scallops/bag was collected in the best sites. The mean number of scallop spat per bag decreased as immersion time increased. Less obvious in Gaspé, this decrease between the autumn and following summer could be as high as 80% in Tracadigache. The distribution patterns changed with sector and year, so it is remains difficult to distinguish a pattern in the vertical distribution of giant scallop spat from 2-8 m off bottom. This kind of opposite result is observed in the temporal collecting success and further analyses and trials may be needed in the future.

Introduction

Depuis le début de ces essais de captage du pétoncle géant en Gaspésie[1-2] au milieu des années 1980, le potentiel d'approvisionnement naturel en naissain a grandement été amélioré. L'approvisionnement en juvéniles est une étape importante de toutes activités d'aquaculture[3-4] mais cette activité est contrainte par les restrictions administratives qui ne cessent de restreindre les transferts entre régions en raison des risques de contamination de transfert d'organismes. Ce fait incite l'industrie à utiliser des sources locales d'approvisionnement afin de pourvoir au développement de leurs activités pectinicoles. C'est pourquoi depuis l'automne 1999, de nouveaux travaux ont été entrepris afin d'évaluer le succès de captage du pétoncle géants le long de la côte de la péninsule de la Gaspésie.

Matériaux et Méthodes

Entre 1999 et 2002, quatre lignes de trois capteurs ont été immergées à 14 sites répartis entre Miguasha et Gaspé; le long de la rive nord de la baie des Chaleurs. Les capteurs ont été immergés peu de temps après la ponte, soit vers la fin août et le début septembre selon les résultats du suivi de l'indice gonadosomatique. Les capteurs, composés de quatre bandes de Netron® insérées dans un sac de type japonais de 3 mm, étaient maintenus à 2 m au-dessus du fond à des sites de près de 18 m de profondeur.

Deux de ces lignes étaient récoltées après quelques mois, entre octobre et décembre, et les deux lignes restantes à l'été suivant, entre juin et août. La première récolte permettait de dénombrer le total des pétoncles spp. présents et la deuxième série des capteurs après 10 mois, permettait de dénombrer par espèce l'abondance des deux espèces de pétoncle présents : pétoncle géant (*Placopecten magellanicus*) et le pétoncle d'Islande (*Chlamys islandica*).

À partir de août 2002, les sites expérimentaux ont été regroupés autour des deux principaux secteurs ayant offert les meilleurs résultats de captage: les baies de Tracadigache et de Gaspé. Afin d'optimiser le potentiel de ces deux secteurs, huit sites par secteur ont été explorés avec des lignes de capteurs identiques aux années antérieures et à un de ces sites, des lignes verticales d'une série de 12 capteurs répartis à quatre profondeurs (2, 4, 6, et 8 m du fond) ont été immergés. À un site, des lignes de trois capteurs maintenus à 2 m du fond, ont été immergées toutes les deux semaines à cinq reprises entre la mi-août et la fin octobre.

Résultats/discussion

Généralement la ponte des pétoncles géants a lieu entre la deuxième semaine et la fin d'août dans la péninsule gaspésienne, toutefois l'indice gonadosomatique varie sensiblement selon les secteurs et l'année du suivi. Les succès de captage moyen mesuré avec les capteurs à la première récolte en décembre variait de 7800 à 13 600 pétoncles/capteur dans les baies de Tracadigache et Cascapédia (TC: Miguasha, St-Omer, Carleton et New Richmond) et de 5100 à 7700 pétoncles/capteur aux sites de la baie de Gaspé (BG: Cap-aux-Os, Douglastown, Haldimand et Fort Péninsule). À partir des capteurs récupérés à l'été suivant, soit après 10 mois d'immersion, nous évaluons que l'on ne récupère par apport aux pétoncles présents à l'automne précédant que 23 % des pétoncles aux sites de TC et 79 % aux sites de la BG.

Malgré de fortes variations annuelles du captage du pétoncle géant, les sites de TC ont maintenu des taux de captage moyens de 1100 à 2200 et ceux de la BG de 1200 à 3000 pétoncles géants/capteur (Fig. 1). Dans ce dernier secteur, le site de Cap-aux-Os a été être exclus, en raison du très faible succès de captage. Les taux de captage obtenus à ce site étaient comparable aux sites du centre de la péninsule entre Bonaventure, Paspébiac, Newport, Grande-Rivière, Coin-du-Banc et Barachois, soit de 99 à 444 pétoncles géants/capteur. Les sites du fond de la baie des Chaleurs, incluant ceux de TC, ont eu les plus fortes proportions de pétoncles géants, soit plus de 80 %, contre seulement près de 50 % dans la BG. Finalement, entre les deux secteurs de TC et BG, se sont la combinaison d'une équivalente et importante fixation de pétoncles dès le premier automne, des différences dans la proportion récupérée de naissain après 10 mois et de la proportion de pétoncles géants très différente qui contribuent à expliquer les taux de captage moyen semblables dans les deux secteurs.

Les résultats de la distribution verticale du captage de naissain de pétoncles de 2003 et 2004, ont données des tendances contradictoires. Le taux de captage moyen est resté supérieur à 750 pétoncles géants/capteur aux quatre profondeurs étudiées et atteint même près de 4000 à 2 m du fond lors du tri en 2003. À ces quatre profondeurs le succès de captage, qui était inférieur dans la baie Tracadigache les deux années; se maintenait entre près de 300 à 2500 pétoncles géants/capteur. En 2004, le succès de captage de Tracadigache a chuté progressivement de près de 550 pétoncles géants/capteur à 2 m jusqu'à près de 250 à 8 m du fond (Fig. 2a). Cette même année, cette tendance n'a pas été observée dans la baie de Gaspé et le taux de captage était d'environ 850 pétoncles géants/capteurs aux quatre profondeurs étudiées.

Les résultats des suivis de la fenêtre de captage des pétoncles de la baie Tracadigache ont montrés que le taux maximum de captage du pétoncle géant se situait à la mi-septembre en 2003 (Fig. 2b). À Gaspé, par contre tous les capteurs immergés entre la mi-août et la mi-octobre ont donné des résultats comparables, soit environ 1400 pétoncles géants/capteur. Les résultats de 2002-2003 indiqueraient toutefois des variations annuelles de la fenêtre de captage.

Les pétoncles d'Islande ont toujours été plus abondants dans le secteur de Gaspé. Il est difficile d'établir une période d'immersion des capteurs qui permettrait de minimiser le captage du pétoncle d'Islande tout en maximisant le succès de captage du pétoncle géant. La position des capteurs dans la colonne d'eau ne permet pas de ségréguer les deux espèces de pétoncle.

Conclusion

Malgré de fortes variations inter-annuelles, les taux de captage de pétoncles géants sont restés élevés dans les deux secteurs des

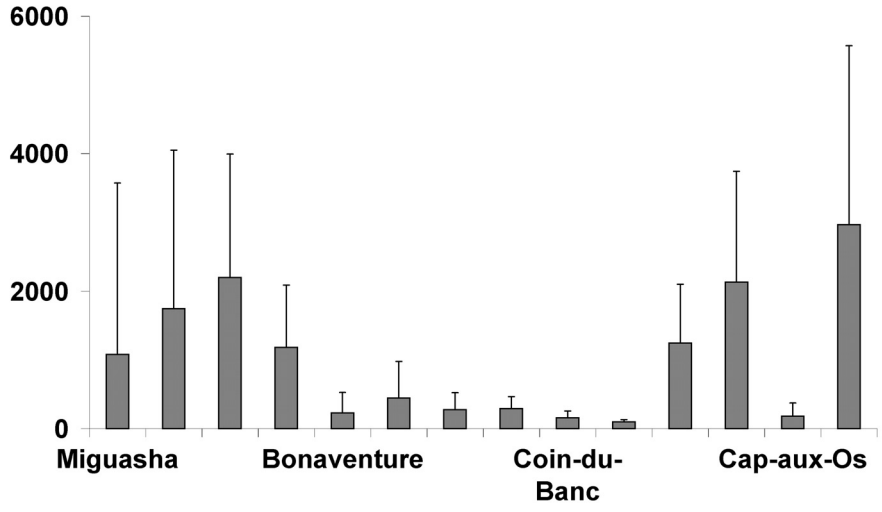

Figure 1. Nombre moyen de pétoncles géant/capteur (± écart type) aux 14 sites de la Gaspésie, de 1999 à 2002 et en 2004 (données de 2003 non intégrées).

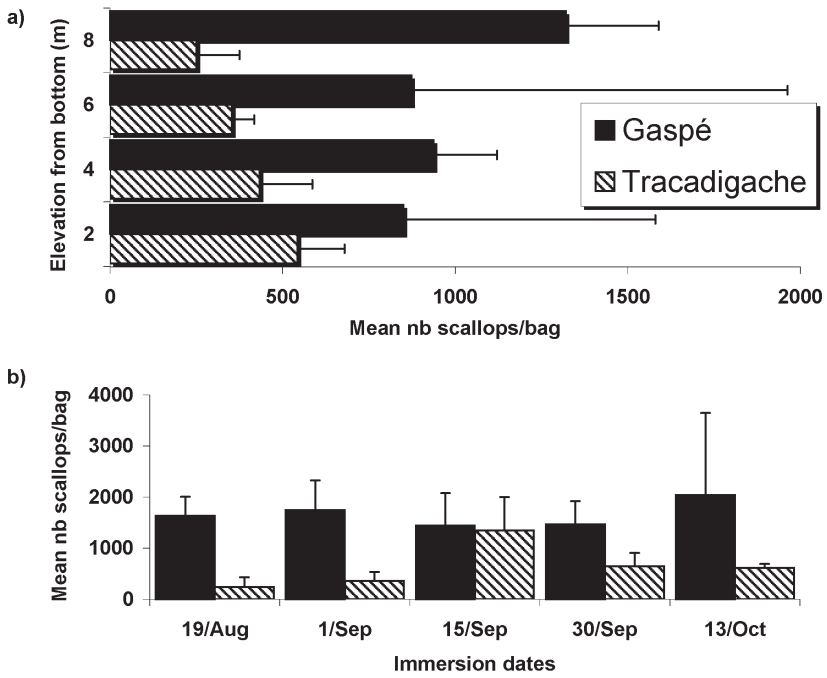

Figure 2.
Nombre moyen de pétoncles géants/capteur (± écart type) à Gaspé et Tracadigache a) selon le niveau des capteurs au dessus du fond et b) selon la date d'immersion en 2003.

baies de Gaspé et de Tracadigache. Entre ces deux secteurs, les résultats ont été inférieurs au nombre de 500 pétoncles géants/capteurs qui sont généralement recherchés par l'industrie. Le succès de captage obtenu dans le secteur de Gaspé était plus élevé au cours des deux dernières saisons échantillonnées. Les résultats contradictoires obtenus lors des deux années des essais ne permettent pas de statuer précisément sur la meilleure profondeur ainsi que la meilleure date d'immersion des capteurs. Les résultats indiquent toutefois qu'une immersion des capteurs les deux premières semaines de septembre à des profondeurs entre 2 et 8 m du fond seraient en mesure de satisfaire aux besoins d'une entreprise pectinicole. Un suivi des tendances sur quelques années permettrait de raffiner les stratégies d'approvisionnement pour les activités commerciales.

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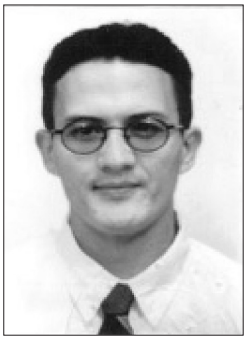
Optimisation de la Production de Microcapsules de Phytase par Séchage par Atomisation



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L'émission du phosphore dans les effluents piscicoles est mise en cause dans le phénomène d'eutrophisation, menaçant l'avenir de la pisciculture au Québec. Le but de ce travail est de réduire les rejets de phosphore par l'encapsulation de la phytase (thermo-instable) et son incorporation dans des régimes à base de protéines végétales chez la truite Arc-En-Ciel. La méthode d'encapsulation par atomisation a été choisie pour sa fiabilité et sa reproductibilité. La BSA (sérum albumine bovin- fraction V) a été utilisée comme protéine modèle, afin de mettre au point la méthode d'encapsulation de la phytase, basée sur l'atomisation de l'alginate. Les rendements de production de microcapsules d'alginate chargées de BSA étaient meilleurs avec une pression de 3 bar vs 4 bar et / ou avec un débit de 0.45 ml/sec vs 0.2 ml/sec. Contrairement au rendement, un débit élevé (0.45 vs 0.2 ml/s) a conduit à la production de microcapsules caractérisées par un moindre relargage de la BSA. L'augmentation de la température d'atomisation a permis l'obtention de microcapsules induisant un pourcentage de relargage de la BSA plus élevé. De cette expérience, trois traitements ont servi pour l'encapsulation de la phytase comportant des températures d'atomisation à espacements égaux (150, 175, et 200ºC). Les rendements de production de microcapsules d'alginate chargées de phytase semblaient similaires entre les traitements (40.52, 40.5, et 39.1%). Le relargage de la phytase dans de l'HCl (pH=1.5) a été faible définissant une activité de la phytase aussi faible. Par contre, le relargage de la phytase dans une solution tampon (pH=5.5) était plus important. Ainsi, la technique de séchage par atomisation permet l'encapsulation de la phytase tout en préservant son activité. Par contre, son relargage dans de l'HCl 0.1N est très faible. Afin d'évaluer le relargage et l'activité de la phytase in vivo, un essai de digestibilité est en cours de réalisation.

Phosphorus emissions in fish farm effluents are implicated in the phenomenon of eutrophication, threatening the future of fish farming in Quebec. The objective of this project is to reduce phosphorus emissions by encapsulating phytase (heat unstable) and incorporating it in plant protein-based diets for rainbow trout. The spray drying encapsulation method was chosen for its reliability and reproducibility. BSA (bovine serum albumin - fraction V) was used as a model protein, in order to perfect the method of phytase encapsulation based on the spray drying of alginate. Production yields of BSA-loaded alginate microcapsules were higher at 3 bar vs. 4 bar and/or a flow rate of 0.45 mL/sec vs. 0.2 mL/sec. Contrary to the yield results, a high flow rate (0.45 vs. 0.2 mL/s) resulted in the production of microcapsules characterized by a lower release of BSA. Increasing the spray drying temperature produced microcapsules inducing a higher percentage of BSA release. In this experiment, three treatments involving equally spaced spray drying temperatures (150, 175, and 200ºC) were used for phytase encapsulation. Production yields of phytase-loaded alginate microcapsules appeared to be similar between treatments (40.52, 40.5, and 39.1%). Phytase release in HCl (pH=1.5) was low, defining an equally low phytase activity. However, phytase release in a buffer solution (pH=5.5) was higher. Thus, the spray drying technique allows for the encapsulation of phytase while preserving its activity. However, phytase release in HCl 0.1N is very low. A digestibility trial is under way to evaluate phytase release and activity in vivo.

Introduction

L'intensification de la production piscicole[(1)] et l'utilisation d'ingrédients riches en phosphore[(2)] ont fini par rendre, au Québec, les effluents des stations piscicoles l'une des sources de pollution hydrique générée par le phosphore[(3)] conduisant à l'eutrophisation des milieux aquatiques[(4,5)].

Une alternative à ce mode d'alimentation est l'usage de sources de protéines végétales à faible teneur en phosphore. Toutefois, ce phosphore est à 75% sous forme phytique[(6)]. Les poissons, comme les animaux d'élevage monogastriques, ne sont pas capables d'utiliser le phytate. Ceci implique la nécessité d'introduire du phosphore inorganique comme additif alimentaire accentuant de ce fait les rejets du phosphore dans les effluents piscicoles.

De nombreux travaux[(7-9)] ont démontré le rôle que pourrait jouer la phytase (enzyme microbienne) dans l'amélioration de la disponibilité du phosphore provenant de sources végétales (phytate). Toutefois, cette enzyme est sensible aux hautes températures générées lors des procédés de fabrication alimentaire[(10,11)]. Ce qui rendu nécessaire la mise en œuvre de technologies adéquates afin de protéger et de maintenir son activité enzymatique.

Au cours des cinquante dernières années, beaucoup de techniques d'encapsulation ont été développées, y compris la séparation de phase, l'évaporation dissolvante, la gélification thermique et ionique, et la méthode de séchage par pulvérisation[(12)]. En particulier, la technique de séchage par atomisation est largement répandue pour la microencapsulation des molécules actives en raison de sa fiabilité, de la reproductibilité des résultats, et de la possibilité de

contrôler la dimension des microcapsules et le relargage des produits encapsulés. Il s'agit en fait d'un processus continu, facile à mesurer et qui dépend légèrement de la solubilité de la molécules active et du polymère[13]. Par ailleurs, les microcapsules obtenues par le séchage par pulvérisation sont, habituellement, exemptes de solvants organiques, contrairement à d'autres méthodes qui engendrent des particules souillées avec des copolymères probablement toxiques.

Parmi de nombreux polymères utilisés comme matrice d'encapsulation, les alginates ont été largement étudiés pour des applications biomédicales dues à leur capacité à former des gels dans des conditions relativement modérées. De ce fait, ils permettent de maintenir l'activité biologique du matériel encapsulé[14]. Les alginates sont un groupe de polysaccharides appartenant à une famille de copolymères binaires disposés linéairement en blocs avec une alternance de sucres mannuroniques et guluroniques[15]. Le but de ce travail est de réduire les rejets de phosphore par l'encapsulation de la phytase (thermo-instable) et son incorporation dans des régimes à base de protéines végétales chez la truite Arc-En-Ciel. La BSA (sérum albumine bovin-fraction V) a été utilisée comme protéine modèle, afin de mettre au point la méthode d'encapsulation de la phytase, basée sur l'atomisation de l'alginate.

Matériel et Méthodes

Réactifs

Le polymère utilisé pour l'encapsulation est un composé comportant du Sodium alginate, des sucres, du trisodium citrate, et du dicalcium phosphate (Manugel L98; ISP corporation, Ayrshire, Scotland, United Kingdom). La phytase microbienne est un acide phosphomonoesterase non spécifique (Natuphos; BASF Canada Corp., Richelieu, QC,). Le dosage des protéines a été réalisé par la méthode Coomassie (Pierce Inc., New York, NY, USA). Les autres produits chimiques ont été achetés chez Sigma (St. Louis, USA).

Paramètres d'encapsulation de la phytase

Une expérience préliminaire fût adoptée pour mettre au point la technique d'encapsulation de la phytase par l'optimisation de la production de microcapsules d'alginate chargées de BSA. Cette dernière a été choisie comme protéine modèle.

Il a été montré qu'un débit élevé (0.45 vs 0.2 ml/s) et/ou une moindre pression (3 vs 4bar) permettent un meilleur rendement de production de microcapsules d'alginate chargées de BSA. Également, une température d'atomisation élevée (200°C vs 175 et 150°C), et/ou un débit de 0.2 vs 0.45ml/s conduisent à l'obtention de microcapsules avec une capacité de relargage de la BSA plus importante. Ces résultats nous ont permis de sélectionner les traitements avec lesquels on a obtenu, simultanément, un meilleur rendement de production de microcapsules et un relargage adéquat de la BSA. Ces traitements choisis comportent des températures à espacements égaux de 150, 175, et de 200°C respectivement pour les traitements T1, T5, et, T10.

En parallèle, des particules d'alginate chargées de phytase ont été conçues par séchage à l'air libre (PSA) à une température de 22°C. Une telle température est supposée préserver la nature native de la phytase. Les deux composés ont été mélangés dans le but de concevoir des particules de 400 FTU/g d'alginate.

Paramètres à quantifier

Les paramètres à analyser sont : le rendement de production des microcapsules qui est défini par le rapport entre la quantité de microcapsules obtenues et la quantité d'alginate utilisée (2% w/v), le pourcentage de relargage de la phytase, l'activité de la phytase, et la température de sortie relevée sur le cadrant de l'atomiseur.

Relargage de la phytase

L'incubation des microcapsules de phytase a été suivie pendant 8 h dans 10 ml de solution d'HCl 0.1 N simulant les conditions de pH stomacal des poissons. Pour tous les traitements, l'incubation a été conduite avec un rapport de 0.04 g de microcapsules/10ml d'HCl 0.1N. Des prélèvements de 100 µl de surnageant ont été effectués tout les 2mn, 30mn, 1h, 2h, 4h, et 8h et ceci après une centrifugation durant 5mn à 12 000 RPM. Le dosage de la phytase a été conduit conformément au protocole de dosage des protéines par la méthode «Coomassie» avec une gamme de détection du réactif de 1 à 25 µg /ml.

Activité de la phytase

L'activité de la phytase a été vérifiée sur le surnageant récupéré après 8 h d'incubation des microcapsules, correspondant à la durée moyenne du transit digestif du poisson à 10°C. Elle a été évaluée par BASF, selon le protocole d'analyse de l'activité de la phytase énoncé par BASF Corporation[16]. L'étude de l'activité de la phytase a été conduite, dans un premier temps, sur les échantillons issus après 8h d'incubation des microcapsules provenant des traitements T1, T5, et T10 dans de l'HCl 0.1N. Par ailleurs, l'activité de la phytase a été évaluée sur la solution issue après 8h d'incubation des microcapsules du T10 en comparaison avec celle issue des particules obtenues par séchage à l'air libre (PSA) dans de l'HCl 0.1N avec ou sans addition d'EDTA. Ce dernier est ajouté comme agent chélateur des ions bivalents. Ce qui permettrait d'ouvrir la structure de l'alginate et de ce fait faciliter la libération de la phytase encapsulée.

De plus, les PSA et les microcapsules issues du T10 ont été incubées dans une solution tampon (substrate buffer) avec ou sans addition d'EDTA. L'EDTA a été ajouté, que ce soit dans de l'HCl ou dans la solution tampon, de manière à obtenir une solution finale de 0.05M d'EDTA.

Dispositif expérimental

Pour l'essai d'atomisation, le rendement de production de microcapsules n'a pas été pris en considération dans l'analyse statistique. Les tests statistiques ont concerné uniquement les données de relargage et d'activité de la phytase.

Concernant les essais de relargage et d'activité de la phytase, deux expériences ont été conduites:

La première expérience a été suivie pour la détermination du relargage et de l'activité de la phytase des microcapsules issues par séchage par atomisation. Elle a été menée selon un dispositif en bloc complet. Les données obtenues n'ont pas fait l'objet d'analyse statistique du fait des très faibles valeurs obtenues.

La deuxième expérience a été menée pour déterminer l'activité de la phytase des microcapsules issues par atomisation (T10) en comparaison avec les PSA. L'expérience est factorielle avec 3 facteurs (2 × 2 × 2):

- Le type d'encapsulation avec 2 niveaux: séchage par atomisation (T10); séchage à l'air libre (PSA).
- La solution de relargage avec 2 niveaux: solution d'HCl 0.1N (pH=1.5) ; solution tampon (pH=5.5).
- EDTA avec 2 niveaux: avec et sans ajout d'EDTA.

L'expérience a été menée en un dispositif en blocs complets, chacun des 3 blocs a compris les 8 traitements complètements randomisés.

Analyse statistique

Les donnés ont été analysées à l'aide de la procédure GLM du logiciel SAS. La comparaison des moyennes a été faite par des contrastes polynomiaux au seuil de 5%.

Résultats et Discussion

Les traitements appliqués semblent aboutir à des rendements similaires de production de microcapsules d'alginate chargées de phytase (40.52, 39.1, et 40.53 respectivement pour le T1, T5, et T10). Les températures de sortie enregistrées avec le T1 et le T10 (66.5 et 65.6°C) ont été relativement meilleures que celle obtenue avec le T5 (74.44°C). Étant donnée que la phytase est thermo-instable son activité est atténuée au delà de 70 °C[10].

Les résultats du relargage de la phytase, issu de l'incubation des microcapsules obtenues par atomisation (T1, T5, et T10) ou des PSA dans de l'HCl 0.1N en fonction du temps, ont été très faibles.

L'activité de la phytase enregistrée, suite à 8h d'incubation des microcapsules chargées de phytase dans de l'HCl 0.1N, a montré des valeurs très faibles de l'ordre de 0.12%, 0.08%, et 0.06% de l'activité attendue (400 FTU/g), respectivement pour les microcapsules issus du T1, du T5, et du T10. Ces faibles résultats pourraient être dus à une perte d'activité de la phytase lors du procédé de séchage par atomisation. En effet, le contact de la phytase avec les hautes températures de séchage (150, 175, et 200°C) pourrait entraîner la perte de son activité[11]. La phytase étant thérmo-instable, son pouvoir de déphosphorylation du phytate pourrait être atténué ou inhibé au contact d'une température supérieure à 70°C[10].

Toutefois, l'activité de la phytase a également été très faible pour les particules obtenues par séchage à l'air libre (22°C). Ceci nous conduit à suggérer que, la phytase n'a pas été libérée par l'alginate et interagit donc avec ce dernier. Par ailleurs, l'activité de la phytase enregistrée suite à son relargage par les PSA dans une solution tampon a été faible en comparaison avec le T10 (P=0.0029) (Fig. 1). Ainsi, contrairement à la forme sphérique des microcapsules issues par atomisation, la structure compacte des PSA semble empêcher le relargage de la phytase. En effet, il semblerait que, plus la phytase est entourée par d'épaisses couches d'alginate plus son relargage serait difficile.

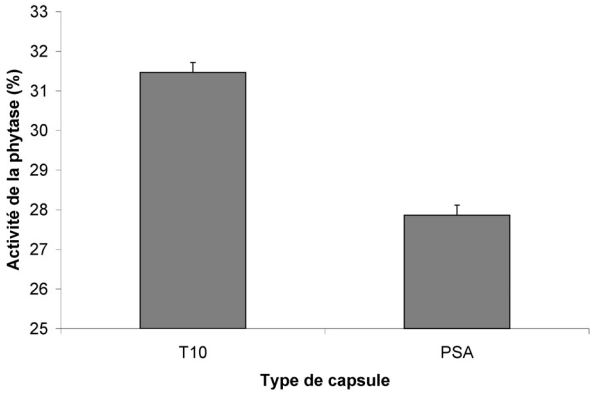

Figure 1.
Relargage de la phytase par les microcapsules obtenues par atomisation et par séchage à l'air libre.

Pour confirmer cette supposition, les essais avec l'EDTA ont été conduits. Le relargage de la phytase, dans de l'HCl 0.1N avec ajout d'EDTA, a montré une augmentation de l'activité de la phytase de 98% et de 96%, respectivement pour le T10 et les PSA par rapport à l'activité enregistrée suite au relargage de la phytase dans de l'HCl 0.1N sans ajout de l'EDTA (Fig. 2).

Cette augmentation marquée de l'activité suite à l'addition de l'EDTA a montré que, le relargage de la phytase ne s'est pas déroulé conformément à celui enregistré au cours de l'expérience préliminaire avec la BSA. En effet, les pourcentages de relargage de la BSA ont été de 74.42, 75.30, et de 74.32% respectivement pour T1, T5, et T10 et ceci après 8h de relargage des microcapsules de BSA dans de l'HCl 0.1N.

Toutefois, l'application de l'EDTA a permis un moindre relargage de la phytase conduisant à une très faible augmentation de l'activité enzymatique (1.68% et 0.79%, respectivement pour T10 et PSA) par rapport à l'activité attendue (400 FTU/g). Ainsi, l'EDTA a permis une légère décapsulation de l'alginate faisant ainsi augmenter dans une moindre mesure le relargage de la phytase.

Contrairement à l'incubation dans de l'HCl 0.1N, l'incubation des microcapsules et des PSA dans la solution tampon avec ou sans ajout d'EDTA a conduit à une activité de la phytase plus élevée (P<0.001) (Fig. 2). Cette augmentation a été de 60.8% et de 58.27% pour le T10, et de 55.97% et de 52.29% pour les PSA, respectivement avec et sans ajout d'EDTA, par rapport à l'activité attendue (400 FTU/g). Ainsi, l'EDTA a eu un moindre effet sur la valeur du relargage de la phytase par rapport à la solution d'incubation (HCl vs. solution tampon).

Un tel schéma de relargage pourrait suggérer deux hypothèses. La première suggère que, la phytase a été dénaturée lors de son incubation à pH=1.5 (HCl 0.1N). La phytase étant une protéine, elle est de ce fait susceptible à l'hydrolyse ou à la désamination en présence d'un acide fort[17].

Cependant, la phytase est largement incorporée dans les aliments d'élevage de monogastriques[18-23]. Les résultats obtenues sur le plan de l'amélioration de la disponibilité du phosphore phytique et des autres nutriments suite à l'incorporation alimentaire de la phytase, indiquent que, son activité enzymatique est loin d'être inhibée et que la structure protéique de la phytase est aussi loin d'être dénaturée par le pH gastrique des monogastriques.

La deuxième hypothèse, imputerait le faible relargage enregistré dans de l'HCl 0.1N à des liaisons électrostatiques qui auraient pu être établies entre l'alginate et la phytase. En effet, les sites actifs contiennent typiquement des groupes qui sont assujettis à l'ionisation (Arg, Lys, His, Glu, et Asp) et qui déterminent le profil de pH d'activité d'une enzyme[24]. Le point isoélectrique de la phytase est de 4.5[25]. Donc, à pH=1.5 les acides aminés basés à l'interface entre la macromolécule et le milieu aqueux dans lequel ils baignent seront chargés positivement. L'alginate étant chargée négativement, finit par se lier à la phytase par des liaisons électrostatiques. Ces liaisons impliquent les charges négatives des carboxyles de l'alginate et les charges positives des groupements amines de la phytase.

La solution tampon comporte un pH=5 qui maintient la phytase dans un pH supérieur à son point isoélectrique (pH=4.5). Un tel pH a conduit à un renversement des charges globales portées par les différentes formes ioniques des acides aminés, composant la phytase, vers le négatif. Ce qui a permis au complexe phytase-alginate de se repousser libérant de ce fait la phytase. En effet, les polysaccharides qui possèdent des groupes carboxyliques ne complexent pas les protéines globulaires à des valeurs de pH largement supérieures au point isoélectrique des protéines.

Conclusion

Au terme de cette étude, nous pouvons dire que la méthode de séchage par atomisation permet la production de microcapsules d'alginate chargées de phytase tout en préservant son activité. Toutefois, le relargage de la phytase dans de l'HCl 0.1N est limité par les liaisons électrostatiques élaborées (au-dessous du point isoélectrique de la phytase) entre la phytase et l'alginate à pH=1.5.

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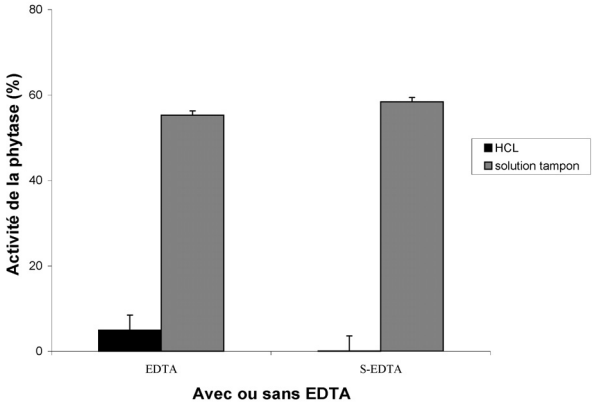

Figure 2.
Effet de la solution de relargage et de l'EDTA sur le relargage de la phytase.

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Effect of Dietary Levels of Herring Meal on Apparent Protein Digestibility by Juvenile Haddock, *Melanogrammus aeglefinus* L.

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A digestibility study was conducted to investigate the effect of incorporating different levels of herring meal into test diets on apparent protein digestibility. Herring meal was incorporated at levels of 10, 20, 30, 40, and 50% to 90, 80, 70, 60, and 50% of a reference diet, respectively. The reference and test diets were fed to the fish to apparent satiation three times daily on weekdays and twice daily on weekends. The fish were acclimated to the experimental diets for one week and fecal collections were made for three weeks. Feces collected in the same bottle from each tank for five days a week were used as one replication for the treatment. Protein digestibility of the reference diet was 94.8%. The increase in incorporation level of herring meal from 10-50% into the test diet had no significant effect on protein digestibility of the feed ingredient, which remained constant at an average of 93.7%.

Nous avons fait une étude de digestibilité visant à établir l'effet de l'apport, dans des rations alimentaires expérimentales, de différentes teneurs en farine de hareng sur la digestibilité apparente des protéines. De la farine de hareng a été ajoutée à des niveaux de 10, 20, 30, 40 et 50 % à 90, 80, 70, 60 et 50 % d'une ration alimentaire de référence, respectivement. Les poissons ont été nourris, trois fois par jour en semaine et deux fois par jour en fin de semaine, des rations expérimentales et de référence jusqu'à satiété apparente. Ils ont été acclimatés aux rations expérimentales pendant une semaine, et des prélèvements de fèces ont été faits pendant trois semaines. Les fèces recueillies dans le même flacon de chaque bassin pendant cinq jours en semaine ont servi de réplique du traitement. Le coefficient d'utilisation digestive des protéines de la ration de référence se situait à 94,8 %. L'augmentation du niveau d'apport en farine de hareng de 10 à 50 % dans la ration expérimentale n'a pas eu d'effet significatif sur le coefficient d'utilisation digestive des protéines, qui est demeuré constant à un taux moyen de 93,7 %.

Introduction

Apparent digestibility coefficient (ADC) values of feed ingredients for fish diets have been indirectly calculated using a mixture of reference diet with an inert external indicator (chromic oxide, Cr_2O_3) and test feed ingredient at the level of 70:30. Cho et al.[1] obtained the ADC values of various feed ingredients for rainbow trout using this same mixture. They collected fecal material from a settling column and suggested that any interaction between the reference diet and the test feed ingredient would not exist. Consequently, the ADC values of feed ingredients for several other aquaculture species (channel catfish[2], Chinook salmon[3], striped bass[4], and red drum[5]) have been determined using the same mixture and a practical fish meal based reference diet. Recently, Cheng and Hardy[6] measured the ADC values of various poultry by-products using the same mixture, however, the reference diet was purified, based on casein-gelatin rather than fish meal.

The incorporation level of various feed ingredients in fish feeds will depend on their physiochemical characteristics. Fish meal is generally kept at more than 30%, while wheat products are generally less than 15% in carnivorous fish feeds. Nonetheless, a conventional 70:30 mixture of reference diet and test feed ingredient is still employed to determine the ADC of feed ingredients. Because a variation in dietary incorporation level may affect the digestibility of each feed ingredient, it is recommended that the ADC values be evaluated based upon their actual incorporation level into a diet. The objective of this study is to investigate the effect of different incorporation levels of herring meal into reference diet on the protein ADC values for juvenile haddock calculated by the equation of Cho et al.[1] and a new equation by Forster[7].

Materials and Methods

The reference diet was formulated to contain 48% crude protein and 15% lipid with 17 MJ digestible energy[8] (as-fed basis). After the reference diet was thoroughly mixed, the test feed ingredient, herring meal (HM), was incorporated at levels of 10, 20, 30, 40, and 50% (w/w basis) to 90, 80, 70, 60, and 50% of the reference diet, respectively. All diets had Cr_2O_3 incorporated at 1%. The test diets were designated as HM10, HM20, HM30, HM40 and HM50 following mixed levels of reference to test feed ingredient. Before starting the feeding trial, the fish were acclimated to the experimental conditions for 3 weeks. During this period, they were fed a commercial diet (Shur-Gain Feeds

Composition (g/100 g DM)	Diet					
	Reference	HM10	HM20	HM30	HM40	HM50
Diet						
Protein	50.64	53.96	56.91	59.95	63.28	66.53
Cr_2O_3	1.04	0.99	0.96	0.76	0.69	0.56
Feces						
Protein	12.67	14.80	17.15	19.66	21.05	21.39
Cr_2O_3	5.05	5.13	5.35	4.51	4.14	3.17
Protein ADC (%)	94.8	94.7	94.6	94.5	94.4	94.4

Table 1.
Composition and apparent protein digestibility coefficients (ADC) of the reference and test diets.

Haddock Ration) having 51.6% crude protein, 15.7% lipid and 23.6 MJ/kg gross energy. Following a 24-h fasting, 240 fish (mean weight, 49.4 g) were randomly distributed into each of six 100-L capacity tanks (40 fish/tank) and bulk-weighed. Water temperature was maintained thermostatically at 12.2 ± 0.4°C and dissolved oxygen was maintained above 7.5 mg/L during the whole period of the experiment. During the four-week experimental period, fish were hand-fed to apparent satiety three times daily during the week (0900, 1200, 1500 h) and twice daily on weekends (0900, 1200 h). Following one week of feeding, fecal collections were made for 3 consecutive weeks. Thirty minutes after the final feeding of the day, the drain pipes and fecal collection columns were thoroughly cleaned with a brush to remove feed residues and feces from the system. The settled feces and surrounding water were carefully collected into 250-mL centrifuge bottles each morning (0830 h). Feces were free of uneaten feed particles and considered to be a representative sample of the feces produced throughout the 17 hour period.

Apparent protein digestibility coefficients for the reference and the test diets were calculated according to Maynard and Loosli[9]. Using these data, protein ADCs in herring meal were then calculated using the equations of Cho et al.[1] and Forster[7]. Protein ADC values were calculated based on the actual mixing levels of the feed ingredient (i) and the reference diet (100-i) taking into account the dry matter contents of herring meal (91.9%) and the reference diet (91.7%) and also the relative contributions of protein from the reference diet and the test feed ingredient to the total protein content of the diet.

Table 2.
Apparent protein digestibility coefficients (ADC) of herring meal* (*Values are means ± SE of triplicate groups; ns = nonsignificant (*P* > 0.05)).

Results and Discussion

Incorporation level of herring meal had no significant effect (*P* > 0.05) on protein ADC of the diet (Table 1) and they averaged 94.6% (range, 94.4-94.8%). In terms of protein ADC of the herring meal test ingredient, the results were the same regardless of the equation used to calculate protein ADC (Table 2). The incorporation level of herring meal had no significant effect (*P*>0.05) on protein ADC of the herring meal and they averaged 93.7% (range, 93.5-93.9%) when calculated by the equation of Cho et al.[1] and 94.0% (range, 93.9-94.1%) when calculated by the equation of Forster[7].

In contrast to the finding that protein digestibility decreases with decreasing incorporation levels of protein sources like fish meal and casein and an increase of starch in diet[8], the values maintained constant in the present study. The results suggest that protein digestibility is not limited by dietary levels of non-nitrogenous components[11,12]. The protein ADC value of herring meal obtained here (93.7%) is in good agreement with those reported using the 80:20 mixture by Watanabe et al.[13] for rainbow trout (93.1%), carp (92.6%), tilapia (92.6%), and Ayu (93.1%). However, the values were slightly lower than those obtained previously using the 70:30 mixture for haddock (95.9%)[14] and grouper (98.0%)[15], respectively. Such variation may be due to difference in fish meal quality and composition of the reference diet.

Fish meal is the best protein source for fish feed formulation. It is incorporated into carnivorous fish diet at levels of 30-60%, providing more than 50% of the total dietary protein. However, ADC of fish meal and other feed ingredients has not been widely studied to date with respect to the fact that the incorporation level could affect the digestibility values. In contrast to our results, De Silva et al.[16] reported that the ADC values for dry matter and protein decreased with increasing levels of incorporation of test feed ingredient (leaf meal) in the reference diet. Spyridakis et al.[17] also found that protein ADC varied with dietary inclusion levels of test feed ingredients. However, Gomes da Silva and Oliva-Teles[18] did not find any significant difference in the ADC values obtained from two incorporation levels (15 and 30%) of the feed ingredients. Even though more re-

ADC (%)	Diet					Average
	HM10	HM20	HM30	HM40	HM50	
Cho et al.[1]	93.5±0.8	93.6±1.2	93.6±0.5	93.8±0.7	93.9±1.2^{ns}	93.7±0.4
Forster[7]	94.0±0.5	94.0±0.9	93.9±0.4	94.1±0.5	94.1±1.0^{ns}	94.0±0.3

search remains to be done, the present results demonstrated that the digestible protein content of herring meal could be additive in juvenile haddock diets once the ADC values are obtained.

Conclusions

It is concluded that the incorporation level of herring meal into the reference diet does not affect the protein ADC values in juvenile haddock diets and that the two commonly used equations for calculating protein ADC result in the same values for highly digestible feed ingredients like herring meal. The protein ADC values remained constant at an average 93.7% (range, 93.5-93.9%) when calculated by the equation of Cho et al.[1] and 94.0% (range, 93.9-94.1%) when calculated by the equation of Forster[7].

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Growth and Survival Improvement of Spotted Wolffish (*Anarhichas minor*) during First-Feeding: A Nutritional Approach

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Spotted wolffish has been recognized as a species of high interest for aquaculture in Quebec's coastal waters. Despite commercial production in northern Norway, this species still requires research for the accurate determination of its specific needs. Subjects of concern include the optimization of growth rates and survival at first-feeding. Adding protein hydrolysates and astaxanthin in the feed could potentially enhance early juvenile growth and survival. Larvae of many fishes including the wolffish begin exogenous feeding before the digestive tract is fully developed. Protein hydrolysates are "pre-digested" polypeptides and are more easily assimilated. The present study is aimed at the determination of the physiological and metabolic responses of wolffish juvenile fed different concentrations of protein hydrolysates and astaxanthin. Metabolic capacity will be evaluated through the measurement of the activity of several metabolic enzymes (aspartate amino-transferase, cathepsin, citrate synthase, lactate deshydrogenase, pyruvate kinase); digestive capacity will be assessed by measuring the activity of trypsin. Our hypothesis is that protein hydrolysates should enhance performances if digestive capacity is a major factor limiting growth rate during early stages.

Le loup tacheté est reconnu comme une espèce présentant un grand intérêt pour l'élevage dans les eaux côtières du Québec. Malgré qu'il fasse l'objet d'un élevage commercial dans le nord de la Norvège ses besoins spécifiques demeurent encore partiellement inconnus. L'optimisation du taux de croissance et de la survie à la première alimentation s'inscrivent parmi les sujets à éclaircir. L'ajout d'hydrolysats de protéines et d'astaxanthine dans les aliments pourrait potentiellement stimuler la croissance et accroître la survie des juvéniles. Les larves de nombreuses espèces de poisson, dont le loup, commencent à s'alimenter avant que le tube digestif ne soit complètement développé. Les hydrolysats de protéines, des polypeptides « pré-digérés », sont plus facilement assimilés. La présente étude vise à déterminer les réactions physiologiques et métabolique des juvéniles de loup tacheté nourris avec différentes concentrations d'hydrolysats de protéines et d'astaxanthine. La mesure de l'activité de plusieurs enzymes métaboliques (aspartate aminotransférase, cathepsine, citrate synthase, pyruvate kinase, lactate déshydrogénase) permettra d'évaluer leur capacité métabolique alors que la mesure de l'activité de la trypsine permettra d'évaluer la capacité digestive. Selon notre hypothèse, les hydrolysats de protéines devraient accroître la performance si la capacité digestive est un facteur limitant fortement la croissance chez les jeunes stades de loup tacheté.

Introduction

Spotted wolffish has been recently identified as a species at risk[1]. Aquaculture production could contribute to the reduction of the pressure exerted on the natural populations by fisheries but could also contribute to the diversification of the economy of areas touched by the collapse of fisheries. Quebec's mariculture industry would benefit greatly from a diversification of its production because the restricted number of species on which the industry actually relies confers a very high level of vulnerability (diseases, competition, collapse of the markets, loss of interest of the consumer)[2]. A study examining the aquaculture potential of various species was carried out in 2002, and the Atlantic wolffish (*Anarhichas lupus*) and spotted wolffish (*A. minor*), were ranked among very high in the final classification[3]. The advantages of raising these fish species resides first in their adaptation to relatively cold water and the low complexity of its larval-juvenile rearing period . The larvae can be fed with formulated food the day of hatching (23 mm, 0.08 g)[4] and are at a relatively advanced stage; the juvenile stage is reached after a few days[5]. This has a great advantage for aquaculture because in other species the larval stage is often fragile and prone to high mortality rates. An international workshop on the development of aquaculture of wolffish was held in Rimouski in 2002[6], which allowed identification of the research priorities necessary to the development of commercial aquaculture of this species. The axes of research to be prioritized are optimization of nutritional and rearing conditions necessary to the stabilization of the performances of the early stages. This species shows very variable rates of survival during the incubation period (0-78%) and during first-feeding (18-50%)[7]. This can be attributed to feed formulation, quality of the eggs and of juveniles, or the development of digestive capacities[7]. Our approach will be to give different formu-

lation of feed at first-feeding ant look at the growth rate, survival, and digestive and metabolic capacities of the fish.

Objectives

- Enhance growth and survival of young stages of wolffish:
  - Evaluate the impact of adding protein hydrolysates and astaxanthin to the feed on survival, growth rate and metabolic and digestive capacity
  - Evaluate the effect of temperature on survival, growth rate and metabolic and digestive capacity
  - Study the interaction between feed and temperature
- Establish links between digestive and metabolic capacity and growth rate.

Materials and Methods

The experiment will be performed at the Centre aquacole marin de Grande-Rivière (CAMGR). Spotted wolffish eggs were incubated at 6°C and hatched in mid November. The eggs originated from Troms Steinbit (Tromsø, Norway), from six different families. At hatching, the fish were randomly distributed among 36 tanks (3 replicates per temperature per diet; approximately 50 fish per tanks). The first week following hatching, survival of the different families was high (>70%). Temperatures tested were 4, 8, and 12°C and feed formulations included a control group, 10 % protein hydrolysates and 100 ppm astaxanthin, 20% protein hydrolysates and 110 ppm astaxanthin, and 110 ppm astaxanthin. The feed was produced at the laboratory facilities of Ifremer-Brest (France).

Five fish per tank will be taken at regular interval (day 0, 5, 15, 30, 45) in order to carry out measurements. These individuals will be weighed, measured, and preserved in a freezer at -80°C until analysis. Enzymatic activity of trypsin, aspartate amino transferase (AAT), pyruvate kinase (PK), lactate deshydrogenase (LDH), cathepsin, and citrate synthase (CS) will be measured in whole fish. The activity of those enzymes will be measured in spectrophotometry in our laboratories. Free amino acids (FAA) will be measured using HPLC techniques.

Discussion and Expected Results

Diet can have a great influence on growth rate[8]. For this purpose, adding protein hydrolysates (pre-digested polypeptides) to food allows an enhancement of the performances of juveniles and the digestibility of the feed[9]. Indeed, in several fish species (including the wolffish) exogenic feeding begins before the digestive system is completely functional. The digestion of intact proteins is thus more difficult[9]. The hydrolysates present a greater bioavailability(10) and improve growth by stimulating the prey capture and digestive functions (stronger activity of trypsin and pepsin)[11]. Studies show that hydrolysates added in various proportions to food increase survival and growth of the larvae of carp[12,13] and common bass[14,15].

Astaxanthin is widely used in the aquaculture industry as a pigment to color the flesh of salmonids. Therefore, in the present study, we will add astaxanthin in the feed to improve performances of the fish. When an organism is subjected to stress, a sudden lack of oxygen causes abnormal reactions in the metabolic pathways, resulting in an overabundance of monovalent oxygen[16] and other reactive oxygen compounds. Those can impair cellular constituents such as membranes and DNA[17]. Carotenoids can inactivate free radicals produced from normal cellular activity and various stressors[18]. Astaxanthin is a carotenoid that has shown to have a very strong antioxydant activity; 100 times greater than α-tocopherol[19]. It has also been recognized that astaxanthin improves the immune status of various species of marine organisms[20,21].

High growth rates occurring in larvae should be sustained by sufficient supply of amino acids which is energetically costly. The enzymatic activity of PK, LDH, and CS will give an idea of the metabolic capacity of the larvae or its capacity to generate ATP. The enzymatic activity of trypsin, cathepsin, and AAT will be a good indicator of protein digestion turnover and oxidation, respectively. It is expected that the hydrolysates and astaxanthin will improve growth and survival of the juveniles. If hydrolysates improve growth, it can be concluded that digestive capacity is the factor limiting growth rate and that wolffish early stages have a specific ability to digest peptides, as it has been shown in other fish species.

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Effect of Dietary Lipid on Prevalence of Fatty Liver Condition in Juvenile Haddock, *Melanogrammus aeglefinus* L.

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Haddock accumulate a high amount of dietary lipid in the liver (>65% lipid, wet weight). The hepatosomatic index (HSI) of farmed haddock may exceed 20 % when the diets contain high amounts of lipid (>12%) and this may affect the overall health of the fish. The present experiment was designed to investigate the effects of several dietary factors (i.e., medium chain and conjugated fatty acids, cholesterol and phospholipid supplements) on minimizing abnormally high lipid accumulation in haddock liver. Duplicate groups of juvenile haddock (7.92 ± 0.06 g, mean initial weight) were fed one of five experimental diets where herring oil was partially replaced with conjugated linoleic acid (CLA), soy lecithin (SL), coconut oil (CO), or cholesterol (CHOL). At the end of the 9-week trial, CLA-fed fish had significantly lower percent weight gain than all other diets (401.51 ± 9.30 %). The hepatosomatic index (HSI) for the CHOL fed fish (10.71 ± 0.12 %) was significantly lower than that of the control diet (11.44 ± 0.14 %). Specific growth rate of fish fed CLA was significantly lower than all other diets (2.21 ± 0.04). No significant differences were found between diets for feed conversion ratio (0.64 ± 0.01 g feed/g gain). Effects of partial replacement lipid source on growth, liver, and muscle lipid level as well as total blood cholesterol are also presented.

L'aiglefin accumule de grandes quantités de lipides alimentaires dans le foie (teneur en lipides > 65 %, poids humide). L'indice hépatosomatique (IHS) de l'aiglefin d'élevage peut dépasser 20 % lorsque le régime alimentaire contient des teneurs élevées de lipides (> 12 %), ce qui peut nuire à la santé du poisson. La présente expérience visait à déterminer les effets de plusieurs facteurs alimentaires (acides gras conjugués à chaîne moyenne, cholestérol et compléments de phospholipides) pour ce qui est de minimiser l'accumulation anormalement élevée chez l'aiglefin de lipides dans le foie. Des groupes doubles d'aiglefins juvéniles (poids initial moyen de 7,92 ± 0,06 g) ont été nourris d'un de cinq aliments dont le contenu en huile de hareng avait été partiellement remplacé par de l'acide linoléique conjugué (ALC), de la lécithine de soja (LS), de l'huile de coco (HC) ou du cholestérol (CHOL). À la fin de l'expérience de 9 semaines, les poissons nourris d'ALC avaient pris significativement moins de poids en pourcentage que tous les autres (401,51 ± 9,30 %), alors que l'IHS des poissons nourris de CHOL (10,71 ± 0,12 %) était significativement moins élevé que celui des poissons du groupe témoin (11,44 ± 0,14 %). Par contre, le taux de croissance spécifique des aiglefins nourris d'ALC était significativement moins élevé que celui de tous les autres groupes (2,21 ± 0,04). Aucune différence significative n'a été relevée entre les régimes alimentaires pour ce qui est de l'indice de consommation (0,64 ± 0,01 g d'aliments/g gain en poids). Les effets du remplacement partiel de la source de lipides sur la croissance, la teneur en lipides dans le foie et les muscles, ainsi que sur la concentration totale du cholestérol sanguin sont aussi présentés.

Introduction

Haddock has been recognized as a potential species for marine aquaculture. Abnormally high accumulation of lipid in the liver, or "fatty liver condition", has been identified as a major constraint facing haddock culture in Atlantic Canada. Fatty livers and hepatosomatic indices (HSI) exceeding 20% have been observed in haddock fed diets with high lipid content (>12%). Also, from field observations, it is apparent that the fatty liver condition affects the overall health and growth of the fish. The underlying cause of this problem needs to be explained[(1)]. It is known that gadoid fish store the majority of available dietary energy as lipid in the liver[(2)]. In cultured haddock, total liver lipid levels can reach >65% when fish are fed a dietary lipid level of 12%. However, total muscle lipid (generally at levels less than 1%) is not affected by dietary lipid content[(1)].

Limited information is available on lipid metabolism in fish. Very low-density lipoproteins (VLDL) are the major transporters of storage lipid (triacylglycerol) out of the liver to extra-hepatic tissues[(3)]. As further evidence, muscle lipid level has been positively correlated with VLDL-triacylglycerol level in the plasma[(4)]. In haddock, high-density lipoproteins (HDL) have been identified as the predominant lipoprotein whereas VLDL concentration was relatively low (<50 mg/dL)[(1)]. The red muscle of haddock possesses a higher specific activity for ß-oxidation than white muscle or liver[(5)]. White muscle, however, comprises more than 50% body weight in haddock and is considered the most significant muscle for lipid catabolism. These findings have led to the conclusion that the trans-

port of lipid from the predominant storage site, the liver, to the catabolic site, the muscle, is low in haddock.

Previous reports have shown that supplementation of lipotropic factors such as choline, methionine, and carnitine, or an increase in inositol, chitin, or fibre content, did not have a significant effect on the reduction of fatty liver condition or HSI in haddock[6]. Certain dietary lipids and fatty acids are known to influence liver lipid accumulation in fish and terrestrial animals[7-9], however their effectiveness has yet to be tested on gadoids, in particular haddock. The main objective of this study was to examine the effects of conjugated and medium chain fatty acids, phospholipids, and cholesterol supplementation in reducing the HSI and prevalence of fatty liver condition in haddock.

Materials and Methods

Diet preparation

The formulation of experimental diets was based on the known nutrient requirements of salmonid and marine finfish[10]. The diets contained 51% protein and 14% total lipid with 17 MJ digestible energy per kg. A dietary lipid level of 14% lipid was selected for experimental diets to induce fatty liver condition in the fish. Herring oil was used as the primary lipid source in order to meet the essential fatty acid requirements of marine fish[10]. The control diet (CTRL, Diet 1) was formulated to contain 14% lipid in the form of herring oil. The following four additional experimental diets were used: Diet 2 – conjugated linoleic acid (CLA), 1.5%; Diet 3 – soy lecithin (SL; source of phosphatidylcholine), 4%; Diet 4 – coconut oil (CO; source of medium chain fatty acids), 4%; and Diet 5 – cholesterol (CHOL), 2%. The varying amounts of the four lipid supplements were replaced at the expense of herring oil in Diets 2 to 5.

Experimental conditions

A feeding trial was conducted at the National Research Council – Marine Research Station (NRC-MRS) at Sandy Cove, Halifax, Nova Scotia. Forty-seven juvenile haddock (7.92 ± 0.06 g, mean initial weight ± SE) were randomly allocated into one of 10 experimental tanks and acclimated to experimental set up for 10 days prior to the start of the feeding trial. During this time they were fed to satiation on a commercial 2.0-mm haddock pellet (EWOS) three times daily (0830, 1230, 1630). During the 63-day experimental period, fish were hand-fed to satiation three times daily during the week (0830, 1230, 1630) and twice daily on weekends (0800, 1200). Feed consumption was recorded weekly. A flow-through system of filtered seawater (30 ppt) was supplied to each tank at a rate of 3 L/min. Photoperiod was kept at 12 h dark: 12 h light. Dissolved oxygen and temperature were monitored daily (10.4 ± 0.2 mg/L; 11.7 ± 0.1°C).

Sampling and analysis

Fish were group-weighed initially and at 3-week intervals (following a 24-hour fast) to determine percent weight gain. Initially, twenty-five fish were randomly sampled for hepatosomatic index (HSI), whole body composition, tissue fatty acid analysis, and histological analysis of the liver. At week 6, two fish per tank were sampled for HSI. At the end of the trial (Day 63), twenty fish from each diet group were randomly sampled for HSI, whole body composition, tissue fatty acid, and histological analysis of the liver.

Eight fish from each diet were analyzed for total liver lipid[11] and muscle lipid content[12]. Total blood cholesterol was measured by spectrophotometer using a clinical kit from BioQuant (San Francisco, California).

Results and Discussion

Haddock fed the CLA-supplemented diet had a significantly lower percent weight gain ($P < 0.05$) than fish fed all other experimental diets (Table 1). A decrease in weight gain after feeding 1% CLA has been reported in hybrid striped bass[13], however other workers have observed either an increase or no effect on growth rates of yellow perch, channel catfish, and Atlantic salmon when fed a diet containing 1% CLA[14-17]. This indicates a species-specific growth response.

The HSI of fish fed CHOL diet was significantly lower than that of the control diet yet, the only significant difference noted in terms of total liver lipid content was in the CLA supplemented group. Total liver lipid was significantly lower in CLA-fed fish than in all other dietary treatments (Table 1). In hybrid striped bass and yellow perch, liver lipid levels were reported to be significantly reduced with the addition of CLA to the diet[13,15]. There was no significant difference found in total muscle lipid content between any of the dietary treatments, however there was a trend towards lower levels in the fish fed the SL diet. Not surprisingly, analysis of blood cholesterol levels revealed a significantly elevated level of blood cholesterol in fish fed the CHOL diet (Table 1).

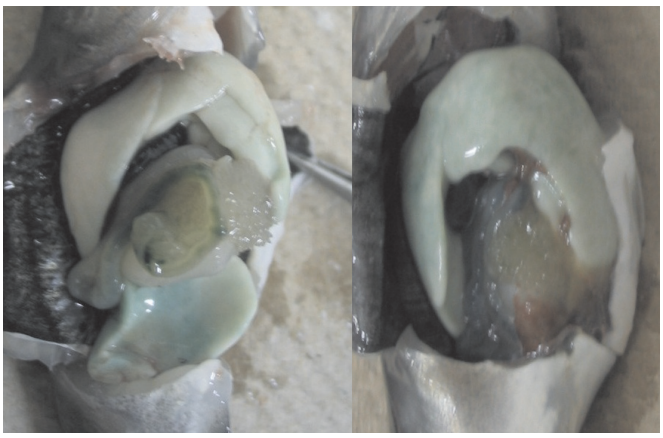

Figure 1.
Discolouration of haddock liver after 6 weeks (left) and 9 weeks (right) of feeding experimental diet where herring oil was partially replaced with sunflower oil as a source of conjugated linoleic acid.

Diet	Percent Weight Gain[(ii)] (g/fish)	HSI[(iii)] (%)	Total Liver Lipid[(iv)] (%)	Total Muscle Lipid[(v)] (%)	Total Blood Cholesterol[(vi)] (mg/dL)
Control	555.94 ± 1.24[a]	11.44 ± 0.14[a]	56.86 ± 1.62[a]	1.04 ± 0.10[a]	129.35 ± 3.79[a]
CLA[(i)]	401.58 ± 9.30[b]	10.85 ± 0.24[ab]	36.18 ± 1.74[b]	0.98 ± 0.05[a]	132.49 ± 14.72[a]
Soy lecithin	586.25 ± 13.47[a]	11.07 ± 0.13[ab]	55.38 ± 1.31[a]	0.82 ± 0.05[a]	106.41 ± 16.35[a]
Coconut oil	565.40 ± 0.56[a]	10.90 ± 0.18[ab]	56.86 ± 1.63[a]	0.91 ± 0.07[a]	128.08 ± 16.20[a]
Cholesterol	598.83 ± 2.00[a]	10.71 ± 0.12[b]	53.58 ± 1.00[a]	1.07 ± 0.06[a]	305.27 ± 53.62[b]

[(i)] Conjugated linoleic acid; [(ii)] Average initial weight 7.92 ± 0.06 g, n=25; [(iii)] Hepatosomatic index = 100 × wet liver wt./body wt.; HSI of initial fish 6.13 ± 0.18%; n=25; [(iv)] Total liver lipid = 100 × extracted lipid wt. (g)/sample wt. (g); n=8; [(v)] Total muscle lipid = 100 × extracted lipid wt. (g)/sample wt. (g); n=8; [(vi)] Total blood cholesterol, n=3

Table 1.
Growth, feed utilization, hepatosomatic index (HSI), tissue lipid, and total blood cholesterol levels of haddock fed various dietary lipid sources for 9 weeks. Values (mean ± SE) in the same column containing different superscripts were significantly different ($P < 0.05$).

Of particular interest was the occurrence of a green discolouration of the liver in approximately ninety percent of the haddock fed the CLA diet (Fig. 1). The cause and mechanism of this green discolouration has not yet been investigated in gadoid fish. A similar condition in red sea bream has been linked to a taurine deficiency[(18)].

Conclusions

Cholesterol supplementation in haddock diets significantly reduced HSI compared to those fed the control diet, however total liver lipid content was not affected. Fish fed the CLA diet exhibited significantly lower weight gain than fish fed other experimental diets. Additional research is underway to determine tissue (liver, muscle, and blood) fatty acid composition including the CLA concentration as well as the pathological changes linked to experimental diets. Future studies will involve a close examination of the effects of CLA on liver function and lipid accumulation as well as the role of cholesterol in lipid metabolism of haddock. Research in these areas will provide some clues regarding the influence of dietary factors in pathogenesis of fatty liver in gadoid fish.

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The Efficacy of Oral Immunostimulants in Enhancing Resistance against Microsporidiosis in Juvenile Atlantic Cod (*Gadus morhua* L.)

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The intracellular parasite *Loma morhua* (Microsporidia) can induce severe pathology in juvenile Atlantic cod (*Gadus morhua* L.), characterized by the formation of conspicuous white xenomas in the gills, heart, and spleen. In the absence of suitable chemotherapeutants and given the potential problem of microsporidiosis to a commercial cod aquaculture industry, studies are ongoing to determine the efficacy of various commercial immunostimulant products as prophylactic treatments. In the present study, juvenile, hatchery-reared cod were fed a formulated gadoid diet treated with one of four immunostimulant products, while control groups received untreated feed. Immunostimulant formulations were based on one of three product classes: beta-glucans, bioflavinoid, or nucleotide supplements. To evaluate product efficacy, growth rates were monitored, and random, peripheral blood samples were analyzed for lymphocyte density as an indicator of immune system activity. Finally, the cod were exposed to the infective spores of *L. morhua*, and the trials were terminated either two (Trial 1) or six weeks (Trial 2) post-challenge. Increased terminal lymphocyte densities were found in all beta-glucan groups for both trials and decreased prevalence of heavy to severe infections with *L. morhua* for both beta-glucan and nucleotide supplement groups in Trial 2.

Le parasite intracellulaire *Loma morhua* (Microsporidia) peut causer de graves maladies pathologiques chez les juvéniles de la morue franche (*Gadus morhua* L.), caractérisées par la formation de xenomes blancs proéminents dans les branchies, le cœur et la rate. En l'absence d'agents chimiothérapeutiques adéquats et étant donné le grave problème que peut poser la microsporidiose pour l'élevage commercial de la morue franche, des études sont en cours en vue d'établir l'efficacité de divers immunostimulants commerciaux à titre de traitements prophylactiques. Dans le cadre de la présente étude, nous avons nourri des morues d'élevage juvéniles d'une ration composée pour gadidés à laquelle avait été ajouté un de quatre immunostimulants, et les groupes témoins, d'aliments non traités. Les immunostimulants contenaient l'une de trois classes de produits : béta-glucane, bioflavinoïde ou nucléotide. Pour évaluer l'efficacité des produits, nous avons contrôlé les taux de croissance et analysé des échantillons de sang périphérique prélevés au hasard afin d'établir la densité des lymphocytes à titre d'indicateur de l'activité du système immunitaire. En dernier lieu, nous avons exposé les morues aux spores infectieuses de *L. morhua*, puis mis fin aux essais soit deux (essai 1) ou six semaines (essai 2) après l'exposition à celles-ci. Tous les groupes des deux essais nourris d'un complément de béta-glucane ont montré des densités terminales accrues de lymphocytes, tandis que les groupes nourris d'un complément de béta-glucane et de nucléotide dans l'essai 2 ont montré une prévalence moindre d'infestation importante à grave par *L. morhua*.

Introduction

Microsporidiosis has emerged as a potential problem[1] in the culture of juvenile Atlantic cod, (*Gadus morhua* L.). The causative agent in cod is the intracellular parasite *Loma morhua*[2], a taxonomic relative of *Loma salmonae*, which causes microsporidial gill disease in Onchorhynchid salmonids[3-5]. Infections are common in vascularized tissue such as the gills, heart, and spleen[4,6] but may also appear in the kidney or digestive tract. After invasion of the host cell, *Loma* undergoes sporogony, resulting in cellular hypertrophy and the characteristic xenoma that, when mature, contains thousands of infective spores. The presence of these xenomas can impair the growth of juvenile cod, or in cases of heavy infection in the gills and/or heart, cause death[1].

The use of commercial vaccines on juvenile cod is now questionable as recent evidence has indicated that Atlantic cod do not exhibit the specific immune response typical to teleost fishes[7,8]. This, in addition to the absence of a suitable chemical treatment for microsporidiosis in juvenile cod, is the rationale behind the current study which uses oral immunostimulants to enhance the non-specific immune response in cod. The immunostimulant formulations used in this study fall into one of three categories: (i) Beta-glucan (BG) formulations have glucans derived from brewers yeast combined with a vitamin mixture; (ii) Bioflavinoid (BF) formulations are extracted from citrus fruit; and (iii) a nucleotide supplement pre-mixed (PM) marine diet. The efficacy of these formulations in enhancing resistance of juvenile cod to microsporidiosis was evaluated by exposing the cod to infective spores of *L. morhua*.

Materials and Methods

Husbandry and feeding protocol

For each trial, the cod were held in a temperature-controlled (10°C), UV-filtered, 2000-L seawater recirculation system at the aquaculture facility of the Marine Institute of Memorial University of Newfoundland. After acclimation, the cod were randomly assigned to treatment groups in two-tank replicates and fed a standard cod diet treated with one of several immunostimulant formulations:

Trial 1 – Beta-Glucan, standard dose (8 mg/g; BG), Beta-Glucan ½ dose (4 mg/g; BG/2), Bioflavinoid (BF), Beta-Glucan + Bioflavinoid (2.5 mg/g; BG+BF).

Trial 2 – Beta-Glucan ½ dose (4 mg/g; BG/2), Beta-Glucan double dose (16 mg/g; BG2×), and a nucleotide pre-mixed diet (PM).

Standard dosages refer to those recommended by the manufacturer for use with rainbow trout, *Oncorhynchus mykiss*.

The BG- and BF-treated feeds were prepared by coating the standard cod diet with sterile fish oil and then mixing the appropriate immunostimulant concentration in powdered form. The PM, nucleotide-supplement diet, required no preparation. Control treatments were prepared by coating the feed with the same sterile fish oil alone. The feeding regime followed manufacturers' recommendations. The BG- and BF-based products used a 2-week feeding period (start day 28, Trial 1; day 13, Trial 2), followed by four weeks of standard diet, then a second 2-week period of treated feed. After exposure to *Loma*, the cod received a third, 2-week-treated feeding. Oil-coated control feed was fed in this same manner. The PM diet was fed for 8 consecutive weeks as recommended. It was given three weeks prior to a known stress event (e.g., a sampling date), and continued for five weeks afterward. An additional 2-week feeding of PM feed was also administered after exposure to *Loma*.

Sampling

Initial (day 0) length and weight measurements were taken for all cod under anaesthesia (MS-222 @ 45 mg/L) and subsequent measurements were performed on random samples of 15 cod from each tank, while blood samples were taken from a random sub-set of 5 anaesthetized cod. Length and weight measurements were used to calculate and analyze mean weight, condition factor, feed conversion ratio, and specific growth rate. Non-lethal blood samples were taken from the caudal vein (1-mL heparinized syringes, 23G needle) and were used to prepare blood smears (stained with Giemsa). Three random fields of view (1000×) were examined and counts were made of erythrocytes and lymphocytes to calculate lymphocyte density (index ratio of lymphocytes to erythrocytes).

Infection protocols

Exposure of juvenile cod to the infective spores of *Loma* was achieved using macerated tissue from the gills, heart, and spleen of infected cod.

In Trial 1, aliquots of macerated tissue were added to each tank during a typical feeding period on day 123 to induce the ingestion of the spores. Many cod were observed purposely ingesting tissue particles. This trial was terminated 17 days after exposure. A gross necropsy was performed on all fish and the gills, heart, and spleen were removed from ten cod per treatment and fixed in 10% formalin for histological examination (Hematoxylin and Eosin staining; H&E).

In Trial 2, all juvenile cod were anaesthetized and 0.2-mL aliquots of macerated tissue were injected directly into the stomach, per os on day 96. In this procedure, a 1-mL syringe was fitted with a tip that was cut from a disposable 1-mL pipette. This method allowed for much greater control over the amount of infective tissue delivered to each fish, and there was no obvious increase in mortality associated with the procedure. This trial was terminated 42 days after exposure. A gross necropsy was performed on all fish and the gills, heart, and spleen were removed from any fish not showing macroscopic signs of infection and fixed in 10% formalin for histological examination (H&E). Livers were also removed and weighed to calculate hepatosomatic index (HSI). The intensity of *Loma* infection was quantified by counting the number of xenomas present per gill arch of a given fish (light:<5 per gill arch; moderate, 6-20; heavy, >20; and severe, innumerable xenomas, possibly fused xenomas.

Data analysis

Statistical analysis was performed using Minitab® 13 for Windows.

Results and Discussion

Growth

Trial 1: Initial mean weight for this trial was 4.2 g. There was no significant difference in mean weights of any treatment as compared to the control group at any point during the trial (General Linear Model, Analysis of Variance with Dunnett's test for comparison to a control). There was, however, a marked decrease in the growth rate of cod in the BG group after day 90. This may support the idea of suppressed growth in fish that are over-fed beta-glucan-based supplements. This was the rationale for including the double-dose beta-glucan treatment (BG2×) in Trial 2. All treatments, except the controls, showed a decrease in growth rates after exposure to *Loma*.

Trial 2: Initial mean weight for this trial was 20.5 g. There was no significant difference in the mean weight of any treatment compared to the control (GLM-ANOVA with Dunnett's test) but the obvious decrease in growth rate after day 35 for cod in the BG2× group is further evidence that over-feeding of the BG-based immunostimulant suppresses growth. Once again, a decrease in growth rate after exposure to *Loma* was evident. However, in this trial, the growth rate for the control fish also decreased. One possible explanation for this is the intrinsic shift in metabolic energy that likely occurs when the immune system attempts to fight the *Loma* infection, resulting in reduced growth. It may also be possible that if any of these immunostimulant formulations can facilitate a reallocation of energy into boosting the nonspecific immune response, there would be that much less energy to go into growth after a pathogen is introduced.

Lymphocyte density

Trial 1: All treatment groups showed significantly higher lymphocyte densities (Fig. 1, G1) than the control by day 90,

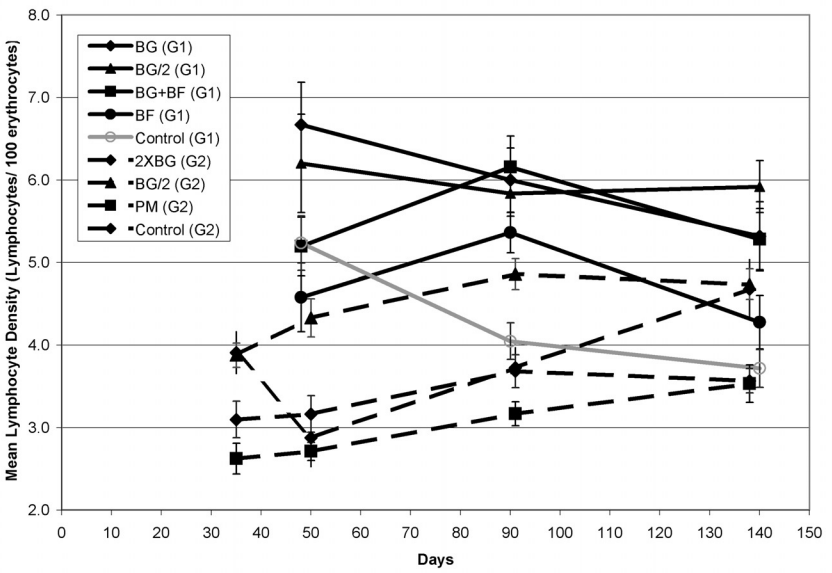

Figure 1.
Mean lymphocyte density for Trial 1 (G1) and Trial 2 (G2) juvenile Atlantic cod fed various immunostimulant formulations. (*mean lymphocyte density = # lymphocytes per 100 erythrocytes).

(GLM-ANOVA with Dunnett's test) and this was also true for the terminal sample at day 140 for all treatments except the bioflavinoid (BF) group. The half-dose beta-glucan treatment (BG/2) showed the least variability in lymphocyte density over time and the density for the BG treatment showed a fairly steady decline from initial to terminal sampling.

Trial 2: Again, the BG/2 treatment group had a mean lymphocyte density significantly higher (GLM-ANOVA with Dunnett's test) than the control (Fig. 1, G2) throughout out the trial. The final density for the BG2× group was also significantly higher but was quite variable throughout the trial.

Prevalence of infection

Trial 1: Although there were no macroscopic infections of *Loma* found, histological examination did reveal presporogonic xenomas in the gill tissue of several cod from all treatment groups. 20-40% of the sections from all treatment groups revealed presporogonic infection.

The appearance of these presporogonic infections in the histology samples provided the evidence that clinical infections of *Loma* could be induced using the concurrent feeding procedure. However, it is impossible to ensure that all fish are provided with the same level of exposure to infective spores, thus there was no inference made as to the effectiveness of the treatments used in Trial 1 in enhancing resistance to infection.

Trial 2: Overall prevalence of infection for fish that survived to the termination of the experiment was highest in the control group (Fig. 2). Half of these showed heavy to severe levels of infection, and half light to moderate. It is important to note that the longer the cod retain the infection in the light to moderate range, the greater the chances of survival as they appear to be somewhat able to overcome lighter infections once they reach a

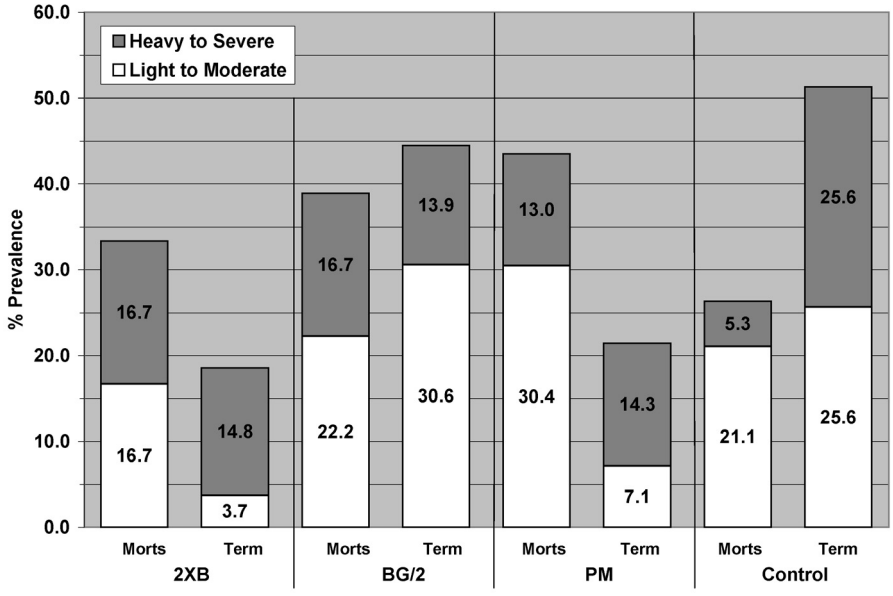

Figure 2.
Prevalence and intensity of *Loma morhua* infection in post-exposure mortalities and surviving Trial 2 juvenile Atlantic cod.

weight of 60-70 g[9]. Therefore, the higher proportion of infected fish with light to moderate infections in the BG/2 treatment may indicate some measure of delay in the progression of the intensity of infection. However, it would be preferable that the overall prevalence is reduced as well.

The reduced prevalence of infection for the PM and BG2× treatments for surviving fish is promising, but the reduced growth rate for the BG2× group is cause for concern, especially in a commercial aquaculture setting.

A potential problem regarding the applicablilty of the PM treatment in an aquaculture setting is the high prevalence of light to moderate infections in post-exposure mortalities.

All treatment groups showed an increase in mortality after being exposed to *Loma*. The control group showed an increased mortality of 1.6% per week; BG2×, 2.2% per week; BG/2, 1.4% per week; and PM, 3.2% per week. The increase observed for the treatment group fed the PM diet may be indicative of a problem beyond the infection itself. If the PM treatment is capable of enhancing growth, it may be at the expense of immunocompetency, as inferred from the lower lymphocyte densities (Fig. 1). In addition, it is possible that if there is a shift in the normal energy budget of the fish towards growth in this treatment, the higher levels of metabolic stress when challenged by a pathogen, may over-burden that energy budget to the point of mortality, as inferred from the high percentage of mortalities with light to moderate infections, which should not be fatal in most cases. Thus, the disease state itself, may only be partially responsible for the increased mortality in this group.

Summary

All beta-glucan-based (BG, BG2×, BG/2, and BG+BF) treatments show some promise as potential immunostimulant feed additives, but as evidenced by the results of this study, there is still much work to be done with regard to finding a "cod-specific" dosage and in the case of the BG+BF group, infection studies. The BG/2 treatment has thus far shown to be the best of the four BG-based products with good growth, consistently high lymphocyte densities, and an apparent delaying effect on the progession of infection of *L. morhua*. There is however, some concern as to the relatively high overall prevalence of infection in this group. Additional infection trials and feeding regime studies are planned for all of the treatment groups used in this study.

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Simplified Illustrated Sea Lice Identification Guide for *Lepeophtheirus salmonis* and *Caligus clemensi* in British Columbia, Canada

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Due to the scope of interest in sea lice and their effect on wild and farmed salmonids in the Pacific Ocean on the west coast of Canada, there are a large number of interest groups involved in monitoring sea lice numbers on farmed and wild Pacific salmonids. Even though there are taxonomic keys available for the identification of sea lice, there have been some issues with the correct identification of the two most relevant marine copepods that parasitize salmonids in British Columbia. This simplified taxonomic key, for the first time, provides an easy to use visual key that describes the two parasitic marine copepods that have the largest impact on wild and farmed salmonids in British Columbia.

En raison des préoccupations que soulèvent les poux du poisson et leurs effets sur les salmonidés sauvages et d'élevage de la côte Ouest du Canada, de nombreux groupes d'intérêt participent à la surveillance du nombre de poux sur le saumon du Pacifique sauvage et d'élevage. Des clés taxinomiques sont disponibles pour l'identification des poux du poisson, mais ils ont eu de la difficulté à identifier correctement les deux copépodes marins les plus souvent à l'origine des infestations chez ces salmonidés. La présente clé taxinomique illustrée simplifiée permettra de facilement identifier ces deux copépodes parasites.

Introduction

In British Columbia, and other regions around the world where salmon are indigenous, the term sea lice, refers to the parasitic copepods that are often found on both wild and farmed salmonids. There is, even within communities that encounter these copepods frequently, some confusion as to what they really are. Amongst some sport salmon fisherman and even salmon farm workers, harmless Cumacean shrimps (closely related to copepods) that exhibit curious swarming behaviour during periods of elevated water temperatures in mid summer, have been confidently identified as sea lice. However, in reality there are only two species of parasitic marine copepod that pose a potential threat to both farmed and wild salmon in British Columbia, *Lepeoptheirus salmonis* and *Caligus clemensi*, although *L. cuneifer* is common on farmed salmon in some regions[1].

Copepods of the genera *Caligus* and *Lepeophtheirus* naturally affect many species of salmon. There are 14 species (2 species of *Caligus* and 12 species of *Lepeophtheirus*) of sea lice parasitizing many marine fishes in British Columbia[2]. However, the common species of sea lice positively identified to affect various salmonid species in BC are *C. clemensi*, *L. salmonis*, and *L. cuneifer*.

Caligus clemensi[3], is a copepod species not usually associated with farmed salmon, but often found on wild salmon (In other regions of the world, other species of *Caligus* are of more importance to both farmed and wild salmon fisheries e.g. *C. rogercresseyi* in Chile). These are very mobile parasites that abandon their host very quickly in the event of handling. Hence there is some evidence that the abundance of *Caligus* spp. on wild salmon has been severely underestimated[4]. *Caligus* spp. are not species-specific and can be found on a wide range of fish, commonly on herring in large numbers, hence the common name, herring louse.

The second copepod that poses a threat to salmonids is *L. salmonis* (Krøyer). Also commonly known as the salmon louse, *L. salmonis* is a parasitic caligid copepod[5,6]. Commonly found

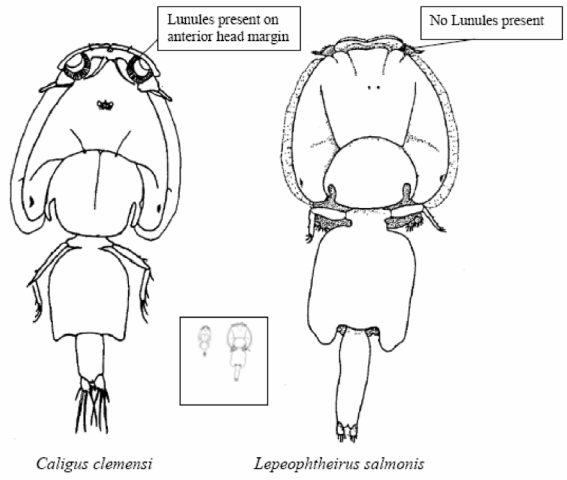

Figure 1.
External anatomy of the distinguishing features between the genus *Caligus* and the genus *Lepeophtheirus*. Dorsal view shown. Small inset shows relative size difference.

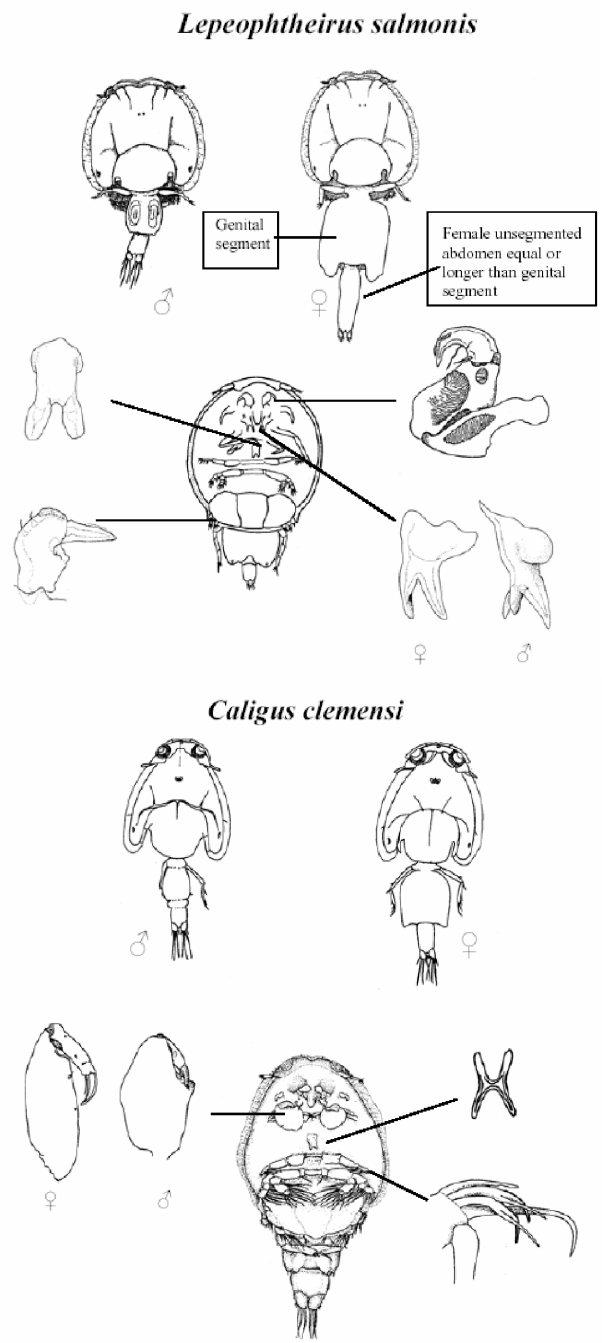

Figure 2.
External anatomy of the males and females of *Caligus clemensi* and *Lepeophtheirus salmonis*. Dorsal views shown (A). For ease of reference, the ventral view (B) depicts generalised morphology of the genus *Lepeophtheirus* and genus *Caligus* respectively with morphology specific to *L. salmonis* and *C. clemensi* males and females shown in detail. (Redrawn and modified from Parker and Margolis[3], Parker et al.[13], and Kabata[2]).

in the Pacific and Atlantic Oceans[2], it parasitizes both farmed[7,8] and wild[9] salmonids. Epizootics in salmon via either primary lesions or secondary infections[10], lead to stress, impaired performance, reduced physiological ability[11,12], and in extreme cases death.

Hence, in the context of salmon aquaculture and wild salmon in British Columbia, Canada, it is *Lepeophtheirus salmonis* that impacts both wild and farmed salmonid populations. It is intended to provide here, to both salmon growers and NGOs, an easy to follow functional illustrated guide for the accurate identification of adult, male and female *Lepeophtheirus salmonis* and *Caligus clemensi*.

Key to Genera

Due to morphological differences in life stage and maturity, in this key we will only be examining gravid adult females (easily identified due to presence of egg strings). *C. clemensi* and *L. salmonis* differ somewhat in appearance. *C. clemensi* is smaller (roughly a quarter of the size), lighter in colour and has short egg strings with a slight brown or red colour. However, the main distinguishing feature between the two groups is the presence of a pair of lunules, the round anteroventral sucker-like structures on anterior head margin (frontal plate) of genus *Caligus*. These structures are absent on genus *Lepeophtheirus*. These key differences between the genera are shown in Figure 1.

As there are twelve species of *Lepeophtheirus* present in British Columbian coastal waters, identification to the species level is important. On the dorsal surface of *L. salmonis*, the feature that distinguishes this species from others is the genital segment is the same length or shorter than the abdomen, which in the adult males and gravid females of this species, is unsegmented. Other features of this species which can aid identification from other *Lepeophtheirus* spp. are differences in the sternal furca, the second antennae, and the basal spine of the exopod of the 3rd leg. The first maxilla displays structural differentiation between sexes.

It is somewhat easier to differentiate between the species of the genus *Caligus* found in British Columbia, as there is only the one species described (although the presence of a second species is suspected). On the dorsal surface, the genital segment of the female appears segmented from the abdomen, and the females have a well developed genital segment compared to the males. On the ventral surface, the sternal furca and first leg of *C. clemensi* are distinct. In addition, the maxilliped of the males is somewhat wider and shorter than that of the females.

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Proteomic Identification of Acute Phase Reactants in Plasma of Rainbow Trout (*Oncorhynchus mykiss*)

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The major acute phase proteins that were induced in plasma of rainbow trout (*Oncorhynchus mykiss*, Walbaum) were characterized following the induction of inflammation by intraperitoneal (i.p.) administration of purified *Aeromonas salmonicida* lipopolysaccharide in Freund's incomplete adjuvant (LPS/FIA) or a commercial oil-based *A. salmonicida* vaccine. Plasma proteins were compared by two dimensional polyacrylamide gel electrophoresis (2D-PAGE) and densitometry. In one experiment, plasma samples were compared between treatment and control groups in which fish were terminally bled. In another experiment, individual fish were sampled repeatedly. In both protocols, a 36 kDa protein was increased up to 13-fold and several proteins were detectable that had not been previously. In all fish treated with LPS/FIA, a 9.5-kDa protein was consistently increased an average of 75-fold in plasma. The only proteins from this panel that were similar to previously identified proteins using mass peptide fingerprints were pre-cerebellin-like (p24kDa) and transferrin (p37kDa) which were increased and induced respectively, and the constitutively produced apolipoprotein A-I-1 (28kDa). These studies identify several plasma proteins that might be useful acute phase indices of inflammation.

On a caractérisé les principales protéines de phase aiguë produites dans le plasma de la truite arc-en-ciel (*Oncorhynchus mykiss*, Walbaum) à la suite du déclenchement d'une inflammation par administration intrapéritonéale d'un lipopolysaccharide purifié d'*Aeromonas salmonicida* émulsifié dans un adjuvant incomplet de Freund (LPS/AIF) ou d'un vaccin commercial en suspension huileuse contre *A. salmonicida*. Nous avons fait appel à l'électrophorèse en gel d'acrylamide bidimensionnelle (PAGE) et la densitométrie pour comparer les protéines plasmatiques. Dans le cadre d'une expérience, nous avons comparé des échantillons de plasma prélevés chez des groupes témoins et des groupes expérimentaux de truites saignées à mort. Dans le cadre d'une autre, nous avons prélevé des échantillons répétés de sang chez un certain nombre d'individus. Dans les deux cas, la concentration d'une protéine de 36 kDa a augmenté jusqu'à 13 fois et plusieurs protéines qui étaient jusque là indécelables le sont devenues. Chez toutes les truites injectées du LPS/AIF, la concentration d'une protéine de 9,5 kDa dans le plasma a augmenté sans exception par 75 fois en moyenne. Les seules protéines que nous avons trouvées qui ressemblent à des protéines déjà identifiées par empreinte peptidique massique sont une protéine ressemblant à la pré-cérébelline (24k Da) et la transferrine (37k Da) (la concentration de la première a augmenté et la production de la deuxième a été déclenchée), ainsi que l'apolipoprotéine A-I-1 (28 kDa) constitutive. Ces études ont permis d'identifier plusieurs protéines plasmatiques qui pourraient se révéler des indices d'inflammation de phase aiguë.

Introduction

In vertebrates, the acute phase response (APR) has been defined as a rapid, orchestrated, physiological induced response to injury, infection, trauma and stress[1,2], involving changes in hepatic, vascular, and immune systems. In mammals, which mount a vigorous and rapid APR, the liver is the major organ responsible for adapting and regulating a range of acute phase proteins (APP) involved in host defence and maintaining homeostatis[3]. Many proteins that increase in the plasma (positive APP) result from increased expression and secretion by the liver[4,5]. Those that decrease as a result of reduced expression rather than consumption or loss are referred to as negative APP[4]. Because of the magnitude of the induction or reduction in plasma protein their relative levels can be monitored to gauge the process of inflammatory disease, injury, or stress.

By contrast, studies to date have suggested that fish and other ectothermic vertebrates have a muted APR that is delayed compared to mammals[6,7]. For teleost fish, it is known that low temperatures are non-permissive for an effective adaptive immune response and they therefore must rely on innate immunity and the APR to provide a more immediate first line of defense[8-10]. Proteins now considered by some to be positive teleost APP are SAA[2], CRP[11,12], transferrin[13], pre-cerebellin-like protein[7], and haptoglobin[14]. For some of these, there is little evidence that plasma concentrations increase substantially as a consequence of increases in gene expression in the liver[6,15]. However, there are examples of inducible proteins whose plasma concentrations do increase in fish. For example, CRP increased three-fold over normal serum levels within 24-48 h following i.p. injection of rainbow trout with *Vibrio anguillarum*[16] and 18-fold after formalin

exposure[17], but these changes are relatively minor compared to those expected for CRP in mammals.

The objective of the present research is to determine if 2D-PAGE and sensitive stains can identify constituitive and induced salmonid plasma proteins that might be useful as diagnostic indicators of inflammatory disease.

Material and Methods

Trial # 1 – Individual fish bled multiple times

Fifteen healthy rainbow trout (450-550 g) were acclimatized to individual 60-L tubs for three weeks prior to the experiment. Fish were anaesthetized with 10 mg/L ethyl-4-aminobenzoate (Acros organics, New Jersey, USA) and blood samples for baseline values were obtained by caudal venipuncture as outlined in Table 1. On day 0, five fish were injected i.p. with LPS/FIA (4 mg of purified LPS[18] emulsified with 0.2 ml of FIA) (Sigma-Aldrich Canada Ltd, Oakville, Ontario, Canada); five fish were injected i.p. with 0.4 mL of Lipogen Triple J vaccine (Aqua Health Ltd, Charlottetown, Prince Edward Island, Canada); and five control fish were injected with 0.4 mL of sterile saline. Blood samples were obtained in the same manner on days 1, 3, 7, and 10 (Table 1). Pre-and post-stimulus pooled plasma samples were produced for each fish by combining 20 µL of plasma from each sample. Quantification of total protein from citrated plasma samples was determined in triplicate using the bicinchoninic acid (BCA) assay with bovine serum albumin standards (Pierce, Rockford, IL, USA).

Trial # 2 – Groups of terminally bled fish

Rainbow trout plasma samples used for this portion of the study were derived from previous experiments[19]. Briefly, a separate group of 300-600-g rainbow trout were held in 200-L circular green fiberglass tanks. On day zero, control fish (no injection) were euthanized by a blow to the head and blood was collected via caudal venipuncture. In addition on day zero, sixteen fish were anaesthetized with 50 mg/L TMS and injected i.p. with 0.2 mL Lipogen Triple J (Aqua Health Ltd, Charlottetown, Prince Edward Island, Canada). Sixteen additional rainbow trout were i.p. injected with 0.2 mL of sterile saline. On days 3, 7, 10, and 14, four vaccinated fish and four saline-injected fish were euthanized and plasma samples were collected and processed as described previously (Table 1). A pre-stimulus sample (Table 1) was produced by combining 20 µL of plasma from each of the four untreated fish on day zero. A post-stimulus sample (Table 1) was prepared by combining 20µL of plasma from each of the sample day pools (i.e. day 3, 7, 10, 14). Plasma protein was quantified as above.

Two-dimensional SDS-PAGE (2D-PAGE) and analysis

Protein spots on polyacrylamide gels were visualized using SYPRO Ruby (Molecular Probes) following manufacturers protocols. Pre-and post-stimulus plasma samples of equal concentration were subjected to simultaneous iso-electric focusing in linear immobilized pH gradient strips (IPG) (Immobiline DryStrip, pH 3-10, 13 cm, Amersham Pharmacia Biotech AB, Uppsala, Sweden) using the Ettan IPGphor Isoelectric Focusing system (Amersham). Analysis of 2D-PAGE gels was performed using ImageMaster 2D Elite analysis software (Amersham) to quantify pre- and post-stimulus plasma protein profiles from individual fish (Trial 1) and groups of fish (Trial 2). *Increased* proteins were elevated three-fold or greater between pre- and post-stimulus while *reduced* proteins decreased two-fold or greater. *Constitutive* proteins were those that were not altered and finally proteins that were absent in pre-stimulus gels but present in post-stimulus profiles were considered to be *induced*. Only those proteins that were altered in all fish for a given treatment were considered. Proteins that were increased or induced in fish injected with saline were not included in the acute phase panel.

Mass spectrometry and or N-terminal amino acid sequencing was performed on excised spots (Amino Acid Analysis Facility, Advanced Protein Technology Center, Hospital for Sick Children, Toronto, Ontario, Canada) and sequences were searched for similarity to homologous sequences with Basic Local Alignment Search Tool (www.ncbi.nlm.nih.gov:80/BLAST/).

Results and Discussion

Several proteins were selected to construct a panel of putative acute phase proteins for future work (Table 2). Proteins of particular interest were those that were altered similarly in both trials. Only four proteins from our panel, p24a (precerebellin-like protein), p37 (transferrin), p10.5 (apolipoprotein), and p28a

Table 1.
Experimental design summary: Trial 1. Individual fish (*n* = 5 for all three groups) repeatedly bled before and after i.p. injection of vaccine; or purified *Aeromonas salmonicida* LPS in FIA; or saline. Trial 2. Single terminal blood samples from groups of fish before i.p. injection (control, *n* = 4) or after i.p. injection of saline (*n* = 16) or vaccine (*n* = 16).

	Days of Experiment	Pre-stimulus plasma			Post-stimulus plasma					Total fish
		-9	5	0	1	3	7	10	14	
Trials 1	Saline (*n*=5)	←	–	→	←	–	–	→	-	5
	Vaccine (*n*=5)	←	–	→	←	–	–	→	-	5
	LPS/FIA (*n*=5)	←	–	→	←	–	–	→	-	5
Trial 2	Non-injected (*n*=4)	-	-	*n*=4	-	-	-	-	-	4
	Saline-injected	-	-	-	-	*n*=4	*n*=4	*n*=4	*n*=4	16
	Vaccine (*n*=16)	-	-	-	-	*n*=4	*n*=4	*n*=4	*n*=4	16

(apolipoprotein) could be identified based upon the number of matched peptides, mass accuracy (0.1%) and expected molecular weight (MALDI-TOF-MS) and amino acid sequence homology (ESI MS/MS and N-terminal Edman degradation). The identification of remaining proteins was not clearly determined by mass spectrometry or amino acid sequence analysis or there were no significant homologous sequences identified.

While there are few reported[7,11-19] plasma proteins that increase during the acute phase response in rainbow trout, several proteins in the present study were altered sufficiently to be valid and potentially useful indicators of an APR. Protein p24a (pI 5.3±0.4) which increased an average of 3.5 times in our studies was identified as precerebellin-like protein (Genbank submission # AAF04305) previously described in rainbow trout to be increased in plasma during inflammation [7]. The induced protein p37, identified by ESI MS/MS as rainbow trout transferrin (Genbank submission #5837759), is similar to the truncated form of goldfish serum transferrin that has been shown to induce nitric oxide production from LPS-stimulated goldfish macrophages[20]. The peptide sequence of the p28a constitutive protein was identified as apolipoprotein A-I-1, a precursor (Genebank submission #AAB96972) of a high-density lipoprotein isolated from rainbow trout serum by 1D-PAGE[21]. In addition, the 10.5 protein was also identified as similar to apolipoprotein (Genebank submission #BAB40966) from the Japanese eel, *Anguilla japonica*[22]. Several induced proteins potentially of most interest as acute phase indicators could not be identified. Many of these increased or induced 2D spots from individual and grouped plasma profiles did however have molecular weight and iso-electric points similar to previously published[2,14,16] and theoretical trout plasma proteins. For example, spots visualized at 23kDa, 24 kDa (pI 4.7), and 34 kDa (pI 5.3-5.6) are similar in molecular weight and isoelectric point as haptoglobin, trout CRP homologue, and SAP, respectively, and await definitive identification by mass spectrometry analysis and N-terminal sequencing.

Table 2.
Common 2D-PAGE protein spots identified during the present trials.

		Average Mass (kDa)	Average pI	Trial	Sequence identification	Reference
Increased or induced	p9.5	9.5	4	1 - LPS/FIA	unknown	-
	p10.5	10.5	6.1	1 - vaccine	apolipoprotein	Kondo et al.[22]
	p24a	24	5.3	1 vaccine + LPS/FIA	pre-cerebrellin	Gerwick et al.[7]
	p24b	24	4.9	1 - vaccine	unknown	-
	p24c	24.5	4.6	1 + 2	unknown	-
	p25a	25	4.5	1 - vaccine	unknown	-
	p36	36	5.5	1 + 2	unknown	-
	p37	37	6.2	1 - vaccine	transferrin	Stafford et al.[20]
Decreased	p16d	16	5.5	1 + 2	unknown	-
Constitutive	p28a	28	5.0	1 + 2	apolipoprotein	Babin[21]
	p28b	28	5.8	1 + 2	apolipoprotein	Babin[21]

In mammals, it is well known that Gram-negative LPS is recognized by soluble and membrane bound receptors that initiate the release of various cytokines and under some circumstances, cause wide spread cell and tissue damage leading to multiple organ failure[23,24]. The relative paucity of altered protein expression in LPS/FIA-injected fish in this study mirrors results reported previously[18] using an identical preparation of purified *A. salmonicida* LPS in FIA. The hypo-responsiveness or lack of systemic alterations to *Escherichia coli* LPS in fish has been demonstrated[25]. However, transient hypoferremic responses to *E. coli* LPS[26,27] and *A. salmonicida* bacterin vaccination[19] have been documented suggesting that an adaptive innate response does exist in rainbow trout, but is not manifested by matched changes in plasma proteins such as those induced in mammals. The degree of induction of p9.5 in response to LPS/FIA was dramatic (75-fold), certainly in comparison to most others identified here and from previous studies [14,16,18,19]. This suggests that there are some LPS-inducible proteins in trout but its identity as hepatic origin should be demonstrated before it can qualify as a conventional APP. However, it remains possible that changes of the magnitude observed might be clinically useful indictors of inflammation in farmed salmonids.

As more teleost APP are identified by either directed studies or PAGE analysis, the 2D pattern of serum proteins may become a novel approach to identify markers for innate resistance and indicators of fish health and welfare. Ongoing work includes the generation of rabbit antisera to our APR panel of proteins to allow the development of quantitative assays and examine the site of tissue production. Quantification using enzyme immunoassay will allow examination of temporal variation after selected inflammatory stimuli to fully understand the influence of protocol and individual variation. Moreover, more precise stimuli are also required to dissect the dynamics of teleost APRs.

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Efficacy of Crushed Garlic and Lemon Juice as Bio-Product Treatments for *Ichthyophthirius multifiliis* ('Ich') Infections Among Juvenile Nile Tilapia, *Oreochromis niloticus*

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Ichthyophthirius multifiliis (Fouquet), a ciliated protozoan parasite, causes white spot disease ('ich') on many different freshwater fishes. Previously, these infections were treated with malachite green, a compound now banned on food fish due to its carcinogenicity. As an alternative, "safe" treatment, crushed garlic (*Allium sativum*) and lemon juice (bioflavinols) have been used to control infestations of *I. multifiliis* and other parasites, but their efficacy has not been reported from controlled studies. To determine the toxicity of crushed garlic and lemon juice, two trials were conducted between July 2003 and September 2004. Various biological indices (condition factors, specific growth rates, feed conversion ratios), histopathology, and blood immunology data were used to assess treatment efficacy and determine if there were any sub-lethal effects on the fish. Crushed garlic (3 g/L) in a continuous static bath exposure was the most effective treatment, whereas the lemon extracts were least effective and resulted in unfavourable water conditions (reduced pH).

Ichthyophthirius multifiliis (Fouquet) est un protozoaire cilié à l'origine de l'affection parasitaire appelée ichtyophtiriose ou maladie des points blancs chez de nombreuses espèces de poissons dulcicoles. On traitait autrefois cette maladie avec du vert malachite, composé qu'il est maintenant interdit d'utiliser sur du poisson destiné à l'alimentation humaine à cause de sa cancérogénicité. Comme traitement de remplacement « sans danger », on a utilisé de l'ail (*Allium sativum*) écrasé et du jus de citron (bioflavonoïde) pour lutter contre les infestations par *I. multifiliis* et d'autres parasites, mais leur efficacité n'a pas été établie dans le cadre d'études menées dans des conditions contrôlées. Afin d'établir la toxicité de l'ail écrasé et du jus de citron, nous avons fait deux essais entre juillet 2003 et septembre 2004. Nous avons utilisé divers indices biologiques (coefficient de condition, taux de croissance spécifique, indice de consommation), ainsi que des données histopathologiques et immunologiques, pour évaluer l'efficacité des traitements et établir s'ils avaient des effets sublétaux sur les poissons. L'exposition statique continue des poissons atteints à de l'ail écrasé (3 g/L) s'est révélée le traitement le plus efficace, alors que l'exposition à du jus de citron l'était nettement moins, en plus de résulter en des conditions défavorables du milieu (réduction du pH).

Introduction

Tilapia (Family Cichlidae) are freshwater fish native to Africa and are now cultured in many parts of the world[1]. Several species of tilapia are cultured globally; this group of fishes is growing in importance as species for both domestic consumption and for export[2]. Under the original extensive or semi-intensive pond culture systems, tilapias were relatively more disease resistant than many other fish species. However, under intensive culture methods, stress due to high stocking densities and high organic matter load through manure fertilisation and feeding increase the chances of parasite and disease development such as white spot disease or 'ich'. Ichthyophthiriasis is caused by an external protozoan parasite, *Ichthyophthirius multifiliis*, which invades the skin and gills of freshwater fish[3]. 'Ich', a generalist parasite, has a monoxenous (one-host) life cycle consisting of an infective theront, a parasitic trophont, and a reproductive tomont[4]. Killing the infective theront or the detached trophont with various antiprotozoal drugs can stop the reproductive cycle and prevent the spread of the disease to other fish[4]. Currently, the only FDA-approved chemotherapeutant in many countries is formalin. However, its use is not practical in developing countries such as Malawi due to its cost and other problems associated with handling. The objective of the present study was to evaluate toxicity of crushed garlic and lemon juice on free-swimming theronts and see if further development of the diseases was prevented or mitigated and to examine if there were any sub-lethal effects on the fish.

Materials and Methods

Experimental animals

The trials were conducted at the Marine Institute of Memorial University of Newfoundland between July 2003 and October 2004. Two groups of juvenile tilapias (n=1000, ~0.5g) from Northern Tilapia hatchery in Ontario were stocked in July 2003 and February 2004 to be used for the first and second trials, re-

spectively. The fish were stocked in a holding tank fitted with a heater and mechanical filter. The fish were fed trout starter feed at the rate of 5% body weight per day. Standard water quality tests (pH, ammonia, dissolved oxygen, nitrite) were monitored daily and water was changed when water quality deteriorated (i.e., increased levels of ammonia and nitrite).

Source of 'ich' and preliminary trials

'Ich' infected fish were bought from a local anonymous pet store. The fish, 3 goldfish (*Carrasius auratus*) and 1 molly (*Poecilia sphenops*) were stocked in a holding tank (18 L), fitted with a heater and a mechanical filter. The temperature was set at ~ 23°C to speed up 'ich' multiplication. The fish were fed trout starter feed at 2% body weight per day. Preliminary trials, modified from Buchmann et al.[5], were conducted in September 2003 to determine the 'effective' concentration of the bio-products, prior to disease challenges. These preliminary trials revealed what concentrations of the bio-products were toxic to infective stages of *I. multifiliis*.

Challenge Trial 1

Tilapia (n = 25 fish/tank, range: 28-29 g) were stocked in 12, 54-L aquaria, fitted with heaters and filters. The fish were fed trout commercial feed (1.0 mm particle size) twice daily at 3% body weight per day. Standard water quality tests (as previously described) were conducted daily and 20% of the water was changed every three days to avoid excessive ammonia build up in the system. Filters were cleaned every three days to remove faecal material and excess feed clogging the cartridges. The bottom of the aquaria were siphoned daily to remove uneaten feed and faecal material. 'Ich' theronts (~264 000 swarmers/fish) were introduced in the 12, 54-L experimental tanks. Theront concentration was estimated by pipetting 0.1 mL of the 'ich' infected water into a Petri dish and counting the theronts with a dissecting microscope (40× magnification). The number was extrapolated to a 1-L volume to determine the final concentrations and prepare challenge exposures. The challenge exposures were prepared by adding 6 litres of 'ich'-infected water to each of the twelve aquaria. The four treatments (in triplicate) – crushed garlic (3 g/L), lemon juice (5 g/L), garlic plus lemon (2 g/L), and the control treatment – were randomly assigned.

Challenge Trial 2

In this trial the fish (n = 20 fish/tank, range: 5-7 g) were stocked among 9 54-L experiment tanks. Husbandry practices as described in Trial 1 were followed throughout the trial period. 'Ich' theronts (~235 000 swarmers/fish) were introduced to six of the nine tanks. The three treatments (in triplicate) – crushed garlic (1.5 g/L), untreated fish, and unchallenged fish – were randomly assigned.

Preparation and application of treatments

The garlic was peeled, sliced into smaller portions, and blended. The required amount of garlic was weighed (0.1 g), mixed with water (1 litre), and sieved using a gauze wire (0.292 mm diameter). The collected garlic liquid was directly applied to the treatment tanks. The lemons were crushed, seeds removed, and weighed (0.1 g). This was also directly applied to the treatment tanks. A mixture of garlic and lemon was prepared and applied as previously described using 50:50 ratio of each product. Crystals of sodium hydroxide were added to the treatment tanks to buffer the reduced pH due to the treatments.

Statistical analysis

The data from both trials (lymphocyte density, neutrophil density, weight measurements (0.1 g), length measurements (0.1 cm), and degree of 'ich' infection) were collected, and from these measurements, several biological indices – condition factors (CF), feed conversion ratios (FCR), specific growth rates (SGR), hepatosomatic indices (HSI), and splenosomatic indices (SSI) – were calculated using Microsoft® Excel. The indices were then transferred to the statistical packages for analysis. The three residual assumptions for independence, homogeneity, and normality governing the use of the calculated P-value were evaluated using appropriate plots[6,7]. Descriptive statistics and analysis of variance (ANOVA) were calculated with the General Linear Model (GLM) being used. Where the treatment means were significant ($P < 0.05$), Tukey's test was used to rank the treatment means. All the statistical analyses were conducted using Microsoft® Excel, Minitab® (version 13.1), and SPSS® for Windows (version 11.0.0) at a 5% level of significance.

Results and Discussion

Trial 1

Mean CF and mean lymphocyte densities were significantly higher ($P < 0.05$) among the treatment groups. However, there were no significant differences in the mean FCRs, mean SGRs, and mean neutrophil densities (P>0.05) (Table 1). The trial also

Table 1.
Mean values (± SD) of some biological indices calculated at conclusion of Trial 1. Common letters denote no significance differences among treatments ($P > 0.05$).

Treatment	Mean CF (± SD)	Mean FCR (± SD)	Mean SGR (± SD)	Mean LD (± SD)	Mean ND (± SD)
Crushed garlic	$1.59^{a} \pm 0.28$	$1.55^{a} \pm 0.63$	$1.31^{a} \pm 0.83$	$3.71^{a} \pm 0.20$	$3.21^{a} \pm 0.53$
Lemon juice	$1.52^{ab} \pm 0.26$	$2.03^{a} \pm 1.38$	$1.11^{a} \pm 0.86$	$2.66^{b} \pm 0.16$	$4.30^{a} \pm 0.69$
Garlic + lemon	$1.51^{ab} \pm 0.16$	$1.75^{a} \pm 2.44$	$1.15^{a} \pm 0.60$	$3.77^{a} \pm 0.23$	$5.30^{a} \pm 0.75$
Control	$1.46^{b} \pm 0.18$	$2.09^{a} \pm 0.94$	$1.02^{a} \pm 0.50$	$1.77^{c} \pm 0.11$	$4.42^{a} \pm 0.93$

Treatment	Mean CF (± SD)	Mean FCR (± SD)	Mean SGR (± SD)	Mean LD (± SD)	Mean ND (± SD)
Crushed garlic	$1.65^{a} \pm 0.02$	$1.40^{a} \pm 0.41$	$1.62^{a} \pm 0.17$	$3.63^{a} \pm 0.26$	$5.46^{a} \pm 1.07$
Un-challenged fish	$1.68^{a} \pm 0.02$	$1.03^{a} \pm 0.86$	$1.83^{a} \pm 0.14$	$4.36^{a} \pm 0.48$	$4.88^{a} \pm 1.67$
Un-treated fish	$1.54^{b} \pm 0.01$	$1.99^{a} \pm 0.17$	$1.50^{a} \pm 0.26$	$1.71^{b} \pm 0.16$	$6.08^{a} \pm 1.24$

Table 2.
Mean values (± SD) of some biological indices calculated at conclusion of Trial 2. Common letters denote no significance differences among treatments ($P > 0.05$).

showed no significant differences in the mean SSIs and HSIs ($P > 0.05$) (results not presented). In general, fish treated with crushed garlic performed better as evidenced by a higher CFs, lower FCRs, and higher SGRs. Crushed garlic treatment also completely removed 'ich' infection and prevented further development of the disease. The gill and skin histopathology didn't reveal pathology associated with either the parasites or the treatments.

Trial 2

In the second trial, mean CF and mean lymphocyte densities were significantly higher ($P < 0.05$) among the treatment groups. There were no significant differences in the mean FCRs, mean SGRs, and mean neutrophil densities ($P > 0.05$) (Table 2). As in Trial 1, there were no significant differences in both the calculated mean SSIs and HSIs (results not presented). The un-challenged fish performed noticeably better as evidenced by the calculated biological indices (Table 2).

Conclusion

The trials have shown that crushed garlic and lemon juice have a great potential as alternative "safe" treatments for white spot disease on tilapias. The crushed garlic in Trial 1 (3 g/L) completely removed 'ich' infections and successfully prevented further development of the parasite after treatment. This concentration could be used to effectively mitigate 'ich' problems in pond culture. Lemon juice, though effective, resulted in tremendous reduction in water pH (7.2 to 4.6) and could have problems with practical use on fish farms where expertise would be needed to buffer the pH after treatments. The concentration of crushed garlic used in Trial 2 (1.5 g/L) failed to completely remove the infection in the treatment tanks. However, besides pH reduction, the products did not induce any noticeable fish behavior and feeding problems and there were no sub-lethal effects associated with the products on the fish. Some areas that may require further research and future considerations include:

- Conducting similar trials under pond culture to compare the results with the laboratory results;
- Quantifying the amount of allicin (the active ingredient) in garlic used for treatments;
- Incorporating allicin in feed to see if it has the same effect as direct application in controlling ichthyophthiriasis infections;
- Testing garlic residues in the flesh of the fish and also to evaluate if garlic treatments affect fish palatability; and
- Evaluating the comparative efficacy of using garlic treatment as a one-time bath dip to the concept of continuous exposure.

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Stress Evaluation in Cultivated Scallops, *Placopecten magellanicus*, and Oysters, *Crassostrea virginica*, using Lysosomal Destabilization Assays

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The neutral red retention assay (NRRA) has proven to be a useful non-lethal stress test in wild and cultured mussels. The development of such assays for use in other commercially important bivalves could benefit the aquaculture industry by allowing a more rapid assessment of culture conditions on species performance. In the present study, the mussel NRRA was modified for the giant scallop, *Placopecten magellanicus*, and eastern oyster, *Crassostrea virginica*. Live oysters held in dry storage at one of two temperatures (0-4ºC; 12-14ºC) for 16 days displayed increasing levels of cellular stress over time, with stress levels significantly higher in oysters kept at warmer temperatures. Scallops were exposed to acute salinity challenges (ambient 32 ppt, 30 ppt, 28 ppt, 26 ppt, 24 ppt) for a 10-day period. Scallop NRRA scores declined slightly over the duration of exposure in all treatments, however the levels were significantly different from the controls in the 24-ppt and 26-ppt treatments only, and varied in a dose dependent manner. The present findings demonstrate that lysosomal destabilization assays provide a useful index of subcellular stress in commercially important bivalves species, and that the assays are sensitive to small environmental changes in the culture environment of the organisms.

Le test de rétention du rouge neutre s'est avéré être un moyen non létal utile pour évaluer le stress chez les moules sauvages et les moules de culture. La mise au point de tests semblables pour étudier d'autres bivalves d'importance commerciale pourrait être avantageuse pour l'industrie aquacole en permettant une évaluation plus rapide de l'effet des conditions de culture sur le rendement de celle-ci. Dans le cadre de la présente étude, nous avons modifié le test conçu pour évaluer le stress chez les moules de manière à pouvoir l'appliquer au pétoncle géant (*Placopecten magellanicus*) et à l'huître américaine (*Crassostrea virginica*). Les huîtres vivantes conservées en milieu sec à deux températures différentes (entre 0 et 4 ºC et entre 12 et 14 ºC) pendant 16 jours ont montré des signes d'une hausse du stress cellulaire au fil du temps, le stress étant significativement plus élevé chez les huîtres conservées à une température plus élevée. Les pétoncles ont été placés dans des milieux de salinité différente ([salinité ambiante] 32, 30, 28, 26 et 24 ppm) pendant une période de 10 jours. Le stress chez cette espèce a diminué légèrement au fil des traitements. Seuls les résultats dans les milieux de 24 et 26 ppm sont significativement différents de ceux du traitement témoin, le stress ayant varié en fonction de la salinité. Les résultats de cette étude montrent que les tests de déstabilisation des lysosomes fournissent un indice utile du stress à l'échelle infracellulaire chez les espèces de bivalves d'importance commerciale et que ces tests sont sensibles aux petits changements environnementaux qui surviennent dans le milieu de culture des organismes.

Introduction

Cellular lysosomes are the site of one of the earliest detectable changes following a stress stimulus in marine molluscs[(1)]. When an animal is stressed, the lysosomal lipid membrane becomes destabilized releasing lytic enzymes into the cytosol of the haemocyte. Such pathological alterations of the lysosomal compartment can be examined in live molluscan haemocytes using the neutral red retention assay (NRRA)[(2)]. The method has been employed to examine pathological changes in the lysosomal compartment in the hemocytes of living organisms subjected to environmental stresses such as hydrocarbon contamination or anoxia[(3)]. The NRRA is an efficient biomarker to make rapid initial assessments of the immune status of shellfish[(1)], and has been adapted and validated recently to measure stress responses in several cultured bivalve species, including the blue mussel[(2)], European oyster (*Ostrea edulis*)[(4)], and the king scallop (*Pecten maximus*)[(5)].

Suboptimal environmental or culture conditions leading to pre- and post-harvest losses in cultured bivalves are difficult to assess and are generally measured at the whole animal level (e.g., scope for growth). These measures can be time consuming, costly, and are often destructive in nature. A possible solution to this problem is to develop user-friendly biochemical tools, such as the NRRA, that industry and researchers can utilize to evaluate stress in shellfish, thus allowing the optimization of the quality of the product, and improvements in production efficiencies[(1)].

Two candidate species of current interest for culture in North America are the sea scallop (*Placopecten magellanicus*) and the Eastern oyster (*Crassostrea virginica*). The objectives of the pres-

ent study were to adapt and evaluate the NRRA as a sensitive indicator of stress in both species with the view of providing useful tools for assessing non-lethal stress under culture conditions.

Materials and Methods

Oyster experiment

Cultured Eastern oysters, *Crassostrea virginica* (60-70 mm shell length), were harvested live from a commercial supplier (Maison Beausoleil, NB), held on ice and shipped via same day air freight to the Marine Institute in St. John's, NL. Once at the Marine Institute, oysters were held in dry-air storage under two temperature regimes: 12-14ºC (stressed group) and 0-4ºC (unstressed control group). A small sampling port (1-2 mm diameter hole) was drilled into individually numbered oysters ($n = 10$) immediately above the adductor sinus to facilitate hemolymph withdrawal. A wax plug was employed in the port between samplings. Oysters were held under these conditions for 16 days and hemolymph sampled repeatedly from individual animals every 3-4 days for assessment of neutral red retention.

Scallop experiment

Cultured sea scallops, *Placopecten magellanicus* (80-90 mm shell height), were obtained from a commercial supplier (Great Maritime Scallop Trading Co., Chester, NS), chilled and shipped via same day air freight. Upon arrival, scallops were placed in recirculating seawater raceways and held at 4-5ºC for a few days prior to experimentation. The scallops were divided into 5 groups of 6 scallops and placed in individual containers at various salinities: 32 ppt (control), 30 ppt, 28 ppt, 26 ppt, and 24 ppt. Water was aerated, changed daily, and scallops were unfed for the duration of the trial (10 days). Hemolymph samples were obtained from each scallop in each treatment every 3 or 4 days for assessment using the NRRA.

Hemolymph sampling

On sampling days, 0.5 mL of hemolymph was withdrawn from the adductor muscle sinus of each animal using a 21-gauge needle filled with 0.5 mL physiological saline. The saline solution for *C. virginica* was adapted from Hauton et al.[5] for *C. gigas.* The saline solution for *P. magellanicus* was based on the formulation for *Pecten maximus* found in Hauton et al.[4]. The hemolymph/saline mixture was transferred to Eppendorf® microcentrifuge tubes. Tubes were inverted and held in a water bath (15ºC) for 15 minutes. The mixture was pipetted in aliquots of 50 µL onto dry Poly-L-Lysine treated slides and placed in a humidity chamber in darkness. 50 µL of neutral red solution was then applied following protocols adapted from Depledge (2000, unpublished lab manual) as outlined in Harding[2].

Slide examination

Each slide was systematically examined under the microscope (400×) every 15 minutes for the first hour then every 30 minutes up to 120 minutes then finally at 180 minutes. The slides were examined for one minute under the microscope in the dark as the neutral red is sensitive to light. Stress was measured by the time it took for 50% of 25 scanned hemocyte cells to release the red dye. Retention time (RT) is expressed in minutes, and the lower the RT, the greater the degree of stress[2].

Results and Discussion

Survival

No mortalities were observed in any of the experiments.

Oysters

Lysosomal neutral red retention time (RT) decreased significantly with the duration of air exposure in Eastern oysters (Fig. 1; ANOVA, $P<0.001$), suggesting increased stress with exposure time. RT was significantly lower in oysters exposed to higher air temperatures (ANOVA, $P<0.001$) indicating the elevated dry storage temperature was more stressful to oysters than lower temperature dry storage conditions. These results are in keeping with previous findings on cultivated mussels exposed to various dry storage thermal conditions[2].

Repeated sampling of oyster hemolymph may have influenced the RT of oysters held under the two dry storage regimes. Oysters held at 2-4ºC without drilled sampling ports displayed significantly higher (t-test, $P < 0.05$) RTs of 114 and 44 minutes at Days 0 and 16, respectively. However, a similar trend was evident in this group as with the two other groups of oysters sampled repeatedly. This, combined with 100% survival of repeat sampled oysters, suggests the repeated sampling procedure did

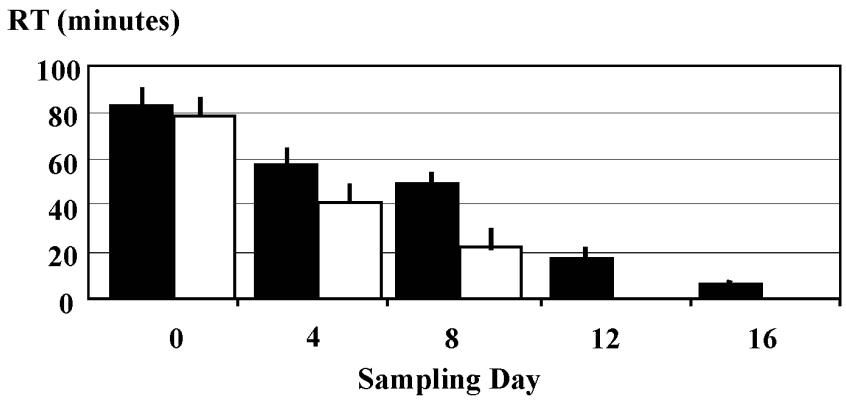

Figure 1.
Neutral red retention time (RT - minutes) in hemocyte lysosomes of oysters, *Crassostrea virginica*, held in dry storage at two temperatures for 16 days. Shaded bars: 0-4ºC, unshaded bars:12-14ºC. Missing unshaded bars on Days 12 and 16 signify 0 minutes. Bars represent the mean + SE, *n* = 10.

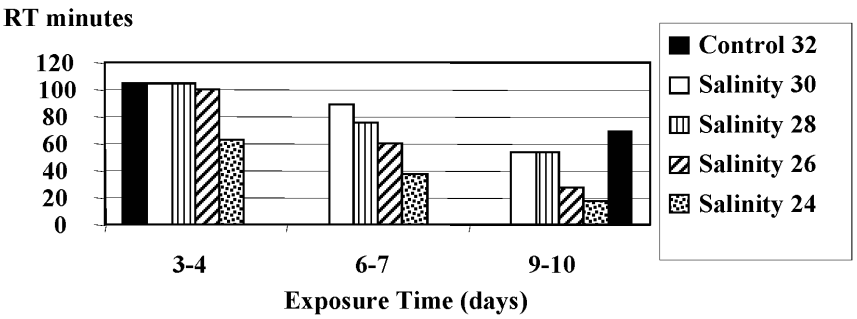

Figure 2.
Change in neutral red retention time (RT – minutes) in the hemocyte lysosomes of *Placopecten magellanicus* exposed to various salinity challenges for 10 days. Bars represent the mean of 6 scallops sampled repetitively.

not contribute substantially to oyster stress. Further work is obviously warranted to clarify this suggestion.

Scallops

Scallops displayed a significant decline in neutral red RT over the 10 day exposure time in all groups including control groups (Fig. 2; ANOVA, $P < 0.001$). Salinity exposure treatments of 28 to 32 ppt were nearly identical in terms of RT response whereas salinities of 24 and 26 ppt displayed significantly reduced RT, suggesting higher stress levels in these animals after just a few days exposure (Fig. 2; Day 3 sampling). Scallops exposed to salinities below 28 ppt produced copious amounts of mucous, a typical stress response in marine molluscs to abrupt environmental insults[6].

Conclusions

Lysosomal destabilization assays using neutral red dye were found to be sensitive indicators of environmental stress in the Eastern oyster (*Crassostrea virginica*), and the sea scallop (*Placopecten magellanicus*).

Oyster responses to thermal challenges were predictable and appeared to be dose-dependent. Similarly, scallops subjected to acute salinity challenges also responded in a dose-dependent fashion with minor salinity decreases showing significant effects on cellular stress in this stenohaline species.

The NRRA allows for non-lethal repeated sampling of marine bivalve hemolymph for stress assays.

The NRRA offers promise as a relatively easy and simple tool for evaluating the influence of various culture conditions on the performance of these candidate species for culture.

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A Comparison of Natural and Artificial Seawater for Algal Growth

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For land-based operations it is important to determine the best source of saltwater. Using imported natural seawater and an artificial mixture of Instant Ocean®, algal growth rates of the species *Isochrysis galbana* (T-iso)*, Chaetoceros muelleri,* and *Pavlova lutheri* were compared. Both treatments were enriched with nutrients and supplied with required amounts of silicate. For *C. muelleri*, there was no significant difference in algae growth until day 13, when the cultures had already begun the crash phase of their growth. For *I. galbana*, there was no significant difference in algal growth until day 6, after which natural seawater produced higher cell densities than Instant Ocean® until the end of the experiment. For *P. lutheri*, there was no significant difference in algal growth between the two water treatments. Economically, it is cheaper in this situation to purchase natural seawater. Although there was a significant improvement with natural seawater during the growth phase for one of the tested species *I. galbana*, an artificial replacement has proven to have similar benefits as natural seawater for the remaining species *C. muelleri* and *P. lutheri*.

Il est important, dans le cas des opérations de culture à terre, d'établir la meilleure source d'eau de mer. Nous avons comparé les taux de croissance des algues *Isochrysis galbana* (T-iso), *Chaetoceros muelleri* et *Pavlova lutheri* dans de l'eau de mer naturelle importée et le mélange artificiel Instant Ocean®. Les deux milieux avaient été enrichis de matières nutritives et des quantités requises de silicate. Dans le cas de *C. muelleri*, nous n'avons observé une différence significative dans le taux de croissance que le jour 13, lorsque les cultures étaient déjà en voie d 'écroulement; dans le cas d'*I. galbana*, que le jour 6, après quoi l'eau de mer naturelle a donné des densités de cellules plus élevées que Instant Ocean® jusqu'à la fin de l'expérience; alors que dans le cas de *P. lutheri*, nous n'avons observé aucune différence significative entre les deux milieux de croissance. Sur le plan économique, dans cette situation, il est moins dispendieux d'acheter de l'eau de mer naturelle. Bien que nous ayons observé une augmentation significative du taux de croissance d'*I. galbana* dans de l'eau de mer naturelle durant la phase de croissance, un milieu artificiel de remplacement s'est révélé, dans le cas de *C. muelleri* et *P. lutheri*, avoir des avantages semblables à l'eau de mer naturelle.

Introduction

Inland aquaculture facilities must make the decision of whether to import seawater from the coast or depend on artificial seawater. The research is performed on a land-locked site at the Nova Scotia Agricultural College in Truro, NS. The seawater options being compared are using synthetic seawater made from the product Instant Ocean® versus trucking natural seawater from the Aquaculture Research Centre, Institute Marine Biosciences, located at Sandy Cove, NS.

There are a variety of saltwater mixtures that are available which can adequately support the growth of algae when properly enriched[1,2]. It is best to test growth in small quantities before committing to a saltwater mix, as a few brands produced for aquarium use are designed to limit algal growth[3]. The synthetic seawater used is Instant Ocean®, produced by Aquarium Systems Inc. According to the company the product Instant Ocean® contains all the necessary trace elements[4].

Natural seawater consists of over 50 known elements[1] which is very similar to the artificial brand Instant Ocean®[5,6]. When the two treatments were compared using Inductively Coupled Plasma (ICP) spectoscopy as a measurement, there was a greater sum of major cations and anions in natural seawater versus Instant Ocean® , and overall tested nutrients were higher in natural seawater[7]. Trace elements for the majority of synthetic mixtures tend to be lower than natural seawater[8], however Instant Ocean® was shown to have higher levels then natural seawater[7].

When analyzing seawater a precise method must be used to avoid inaccurate readings[8]. It is difficult to fully analyze natural seawater as the contents may vary from source to source, as well as the depth in the water column that it is found. The levels of trace elements found would be constantly changing because of natural biological functions[8]. When comparing the two treatments parameters such as length of storage and temperature that it is kept at should also be considered.

Like all living organisms, marine algae are directly influenced by their environment. Toxic levels can be present if the artificial seawater is not properly mixed[9]. The concentration of the seawater will affect the algal growth[11]. In addition to nutrient quality and quantity, the environment of algal growth is also influenced by other parameters such as salinity[2]. Salinity levels of natural seawater vary depending on the season[1,11]. Both the artificial seawater mixture and natural seawater must be tested to ensure that the salinity is correct.

The algae species used in this study included the diatom *C. muelleri* and the flagellates *I. galbana* and *P. lutheri.* These species

are commonly used in commercial shellfish hatcheries and are known as beneficial algal diets for most shellfish species. Harrison et al.[6] had grown a series of species from the *Prymnesiophyceae* class, including *I. galbana* and *P. lutheri* and found there was no difference between natural and artificial seawater.

Materials and Methods

Algae was grown using a batch culture system. This begins from a small axenic stock culture and proceeds by dilution into successively larger culture volumes until the final volume is achieved. Transfers into larger volumes are made before nutrients in the original volume become limiting; thus growth continues exponentially[3]. Salinities were the same for both natural and artificial solutions (32 ppt). Thorough filtration was performed before any imported seawater entered the system. The seawater passed through a 0.22-µm particle filter and then through ultraviolet filters.

Before inoculation of a culture vessel, the flask or carboy is washed, rinsed with 10% HCl and rinsed with distilled water. The vessel is then filled with the salt water of choice, the correct volume of nutrients are added, and for the diatom species, sodium silicate is added for the production of their external shell. Kent Pro-Culture F/2 was added as a nutrient source. HCl was added to prevent precipitation. The vessel was then autoclaved at 121°C under a pressure of 6.82 kg for 35 minutes. After the water cooled, the vessel was inoculated with algae.

Under a flow through transfer hood, over a flame, a volume of 10 mL of stock culture was inoculated into 125-mL Erlenmeyer flasks containing a volume of 50 mL of either transported seawater or Instant Ocean® prepared solution. Daily, the cultures were swirled to keep the algae in suspension. After one week, using the same sterile transfer technique, the cultures were transferred to 1-L flasks containing 500 mL of sterilized solution. The 1-L flasks were grown for one week after which carboys were inoculated. The carboys were injected with pressurized air mixed with approximately 3% carbon dioxide to compensate for any pH changes[12]. The pH was monitored daily and the CO_2 adjusted accordingly so that a constant pH of approximately 7.8 was maintained throughout the trial.

To eliminate any error, external parameters were kept as constant as possible. In addition to injected air pressure the carboys were vigorously shaken and randomly placed on a daily basis. Temperature was kept at 20ºC and the cultures were kept under 24-hour 40W fluorescent lighting in a greenhouse.

Cell counts on the carboys were performed for a two week period every 2-3 days using a Neubauer hemacytometer. The carboy cultures were grown in triplicate for each treatment and two cell counts were performed on each culture. An analysis of variance was performed using statistical analysis with SAS computer software.

Results

For the diatom, *C. muelleri*, there was no significant difference in algae growth between the two treatments until the cultures began to decrease in cell densities after day 10. For *I. galbana* (T-iso), natural seawater produced significantly higher cell densities than synthetic seawater after day 6 of the experiment. For *P. lutheri*, there was no significant difference in algae growth between the two water treatments throughout the experiment (Fig. 1).

Discussion and Conclusion

With *I. galbana* having significantly higher growth in natural seawater, artificial replacements may not always be the best choice. Artificial seawater may be lacking an adequate level of re-

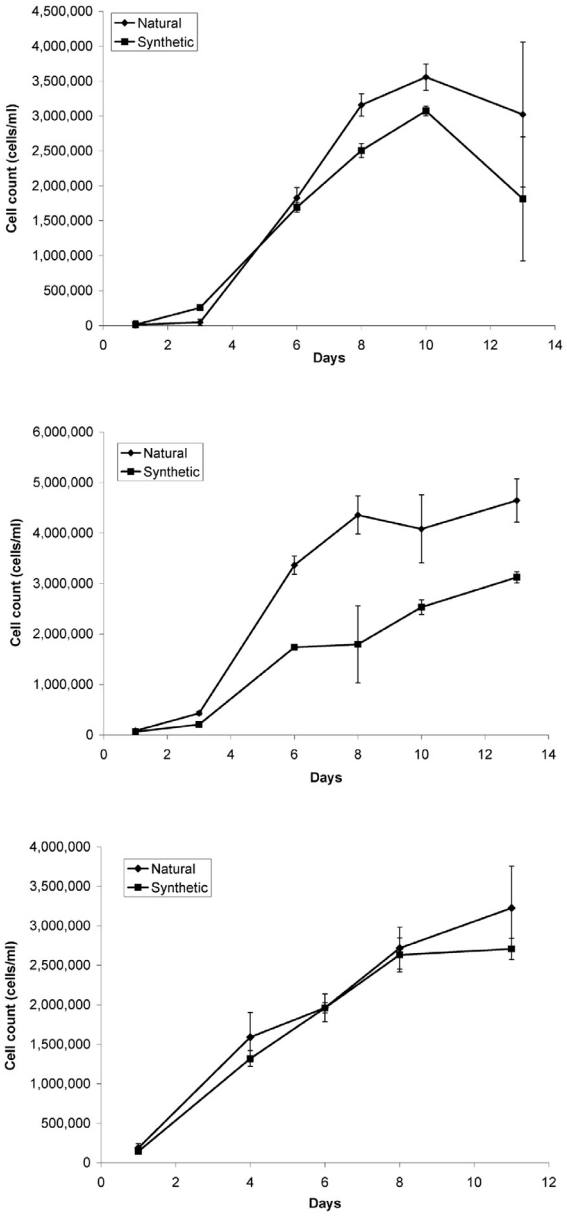

Figure 1.
Comparison of natural and artificial Instant Ocean® seawater using the growth of algal species *Isochrysis galbana* (T-iso; top), *Chaetoceros muelleri* (middle), and *Pavlova lutheri* (bottom).

quired nutrients. A trial preformed in 1980 by Harrison et. al [6] claimed that 77% of algal cultures grew best in natural seawater. This suggests species specific nutrient requirements. The composition of artificial seawater however has since advanced[13]. Comparing natural and artificial seawater, *P. lutheri* did not show a significant difference throughout the trial and *C. muelleri* had no significant difference until the 'crash phase'. With there being no overall significant difference between the treatments the cost is often the deciding factor.

For land-locked aquaculture operations expense impacts the decision of saltwater choice. In this instance ordering Instant Ocean® to our facility, including shipping and taxes is priced at 2.38¢/L while transferring truck loads of seawater from a saltwater site would cost 1.56¢/L. Purchasing natural seawater is more cost effective for this site, which is not always the case for other facilities. Depending on location, it is cheaper for some facilities to purchase an artificial replacement[14].

The species *I. galbana* has shown a significant difference in growth, favoring the natural seawater environment. For the species *P.lutheri* and *C. muelleri* an artificial replacement had similar growth results as natural seawater. With the composition of natural seawater being variable depending on location, depth and season an artificial replacement becomes more desirable if consistency is required. The artificial mixture of Instant Ocean® is found to be an acceptable substitute for natural seawater for the growth of 2/3 species of marine algae tested.

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Potential for Freezing and Freeze-Drying as Methods for Preserving Microalgal Concentrates in Bivalve Hatcheries

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As the human population grows, there is a growing demand for bivalves as they are low in saturated fats and a nutritious source of omega-3 fatty acids. There is also increasing reliance on bivalve aquaculture, which has increased in Canada and around the world over the past two decades, to provide these healthy products for human consumption. The limitation in this industry is that it may not be able to increase its production enough to meet growing demands unless hatcheries are able to supply juveniles to marine grow out farms. Hatchery rearing of bivalves depends on the production of live microalgae which is costly and unpredictable. Hatchery production would be improved if optimal microalgal diets could be concentrated and preserved in frozen or freeze-dried forms. An effective concentrated diet would improve reliability and success of bivalve aquaculture by accelerating growth rate of young bivalves under repeatable and controlled conditions while maximizing return on microalgae-culture investment. In the Maritimes, this microalgal supply problem is particularly evident in aquaculture of the American oyster, *Crassostrea virginica.* A cost-effective frozen or freeze-dried alternative to onsite microalgae would reduce operating costs in hatcheries and improve the efficiency of juvenile oyster production. This paper reviews the literature on the use of concentrated microalgae as food for juvenile bivalves.

L'accroissement de la population mondiale engendre une hausse de la demande pour des bivalves, car ils sont faibles en gras saturés et riches en acide gras oméga-3. Pour satisfaire à cette demande de produits sains, on a de plus en plus recours à la conchyliculture, qui a connu un essor au Canada et à l'étranger au cours des deux dernières décennies. L'industrie ne sera pas en mesure d'accroître suffisamment sa production pour répondre à cette demande croissante à moins que les écloseries soient capables de fournir des naissains aux fermes de grossissement en mer. La culture de bivalves en écloserie dépend de la production de microalgues vivantes, coûteuse et imprévisible. La production en écloserie pourrait être accrue si des rations optimales de microalgues pouvaient être concentrées et conservées sous forme congelée ou lyophilisée, ce qui permettrait d'accroître la fiabilité et le succès de la conchyliculture, le taux de croissance des jeunes bivalves pouvant être accéléré dans des conditions reproductibles et contrôlées et le rendement du capital investi dans la culture des microalgues maximisé. Dans les Maritimes, ce problème d'approvisionnement en microalgues est particulièrement évident au niveau de la culture de l'huître américaine, *Crassostrea virginica*. Une source rentable d'approvisionnement en microalgues congelées ou lyophylisées, qui remplacerait l'approvisionnement sur les lieux, permettrait de réduire les coûts d'exploitation des écloseries et d'améliorer l'efficience de production d'huîtres juvéniles. Nous passons en revue la documentation sur l'utilisation de concentrés de microalgues comme aliments pour les bivalves juvéniles.

Introduction

Aquaculture of fish, crustaceans, and mollusks is growing more rapidly globally than any other food sector. It has increased production by an average compounded value of 9.2% annually between 1970 and 2002, compared to 1.4% for capture fisheries and 2.8% for terrestrial farmed meat. In 2002, mollusks, primarily consisting of bivalves, accounted for 46.2% of world mariculture as well as 6.1% of brackish water aquaculture[1]. Bivalve aquaculture has a bright future due to human nutritional benefits that their products can offer as a part of a healthy diet low in saturated fat and high in omega-3 fatty acids[2,3]. This is compounded by the fact that wild stocks have diminished due to over-collecting, contamination, habitat loss, and disease[4]. As the human population grows the demand for seafood from aquaculture will continue to be an essential element in our food security. Bivalve aquaculture may also most certainly be a key player in ecologically sustainable mariculture. Cultured bivalves, in addition to providing a safe, nutritious, healthy food source, also act as biofilters, improving water quality by removing particulates and some unwanted nutrients from the water column[5].

In Canada, bivalve aquaculture takes place mainly in seawater around the Atlantic Provinces and British Columbia with contributions made by coastal Quebec. PEI makes the largest contribution followed by BC. Between 1986 and 2003 tonnage produced by bivalve aquaculture in Canada has increased five-fold from seven to upwards of thirty-five thousand metric tons. In the Maritimes the greatest tonnage comes from the culture of the blue mussel (*Mytilus edulis*) totaling 18 718 metric tons in 2003 followed by the American or Eastern oyster (*Crassostrea virginica*, Gmelin) totaling 5784 metric tons in that same year[6]. Although Canada makes a modest contribution to world bivalve aquaculture, the products are of excellent quality and some such as "Malpeque" oysters are enjoyed worldwide. The industry has grown in this country over the past two decades and there is potential for more growth in the future[6].

Despite the promising outlook for bivalve aquaculture in Canada and around the world, there is a serious limitation in the in-

dustry. The limiting factor is the lack of a cost-effective means to produce microalgae, the required food for bivalves at all stages of their life cycle[7]. Marine grow out farms rely increasingly on hatcheries to rear larvae and juveniles – also referred to as spat or seed[8] – but for these hatcheries to be feasible they must be able to turn a profit, hence a solution to the microalgae supply problem must be found. A common suggested solution is using wild juveniles, but there are multiple advantages for growers to use reared juveniles over those collected in the wild:

- selective breeding of broodstock in the hatchery gives reared oysters significant advantages over those from the wild, such as genetic improvement for increased growth, improved shell shape and disease resistance, the use of triploids, and the general availability of a variety of cultured species[8];
- the ability to control the food intake of the larvae and juveniles exists in the hatchery enabling maximization of growth[9];
- hatchery rearing is more reliable so juveniles can be provided to marine grow out farms in areas where "spatfall" is low[10];
- by getting a head start in the hatchery, mortality rates of juveniles are lower during their first winter grow out season; and
- in an area such as in the Chesapeake Bay where aquaculture has become a race between disease and attainment of market size rapid juvenile growth is critical[11].

As the bivalve aquaculture industry expands, there is a demand for an alternative food to replace live microalgae that is more economical than present conventional methods[12]. Presently microalgae are cultured onsite and comprise 20 to 50% of total hatchery expenditures and are a major bottleneck in the industry[12,13]. This microalgal supply problem is very evident in the Maritimes in the American oyster aquaculture industry. There is a major problem due to a shortage of natural juvenile (seed) stock. Regrettably, due to the low water temperatures in some areas, setting of oyster larvae in commercially significant quantities does not occur every year. To help solve the problem, Fisheries and Oceans Canada set up an experimental oyster hatchery at Ellerslie, PEI. It developed reliable techniques for juvenile oyster production but the process proved too costly because of the high cost and risks of culturing microalgae on-site to be commercially viable[14], resulting in a "catch-22" situation; the American oyster industry in the Maritimes could benefit from hatcheries but the high cost of producing microalgae for food for larvae and juveniles is not practical in this cold climate. In 2004, there were no American oyster juveniles available anywhere in the Maritimes so growers have to use unreliable wild juveniles or import stock from the US. There is a real need for an inexpensive alternative to on-site microalgal production.

Microalgal Concentrates

There have been numerous studies on the feasibility of alternative diets in the bivalve aquaculture industry that may be more cost effective and reliable than live microalgae. These have included such things as microalgal concentrates[7,15,16] microbound diets[15], microencapsulated diets[17], microgel capsules[18], gelatin-acacia capsules[7], lipid microspheres[18], alternative cellular organisms such as yeast[13,17] and bacteria[19], and other inert diets such as cornmeal, cornstarch[20], wheatgerm[21], and cheese whey[22]. Despite alternatives to microalgae that have exhibited promise as either partial or full replacement diets for some bivalves, the cost of producing and distributing products has generally proved to be too high and the nutritional quality too low to be feasible for commercial hatchery operations[13,15]. A notable exception, however, has been the use of microalgal concentrates[23].

Microalgal concentrates are produced by centrifuging live microalgae in a high-speed centrifuge to remove excess extracellular water resulting in an algal paste or slurry. This paste can subsequently be preserved by refrigeration at 4ºC, spray-drying, freezing, or freeze-drying[7]. The advantage of concentrates are that they are the natural food of bivalves and potentially harmful metabolites that could be in the culture water of microalgae are removed making it safer to use as feed for larvae and juveniles[24].

Refrigerated paste (4ºC) can be purchased from a supply company such as Reed Mariculture, San Jose CA, or be made onsite in the hatchery. The problems with this preservation method are the shelf life is usually limited and to purchase from a central supply company is very expensive, in excess of $500US per kg[25]. This does not provide a long-term solution to the microalgae supply problem and there is concern about reduction of nutritional value and bacterial degradation when stored at 4ºC[26].

Techniques have been developed for the large scale production of marine microalgae under heterotrophic growth conditions, by utilizing organic carbon instead of light as a source of energy thus reducing production costs. Heterotrophic microalgal cultures can attain up to 1000 times higher densities than photoautotrophic cultures which makes them a candidate for preserving by spray-drying. Unfortunately, heterotrophic mass-production techniques has been realized for very few microalgal species[17] and it has been found when most species are grown heterotrophically they undergo major changes in their nutrient composition that result in drastic reduction of omega-3 fatty acids compared to light-grown microalgae[17]. *Tetraselmis suecica* was heterotrophically grown and spray-dried and marketed as Algal 161™ in the1980s. It was thought to be very expensive and only produced moderate growth in bivalves so is no longer on the market[25]. A commercialized heterotrophically grown, spray-dried marine microalgae, *Schizochytrium* sp. (ALGAMAC-2000™, Aquafauna Bio-Marine, Los Angeles, CA) high in the omega-3 fatty acid docosohexanoic acid has shown very good results as a 70% replacement for live microalgae for pennaied shrimp but has not been used widely for bivalves[27].

Freezing microalgae or cryopreservation at -80ºC has potential as a replacement for live microalgae in the hatchery[28]. It is possible to freeze microalgae up to 21 months without significantly affecting fatty acid profiles[29]. High lipid content found in some phytoplankton is thought to protect the cells from injury during freezing, preventing intracellular ice formation[30]. There are some commercially available frozen *Nannochloropsis* spp. and *Tetraselmis* spp. sold at Reed Mariculture but, like the microalgal pastes, are very expensive. Techniques in cryopreservation used in culture collections for the preservation of microalgal strains have been very successful and may have application in bivalve aquaculture[28].

Freeze-drying, also referred to as lyophilization, is the dehydration (intracellular and extracellular) of frozen aqueous material (the microalgal paste) through the sublimination of ice. Thus it is first frozen and then freeze-dried. There has been controversy over how well this method preserves the fatty acid profile. Molina-Grima et al.[26] found that the fatty acid profile remained unchanged after freeze-drying. Cordero and Voltolina[31], however, found that freeze-drying was not adequate for the purpose of long-term preservation of microalgae. At the Nova Scotia Agricultural College, juvenile oysters were fed either live,

freeze-dried or a 50% live/50% freeze-dried mixture of two commercially viable algae species, *Tetraselmis suecica* and *Chaetoceros muelleri.* Very promising results were found with freeze-dried *T. suecica* indicating it could be used as a total or partial replacement for live diets. It actually performed better as a feed freeze-dried than fresh, due to its reduced size when rehydrated making it more palatable for shellfish[16].

Summary

Frozen and freeze-dried diets show the greatest potential as replacement for live microalgae in the bivalve hatcheries. Successful hatcheries could mean the creation of many jobs and juveniles could be made available locally for grow out farms. This in turn could lead to increased bivalve production in aquaculture to help meet the increasing consumer demand. The development of these diets could indeed make American oyster hatcheries feasible in the Maritimes making a contribution to the local economy. In order for this all to happen research and development is needed to create a nutritious frozen or freeze-dried concentrated diet and also to study the physiological response of bivalves to these diets. Hopefully a solution to the live microalgal supply problem will be found and hatcheries will have the technology to remuneratively preserve concentrates during the off season to store for the busy season as a more reliable food source.

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A New Model for Predicting the Expansion of Fluidized Bed Biofilters

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Fluidized-bed biofilters are commonly used for removing ammonia in recirculating aquacultural systems. The nitrifying bacteria grow on the fluidized media (sand or plastic particles) and extract their nutrients from the wastewater passing over the particles. As the superficial velocity of the liquid phase increases or as the density of the particles changes due to biofilm growth, the fluidized particles move apart to keep the drag force exerted by the water equal to the buoyant weight of the particles. This expansion of the bed must be taken into account when calculating the volume of the biofilter required to achieve a certain ammonia removal efficiency. The Richardson-Zaki equation is commonly used to calculate bed expansion but is strictly only valid for spherical particles. A new model is proposed for particles of any sphericity. The model assumes that the porosity of the fluidized bed is a power function of superficial velocity and reaches unity when the superficial velocity is equal to the terminal settling velocity of the particles. The predictions of the new model are in good agreement with published experimental data for sand, carbon, and glass beads. It is also shown that the new model can accurately predict the expansion of down-flow biofilters that fluidize plastic particles lighter than water.

Les biofiltres à lit fluidisé sont communément utilisés pour éliminer l'ammoniac dans les circuits aquicoles fermés. Les bactéries nitrifiantes prolifèrent sur le milieu de croissance fluidisé (particules de sable ou de plastique) et tirent des matières nutritives des eaux usées passant au-dessus des particules. À mesure que la vitesse superficielle de la phase liquide augmente ou que la densité des particules change à cause de la croissance du film biologique, les particules fluidisées se séparent de sorte à ce que la force de traînée exercée par l'eau reste égale à la densité immergée des particules. Il faut tenir compte de cette expansion du lit lorsqu'on calcule le volume du biofiltre requis pour obtenir un certain niveau de rendement d'élimination de l'ammoniac. L'équation Richardson-Zaki est communément utilisée pour calculer le niveau d'expansion du lit, mais elle ne s'applique strictement qu'aux particules sphériques. Nous proposons ici un nouveau modèle applicable aux particules de toute sphéricité. Ce modèle suppose que la porosité du lit fluidisé est une fonction puissance de la vitesse superficielle et atteint l'unité lorsque la vitesse superficielle est égale à la vitesse de sédimentation terminale des particules. Les prédictions issues du nouveau modèle concordent bien aux données expérimentales publiées pour le sable, le charbon et les billes de verre. Nous démontrons aussi que le nouveau modèle peut précisément prédire le niveau d'expansion de biofiltres à circulation descendante qui fluidisent les particules de plastique plus légères que l'eau.

Introduction

Fluidized bed biofilters are widely used in recirculating aquaculture systems because they provide a high biomass concentration, high mass transport rate, high bed voidage and avoid clogging problems[1-3]. Sand, activated carbon, and plastic beads are used as support media for the biofilm. The expansion property of particles has a strong effect on the performance and hydrodynamic characteristics of fluidized bed biofilters. As the density of the particles changes due to biofilm growth or as the superficial velocity of the liquid phase increases, the fluidized bed expands to keep the drag force equal to the buoyant weight of the particles.

The equations obtained by Richardson and Zaki[4], Ergun[5], and Khan and Richardson[6] have been applied for many years to predict fluidized bed expansion. The Richardson-Zaki equation and the Khan-Richardson equations are, however, only valid for spherical particles and highly expanded beds. The Ergun equation, on the other hand, takes sphericity into account, but is only valid for small bed expansions.

The models suggested by Richardson and Zaki, and Ergun are combined in this study to develop a new model for predicting bed expansion. The new model is validated using expansion data for particles of different shape and density.

Literature review

The model proposed by Richardson and Zaki[4] is an empirical model for highly expanded fluidized beds:

$$\frac{u}{u_t} = \varepsilon^n \qquad (1)$$

Several methods have been proposed to calculate the value of n in Equation 1. If particles have a spherical shape and their size

is small compared to the column diameter, the equation suggested by Khan and Richardson[6] applies:

$$\frac{4.8-n}{n-2.4}=0.043Ar^{0.57} \quad (2)$$

To take wall effects into account, Richardson and Zaki[4] suggested the following two equations for calculating the exponent n:

For $1<Re_t<200$

$$n=(4.45+18\frac{d_p}{D_c})\mathrm{Re}_t^{-0.1} \quad (3a)$$

For $200<Re_t<500$

$$n=4.45\mathrm{Re}_t^{-0.1} \quad (3b)$$

These equations were later empirically modified by Cleasby et al.[7] to take particle shape into account:

For $15<Re_t<200$

$$n=(4.45+18\frac{d_p}{D_c})\mathrm{Re}_t^{-0.1}\,\varphi^{(-2.9237\varphi^{0.884}\,\mathrm{Re}_t^{-0.363})} \quad (4a)$$

For $200<Re_t<503$

$$n=4.45\mathrm{Re}_t^{-0.1}\,\varphi^{(-2.9237\varphi^{0.884}\,\mathrm{Re}_t^{-0.363})} \quad (4b)$$

The Ergun equation[8,9] for predicting the minimum fluidization velocity has also been used to predict small bed expansions:

$$150\frac{(1-\varepsilon)\mathrm{Re}}{\varepsilon^3\varphi^2}+1.75\frac{\mathrm{Re}^2}{\varepsilon^3\varphi}=Ar \quad (5)$$

Theory

A more general model that can be applied to particles of any shape and density was developed by applying Equation 1 at the point of incipient fluidization ($\varepsilon=\varepsilon_{mf}$, $u=u_{mf}$):

$$n=\frac{(\ln u_{mf}-\ln u_t)}{\ln\varepsilon_{mf}} \quad (6)$$

or in terms of Reynolds numbers:

$$n=\frac{(\ln\mathrm{Re}_{mf}-\ln\mathrm{Re}_t)}{\ln\varepsilon_{mf}} \quad (7)$$

Both Re_t and Re_{mf} are functions of sphericity, φ, and Archimedes number, Ar. Re_t can be determined from the model recently proposed by Veerapen et al.[10]:

$$\mathrm{Re}_t=\frac{Ar}{18+5.1\,1Ar^{1/2}\exp(-2.13\varphi)} \quad (8)$$

whereas Re_{mf} can be evaluated using the Ergun equation:

$$150\frac{(1-\varepsilon_{mf})\mathrm{Re}_{mf}}{\varepsilon_{mf}^3\varphi^2}+1.75\frac{\mathrm{Re}_{mf}^2}{\varepsilon_{mf}^3\varphi}=Ar \quad (9)$$

The bed porosity at the point of incipient fluidization ε_{mf} is a function of particle sphericity and is determined through experiments or estimated using the Wen-Yu equation:

$$\frac{1}{\varepsilon_{mf}^3\varphi}=14 \quad (10)$$

Alternatively, if the bed porosity at the point of incipient fluidization is known, the Wen-Yu equation can be used to estimate the sphericity of the particles. Sphericity (φ) is the ratio of the surface area of a sphere of equivalent volume to the surface area of the actual non-spherical bioparticle. The sphericity of perfectly spherical bioparticles is one, and the sphericity range of non-spherical particles is from 0 to 1.

Procedure

The tests were performed in an upflow fluidized bed with a diameter of 7.62 cm and in a 480-cm inverse fluidized bed. Particles were fluidized using tap water. Bed expansion was obtained as a function of liquid flow rate from measurements of bed height H:

$$H(1-\varepsilon)=H_{mf}(1-\varepsilon_{mf}) \quad (11)$$

Sand particles, glass beads, and plastic particles were employed as support media inside the columns (see Table 1 for size and densities). The terminal velocity of the particles was measured by measuring the time taken by single particles to cover a known distance within a glass column full of water.

Results and Discussion

The predictions of Equations 7 to 10 are compared with experimental data from several studies in Table 1. The prediction of u_t and u_{mf} are in good agreement with measured values over a wide range of particle sizes, densities and sphericities. The accuracy of the ε_{mf} prediction is, however, not as good especially when sphericity is less than 0.8. For this reason, the predicted n values presented in Table 1 were calculated using the measured ε_{mf} data along with the predicted u_t and u_{mf} values. The n values calculated in this manner are in excellent agreement with reported values as indicated in Table 1. This confirms that the proposed approach for calculating n is valid for a wide range of particles including particles lighter than water.

The predictions of the proposed model (Eqns. 7 to 9) are compared with predictions of well established models for spherical particles in Figure 1. Like the Khan-Richardson model, the proposed model correctly predicts that n should approach 4.8 at small Archimedes number and 2.4 at high Ar values when φ=10. At intermediate Ar values, the predictions of the proposed model are higher than those of the Khan-Richardson and Richardson-Zaki models but appear to be in better agreement with experimental data. This serves as further validation of the proposed model and suggests that the new model is superior to existing models.

The bed expansion results obtained in this study are compared with the predictions of the new model in Figure 2. The data for particles heavier than water were obtained with the upflow fluidized bed whereas the data for the plastic particles were obtained using the inverse fluidized bed. Also included in Figure 2 are the predictions of existing models. As before, the predictions

Material	d_p (mm)	ρ (kg/m^3)	φ	ε_{mf}		u_t (m/s)		u_{mf} (m/s)		n	
				measured	Eq.(10)	measured	Eq.(8)	measured	Eq.(9)	measured	Eq.(7)
sand	0.275[12]	2664	0.90	0.45	0.43	0.0362	0.0369	-	0.00071	3.80	4.50
	0.455*	2660	0.90	0.45	0.43	0.07	0.071	-	0.0029	4.32	4.00
	0.556[11]	2639	0.90	0.41	0.43	0.083	0.086	0.00245	0.00299	4.22	3.75
	0.598[7]	2650	0.77	0.468	0.45	0.0817	0.076	0.005	0.0043	3.38	3.78
	0.85[7]	2650	0.81	0.459	0.45	0.1075	0.1075	0.007	0.0082	3.21	3.31
	1.006[7]	2650	0.73	0.467	0.46	0.1258	0.11	0.009	0.0097	3.17	3.19
	1.463[7]	2650	0.71	0.465	0.47	0.1635	0.13	0.016	0.016	3.00	2.71
	1.962[7]	2650	0.71	0.446	0.47	0.1913	0.15	0.022	0.021	2.63	2.47
flintag	0.526[7]	2620	0.65	0.577	0.48	0.0603	0.0549	0.005	0.0053	4.05	4.25
	0.782[7]	2620	0.61	0.572	0.49	0.0821	0.07	0.0095	0.0095	3.80	3.58
coal	0.505[7]	1460	0.75	0.565	0.46	0.0283	0.0269	0.0015	0.0018	4.50	4.77
	0.815[7]	1460	0.65	0.564	0.48	0.0394	0.0366	0.0024	0.0033	4.38	4.20
glass beads	0.783[7]	2970	0.97	0.43	0.42	0.148	0.147	0.008	0.0087	2.93	3.30
	1.3*	2480	1.0	0.4	0.41	0.17	0.19	0.014	0.013	3.20	2.93
ball bearing	6.35[4]	7740	1.0	0.439	0.41	1.127	1.059	0.136	0.139	2.38	2.50
plastic particles	5.2*	940	0.80	0.43	0.45	0.074	0.063	0.0078	0.0073	2.33	2.75

Table 1.
Physical characteristic of particles and predicted results. (* this study).

of the new model are in good agreement with the data and generally more accurate than those of existing models.

The main disadvantage of the proposed model is that it requires an accurate estimate of ε_{mf}. The Wen-Yu model (Eqn. 10) can be used to estimate ε_{mf} when is greater than 0.8. Otherwise, ε_{mf} should be determined experimentally.

Conclusions

A general model for predicting the expansion of fluidized beds has been validated using data for a wide range of materials. The model correctly predicts the effect of sphericity and can be used with both sinking and floating particles.

Acknowledgements

The financial support of AquaNet, NBIF, and the NB Department of Agriculture, Fisheries and Aquaculture is gratefully acknowledged.

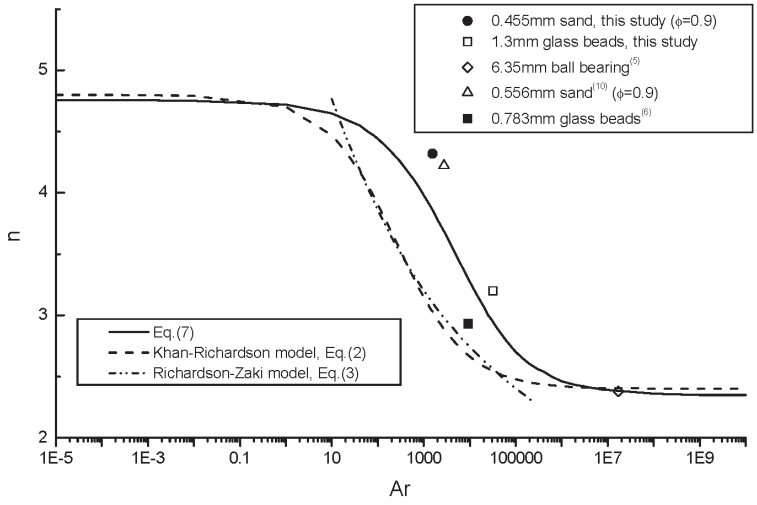

Figure 1.
Prediction of *n* using different models, φ = 1.0, ε_{mf} = 0.42.

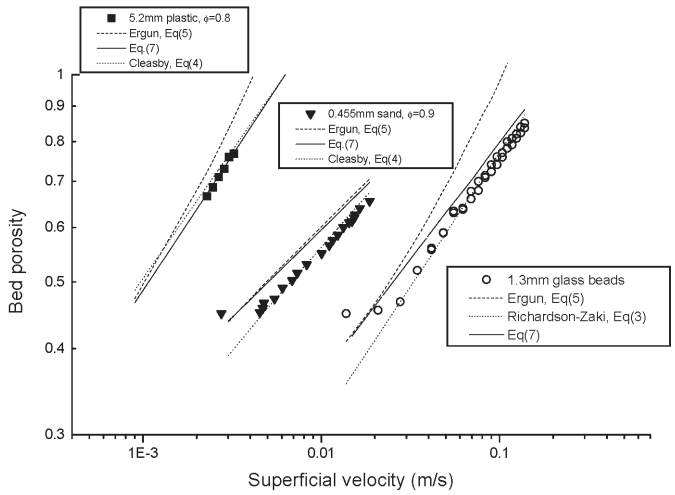

Figure 2.
Prediction of bed expansion using different models.

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Nomenclature

Ar	Archimedes number; $Ar = \frac{d_p^3 \rho_w (\rho_p - \rho_w) g}{\mu^2}$	u	superficial liquid velocity in the fluidized bed biofilter, m/s
D_c	diameter of fluidized bed, m	u_{mf}	minimum fluidization velocity, m/s
d_P	mean equivalent volume diameter of particles, m	u_t	particle terminal velocity, m/s
g	gravity acceleration, m/s^2	n	exponent in Eq.(1)
H	bed height, m	Re_{mf}	Reynolds number based on minimum fluidization velocity
H_{mf}	initial bed height, m	Re_t	Reynolds number based on particle terminal velocity
	Greek letters		
ε	bed porosity	ρ_p	density of support media, kg/m^3
ε_{mf}	bed porosity at the point of incipient fluidization	ρ_w	density of water, kg/m^3
φ	sphericity	μ	viscosity of water, Pas

Consideration of Turbulence in Calibration of Plaster Blocks Used for Flow Measurement

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An inexpensive and simple method for measurement of net water flow is the use of plaster spheres or blocks. The partially soluble plaster blocks dissolve at a rate that is a linear function of water flow. Blocks are weighed prior to and after deployment in the field and the mass lost is correlated to the net water flow past the block. Laboratory calibrations are carried out prior to deployment at known flow rates and temperature to determine the relationship between mass lost and water velocity. These methods provide a rough estimate of flow rates in the field, however in most cases, calibrations are made without regard to the turbulence of the environment that will be studied. Here, we compare calibrations in both turbulent and laminar flow environments at the same net flow rate. Our results demonstrate that the rate of dissolution is significantly higher in turbulence than in laminar flow (P<0.0001); the slope of the linear relationship between percentage mass lost and water velocity was 1.65 for laminar flows (R^2=0.83) and 2.74 for turbulent flows (R^2=0.71). This result shows that calibrations must be carried out carefully to avoid overestimation of flow rates.

L'utilisation de sphères ou de blocs de plâtre est une méthode simple et bon marché pour mesurer le débit net. Les blocs de plâtre partiellement solubles se dissolvent à un taux qui est fonction linéaire du débit. Les blocs sont pesés avant et après l'utilisation sur le terrain, puis la masse perdue est corrélée avec le débit net au-dessus du bloc. Avant la réalisation des travaux sur le terrain, les étalonnages sont faits en laboratoire à des débits et températures connus en vue d'établir la relation entre la masse perdue et la vitesse du courant. Cette méthode donne une estimation approximative des débits sur le terrain, même si, dans la plupart des cas, les étalonnages ne tiennent pas compte de la turbulence dans le milieu étudié. Nous comparons les étalonnages faits dans des milieux soumis à une turbulence et à un écoulement laminaire au même débit net. Nos résultats démontrent que le taux de dissolution est significativement plus élevé en situation de turbulence que d'écoulement laminaire (P < 0,0001); la pente de la relation linéaire entre le pourcentage de masse perdue et la vitesse du courant se chiffrait à 1,65 dans le cas de l'écoulement laminaire (R^2 = 0,83) et à 2,74 en présence de turbulence (R^2 = 0,71). Ceci indique que les étalonnages doivent être faits soigneusement afin de ne pas surestimer le débit.

Introduction

The measurement of rate of water flow is important to the study of marine ecological systems, particularly those involving settlement of invertebrate larvae[1]. A simple and inexpensive method for assessing relative rate of water movement was first introduced by Muus in 1968[2]. The rate of dissolution of plaster balls in seawater is dependant on the rate of water flow past the plaster surface. Muus placed plaster balls of known dry mass into flowing water where a portion of the ball would dissolve. The balls were then removed from the water, dried, and weighed again to determine the amount of dissolution. This technique has been used by many investigators to determine water motion in the field (see Porter et al.[3] for references listed therein).

The preparation of plaster blocks, or clod cards as described by Doty[4], involves casting plaster of Paris (gypsum) in ice cube trays[4]. Although a radially symmetrical shape is preferable for dissolution trials[5], the efficiency and ease that is allowed by using ice cube trays makes this a desirable cast. The success of this method is dependant on accurate laboratory calibration prior to placement of cards into the field. Calibration involves measurement of dissolution of the clods under known flow rates at the same temperature and salinity as those that will be encountered in the field. Rate of dissolution increases with temperature[5], therefore temperature must be matched in the laboratory calibrations to what will be seen the field.

One problem with this method that is rarely adequately accounted for by investigators is the influence of steady flow versus turbulent flow[3]. In many calibrations, flow is steady and smooth, while flows in the field are likely to be more turbulent or mixed. Turbulent flow leads to a greater exchange between a surface and the overlying water[5] and thus greater dissolution of the clod card. Without consideration of this effect, velocity of water would be overestimated when steady flow calibrations are used to evaluate turbulent field flows[3].

In this experiment, both turbulent and steady flow calibrations were carried out at overall flow rates ranging from 0 cm/sec to 4 cm/sec to determine the difference in dissolution rate created by the difference in flow structure.

Materials and Methods

Clod cards were prepared following the methods used by Thompson and Glenn[6]. Plaster of Paris dry mix (produced by

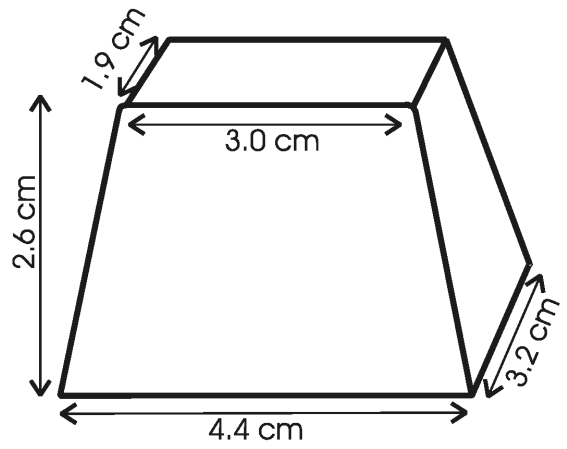

Figure 1.
Dimensions of clod cards used.

DAP Inc. 2002) was mixed 2 parts plaster to 1 part clean cold water. The liquid was transferred into ice cube trays with a baster and the side of the tray was tapped to remove air bubbles. Plastic cocktail swords were inserted, handle down, into each cast to be used later for attachment in the field and for labelling. The plastic swords were used to avoid metal that may corrode and break apart in the field and thus affect the final weight of the clod card. The clod cards were allowed to harden for at least 30 minutes before removal from the tray. After removal from the tray they were dried in a drying oven for 68 hours at 30°C.

Prior to initiation of the experiment, each dry block was pre-weighed and labelled with a unique number. Each block was 4.4 cm by 3.6 cm at the base and 3.0 cm by 1.9 cm at the top and had a height of 2.6 cm. The final dimensions of the clod cards are shown in Figure 1. The weight of each card was 25.39 g (standard error = 0.10 g). Flume tanks were set up with bulk flow of 1, 2, 3, or 4 cm/s. Two tanks were used, each 250 cm long, one 40.5 cm wide and one 35 cm wide, and both were filled to a depth of 10.5 cm. Water supplied to each flume was from a re-circulating system that contains roughly 7600 L of filtered salt water, therefore saturation as the blocks dissolved was not a concern. In each flume, one end contained turbulence while the other flowed smoothly. Laminar flows were achieved by placing a large honeycomb shaped manifold (holes 0.5 cm diameter, 15 cm long) within the flow to constrain it. Both turbulent and laminar flows were confirmed by using dye to visualize the streaklines[7]. At each bulk flow rate 4 blocks were placed in each type of flow for 24 hours. Still water (0 cm/s) calibrations were carried out with 1 block suspended in a 20-L tank with no inflow or outflow for 24 hours. Still water calibrations were repeated 4 times with water replacement each time. All trials were run at a temperature of 13°C. After 24 hours in the water the blocks were retrieved and again placed in a drying oven for 68 hours at 30°C. After drying, each block was weighed a second time to determine mass lost over 24 hours.

Results

The dissolution of the blocks showed a linear relationship between percent mass lost and water velocity for both laminar and turbulent flows (Fig. 2). The slope of the graph of percentage mass lost and water velocity was 1.65 for laminar flows (R^2=0.83) and 2.74 for turbulent flows (R^2=0.71). The dissolution rate of blocks in turbulent flow was significantly higher than in laminar flow at the same bulk flow rate (P<0.0001). The slope of the turbulent flow relationship is greater than that of the laminar flow and therefore the difference in percent mass lost between laminar and turbulent flows is enhanced at higher flow rates. The variability of the data points around the linear relationship is greater for turbulent flow than for laminar flow (lower R^2).

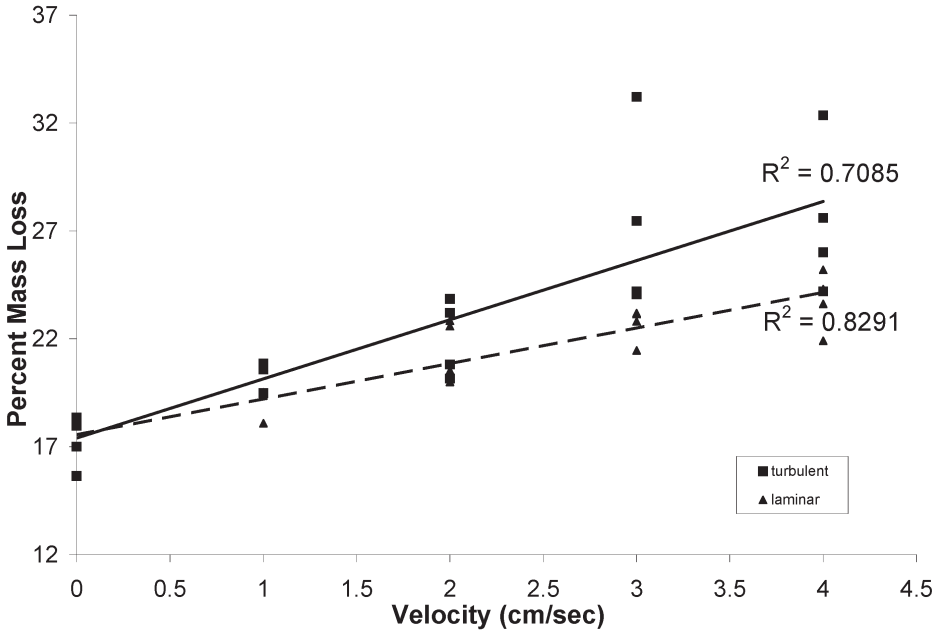

Figure 2.
Percentage mass lost from blocks over a 24-hour period at flows from 0-4 cm/sec.

Discussion

Dissolution of plaster blocks has been used extensively as a method for field estimation for flow rates[8]. The calibration of the blocks prior to placement in the field is crucial to the success of the method. Calibration has been primarily carried out under smooth flow conditions and the observed relationships between dissolution and flow are good in most studies[3]. However, one factor that is often overlooked is the type of flow being investigated and laminar calibrations are commonly applied to turbulent flows in the field[3].

In this experiment, we found that dissolution of the blocks increased with increasing water flow in a linear relationship, as expected. We also found that the rate of increase was higher when the flow was turbulent versus when it was smooth. The difference in dissolution rates between laminar and turbulent flows must be considered when calibrations are carried out. In cases where calibrations are carried out in a laminar environment and field measurements are made in a turbulent environment, the water velocity will be overestimated due to this increase in dissolution. The degree of overestimation is increased at higher bulk flow rates. Another result of this experiment was that in turbulent flows there was greater variability in the relationship between flow and dissolution. This is also important to note when carrying out calibrations that will be used to measure turbulent flows in the field.

Overall, this method of flow measurement is effective and easy to use. If the intended outcome is comparison of sites with similar turbidity, then an appropriate calibration will lead to reliable results. However, if sites to be examined show differences in the level of turbulence, then the effect of the turbulence on the dissolution must be considered.

Acknowledgements

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The Role of Blue Mussel (*Mytilus edulis*), Kelp (*Laminaria saccharina*), and Biofouling on the Dissolved Oxygen Budget of Integrated Multi-Trophic (Salmon-Mussel-Seaweed) Aquaculture

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Dissolved oxygen is one of the water quality parameters important to farm husbandry and environmental impact considerations. Existing dissolved oxygen models have incorporated salmon biomass, hydrography, and benthic components but none have looked at the oxygen demand of mussels, kelps, or fouling organisms in aquaculture. The objective of this project is to better develop a dissolved oxygen budget model for multi-trophic aquaculture sites. Preliminary respiration analyses have been done and show mussel line respiration rates (±SE) of 78±3 mg O_2·kg WW^{-1}·hr^{-1}. Preliminary trials with kelp recorded respiration rates of 60±4 mg O_2·kg WW^{-1}·hr^{-1} and photosynthetic rates of 226±44 mg O_2·kg WW^{-1}·hr^{-1}. Biofouling trials are currently underway and will be completed in the near future.

L'oxygène dissous est l'un des paramètres de la qualité de l'eau important sur le plan de l'élevage et de l'impact sur l'environnement. Les modèles existants de l'oxygène dissous regroupent la biomasse de saumons, des paramètres hydrographiques et des éléments benthiques, mais aucun ne tient compte de la demande en oxygène d'une culture de moules, des laminaires ou des organismes encrassants. L'objectif de ce projet est d'élaborer un meilleur modèle du budget d'oxygène dissous pour les sites de polyculture intégrée. Selon les analyses préliminaires de la respiration, l'intensité respiratoire (± É.-T.) des moules cultivées sur filins s'élève à 78 ± 3 mg O_2·kg (poids humide)$^{-1}$·h^{-1}, tandis que chez les laminaires, elle se chiffre à 60 ± 4 mg O_2·kg (poids humide)$^{-1}$·h^{-1}, alors que le rendement photosynthétique se situe à 226 ± 44 mg O_2·kg (poids humide)$^{-1}$·h^{-1}. Les essais sur les organismes encrassants actuellement en cours seront terminés sous peu.

Introduction

The Quoddy region of the Bay of Fundy has been an area of active aquaculture development for the last two decades[1]. The first successful salmon aquaculture site in this area was initiated in 1978 and since that time development has increased significantly to an estimated 96 farms in operation today in the Southwestern New Brunswick region[1].

The emergence of environmental concerns in the last few years has prompted many in-depth studies into the ecological implications of finfish monoculture[2]. The concerns raised cover a wide range of topics including disease transfer and mitigation, benthic impact, nutrification, suspended solids, and chemotherapeutant persistence.

Some interest of late has focussed on developing an integrated ecosystem approach to aquaculture that will help balance the ecological issues the industry is facing and help generate some additional revenue. Studies to date have shown that "extractive" organisms (e.g., mussels, kelps) cultured along with "fed" organisms (e.g., salmon) can help decrease the possible influence of salmon aquaculture on the environment while producing another marketable product[3,4]. Kidd[5] stated that mussels can grow 20% faster when cultured in the vicinity of salmon cages and seaweeds build up biomass very quickly when cultured at these sites. These findings are encouraging but as with all development there may be drawbacks.

Dissolved oxygen (DO) has been a concern for some of the salmon aquaculture industry in the Quoddy Region particularly during the annual late summer-autumnal minimum in ambient DO concentrations when water temperatures are at a seasonal maximum and there is an associated elevated high demand for oxygen by pre-market salmon[6]. Industry concerns such as decreased production and lower food conversion efficiencies at low DO concentrations have prompted the development of several DO thresholds for the industry in Southwestern New Brunswick. Page and Martin[7] suggested that to minimise the impact of the aforementioned industry concerns, the DO concentration onsite should be maintained above 6 mg/L. The United States Environmental Protection Agency (EPA)[8] indicated that at DO concentrations below 5 mg/L, there is an increased incidence of environmental impacts such as behavioural changes in non-target species. The increased biomass associated with multi-trophic aquaculture may generate an oxygen demand in the localised area of a farm that may result in lowering the farm scale DO concentrations below the desirable thresholds. Exist-

ing dissolved oxygen mass balance models have incorporated salmon biomass, hydrography, and benthic components but none to date have looked at the role played by fouling organisms present on the cages nor the oxygen demand/supply associated with the integration of mussel or kelp culture[6].

The purpose of the research reported here is to investigate the contribution of mussel, kelp and net bio-fouling respiration/photosynthesis to the existing oxygen budget model. This will help give salmon farmers a better understanding of the oxygen sources and sinks on their farms and potentially assist in the development of husbandry protocols for things such as net changing schedules. It will also help develop policies regarding site selection and approved production limit criteria for proposed integrated aquaculture sites.

Materials and Methods

Three study sites were used for this project. They are located in the Bliss Harbour/Back Bay region of Southwestern New Brunswick. They are: Charlie Cove (MF0276; 45° 01' 9.40" N, 66° 51' 57.13" W), Frye Island (MF0028; 45° 02' 25.33" N, 66° 50' 34.33" W), and J.D. Stewart (MF0029; 45° 02' 4.78" N, 66° 49' 56.10" W). All of these sites are odd-year-class sites (farm stocked with salmon smolts in an odd year) and range in exposure from quite sheltered (Frye Island) to relatively exposed (Charlie Cove).

Mussels

In April 2004, mussels were collected in the field and socked using cotton mussel socking. Mussels were socked at an average density of 320 mussels per meter of socking material and held in a holding tank for 48 hours to allow byssal thread attachment. The socks were then transported to the three salmon aquaculture sites via the Canadian Coast Guard vessel Pandalus III. Twelve socks were assigned randomly to individual polar circle cages at each farm site and each was moored at a depth of 5 m.

Trials took place during the summer and fall of 2004. Monthly samples of three mussel socks were collected from each site. Three 10-cm sections were cut from each sock and placed within individual respiration chambers. Chambers were constructed from clear acrylic pipe (30 cm × 95 cm) capped at both ends. A 12-volt Rule™ through-hull pump (360 gph) was attached to each chamber to circulate water. Oxyguard™ temperature-compensated DO probes were placed within each chamber (Fig. 1) and measured DO (mg/L) continuously over a 60-minute trial period. This information was relayed to a PT4 datalogging DO meter. Chambers were hung at a depth of 0.2 m from a floating raft during each trial. Upon termination of the trial, the samples were removed and kept for weight analysis. Control trials were run simultaneously with experimental trials and consisted of chambers housing ambient water. Trials were also conducted on just the shells of the mussel line with all the mussel flesh removed to determine the respiration rate of any fouling organisms present.

Upon returning to the lab, data from the PT4 DO meter was downloaded onto a computer and respiration or photosynthetic rates were determined using linear regression analysis. To allow for an acclimation period the data from the first five minutes of the trial were not used. The last five minutes of data from each trial were also ignored to remove any disturbance effects of dismantling any adjacent chambers at trial termination.

Kelp

Kelp lines consisting of 12-mm polysteel rope were installed horizontally at a depth of 1.5 m around the perimeter of each site using the existing compensator buoys for anchorage. In November 2003, twine seeded with juvenile *Laminaria saccharina* sporophytes were wrapped around these lines for grow out. To determine net oxygen production by the whole kelp, triplicate samples were collected in the summer and fall of 2004 and placed within the same respiration chambers as used for the mussel trials. To determine the respiration rates the chambers were covered with a heavy black tarp to exclude ambient light.

Fouling

Mesh currently used by the industry for smolt (3.5 cm) and premarket fish (6 cm), was cut into 7-cm × 7-cm panels. Flexgard™ antifoulant treatment was applied to half of the panels for both the smolt and premarket mesh. Twelve replicates of each of the four treatments (smolt mesh with/without

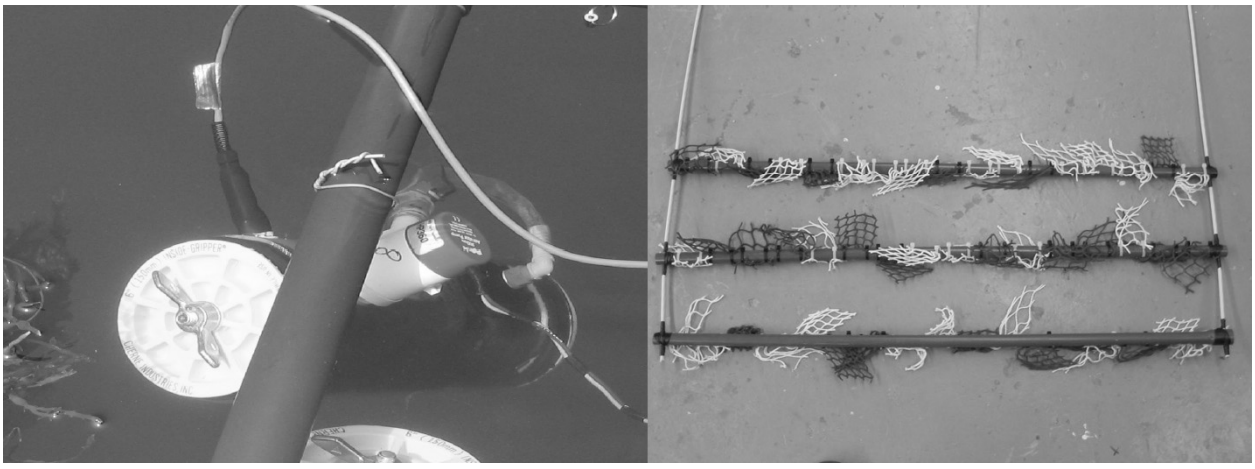

Figure 1.
Respiration chamber (left) and fouling ladders (right) used in these trials.

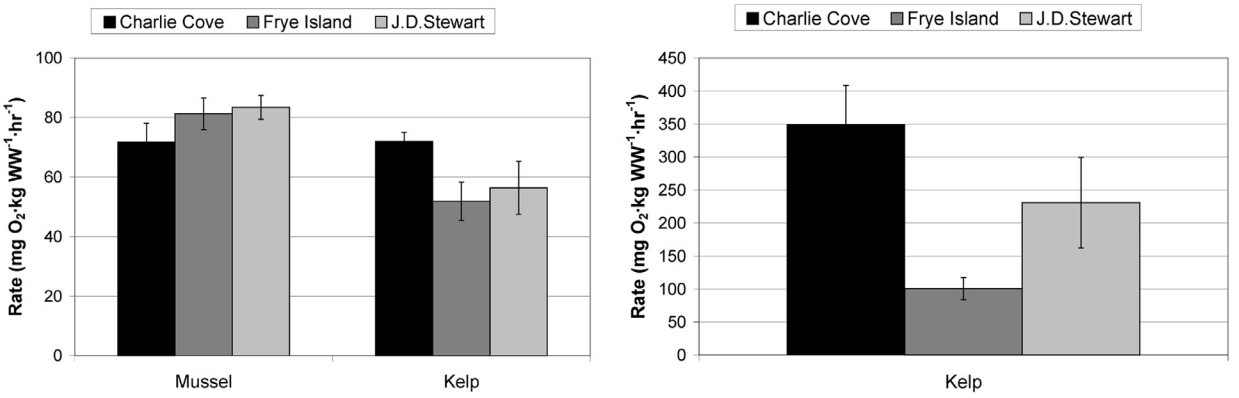

Figure 2.
Respiration rates (left) and photosynthetic rates (right) (± SE) of mussel lines and whole kelps at each of the three sites. (*denotes significant difference using one-way ANOVA, α = 0.05).

antifoulant, premarket mesh with/without antifoulant) were hung randomly at depths of 1 m and 5 m on a cable ladder system. These ladders were constructed of two 5-m sections of 6-mm-diameter coated cable hung vertically with rungs of PVC pipe (20 mm, Schedule 40) at each of the two depths. Mesh panels were attached to the rungs using cable ties and ladders were hung randomly throughout each site (Fig. 1).

Fouling trials are still in the preliminary stages and no data analysis has been completed to date.

Results and Discussion

Preliminary analysis of the mussel and kelp respiration and photosynthesis trials has been completed and the results for each of the three sites are shown in Figure 2. The mussel respiration rates for each of the three sites averaged 78±3 mg O_2·kg WW^{-1}·hr^{-1}. This value is in agreement with other studies focussed on determining respiration rates for individual mussels[9]. The kelp respiration rates were similar over the course of the trials with an average rate of 60±4 mg O_2·kg WW^{-1}·hr^{-1}. These preliminary values seemed high when compared to others in the literature[10,11] but may have been due to the simulated dark conditions or chamber construction[12].

The net photosynthesis trials exhibited high variability with an average of 226±44 mg O_2·kg WW^{-1}·hr^{-1} (Fig. 2). The rate for the Frye Island site was markedly lower than those at the other two sites. This may have been caused by extremely foggy conditions during the course of the trials at this site. These data are still in the preliminary stages of analysis and will be investigated further.

Conclusions and Future Work

Preliminary results show that the weight specific respiration rate of a mussel and/or kelp line (at night) are lower than that of salmon (100 mg O_2·kg WW^{-1}·hr^{-1}.)[13] The influence of this respiration on the dissolved oxygen budget of a multi-trophic site will depend on the relative biomass of each respiring component. In the near future mussel and kelp respiration and photosynthetic trials will be continued, biofouling trials conducted and other components of the integrated mutli-trophic site investigated. Kelp respiration trials have also been conducted during the night time hours and need to be analyzed to determine if the respiration rates determined under simulated dark conditions achieved by the tarp are representative of the respiration rates during actual nighttime hours.

As each component of the integrated multi-trophic site is examined and their contribution to the DO budget is determined, the natural DO dynamics of these sites will be further modelled to help the industry and regulators make informed decisions on how to better manage aquaculture sites. This will ultimately help industry to balance the biomass of each component of a multi-trophic site in order to increase profits, maintain healthy, diversified crops, and maintain DO levels above a desirable threshold, such as the 6 mg/L threshold mentioned above.

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Risk Communication in the Aquaculture Sector: The Role of the Scientist

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Among the many stakeholders involved in the process of risk assessment in the aquaculture sector, scientists – including academics, government researchers, analytical laboratories, and scientific consultants – are often the most familiar with the data generated and used in risk assessments. As such, they have a crucial role to play in both risk assessment and risk communication processes. Scientists bear the ultimate responsibility for accurately characterizing, evaluating, and interpreting the information used to predict health risks. This paper highlights various fundamental roles for scientists in communicating health risk information to aquaculture sector stakeholders. Some examples include identifying state-of-the-art analytical methods and providing clear information on their strengths and limitations; defining standards of data quantity and quality; defining assumptions and uncertainties in the data; highlighting the implications of uncertainties on the characterization of risk; and providing context and perspective on risk by integrating other scientific factors. To ensure that scientific data are not misinterpreted or used out of context, it is vital for scientists to be involved in the communication of this type of information to stakeholders in the aquaculture sector. A current, controversial issue is used as a case example to illustrate these principles. Recent reports in the scientific literature and news media, related to levels of polychlorinated biphenyls (PCBs) in farmed and wild salmon, have had a significant impact on public opinion and consumer behaviour, thereby influencing the sales of farmed salmon in North America and Europe. The role of the scientist in the communication of risk related to this PCB exposure issue is explored.

Parmi les nombreux participants au processus d'évaluation du risque dans le secteur de l'aquaculture, les scientifiques, y compris des professeurs d'université, des chercheurs fédéraux et provinciaux, des laboratoires d'analyse et des experts-conseils scientifiques, sont souvent ceux qui connaissent le plus les données recueillies et utilisées aux fins d'évaluation du risque. À ce titre, ils ont un rôle névralgique à jouer dans le processus d'évaluation et de la communication du risque. Les scientifiques assument la responsabilité ultime pour ce qui est de caractériser, d'évaluer et d'interpréter précisément les informations utilisées pour prédire les risques pour la santé. Nous mettons en lumière les divers rôles fondamentaux des scientifiques au titre de la communication des informations sur les risques pour la santé aux intervenants du secteur de l'aquaculture. Par exemple, ils doivent trouver des méthodes analytiques de pointe et fournir de l'information claire sur leurs forces et leurs limites; définir les normes relatives à la qualité et à la quantité de données; formuler des hypothèses et établir les incertitudes dans les données; mettre en lumière les conséquences des incertitudes sur la caractérisation du risque; et établir le contexte et la perspective de risque en intégrant aux données d'autres facteurs scientifiques. Afin d'assurer que les données scientifiques ne sont pas mal interprétées ou utilisées hors contexte, il est essentiel que les scientifiques participent à la communication de ce type de renseignements aux intervenants du secteur de l'aquaculture. Nous utilisons une question d'actualité controversée comme exemple pour illustrer ces principes. De récents rapports publiés dans des journaux scientifiques et diffusés dans les médias d'information, portant sur les teneurs en biphényle polychloré (BCP) chez le saumon sauvage et le saumon d'élevage, ont eu un impact important sur l'opinion du public et le comportement des consommateurs, ce qui a des répercussions sur les ventes de saumon d'élevage en Amérique du Nord et en Europe. Nous examinons le rôle des scientifiques dans la communication du risque relié à l'exposition au BPC.

Introduction

The current human health risk assessment paradigm used widely in Canada (and in many other countries) is based on frameworks developed and detailed by various North American regulatory agencies, including the US Environmental Protection Agency (EPA)[1] and various other risk assessment guidance documents, updates, and addenda published by the US EPA[2-4], Health Canada[5], the Canadian Council of Ministers of the Environment, and the Ontario Ministry of Environment (OMOE)[6]. A brief overview of each step in the paradigm (outlined in Fig. 1) is as follows.

Problem Formulation involves the definition of the management goal of the risk assessment, characterization of key issues, identification of chemicals of concern, potential receptors, and exposure pathways of concern.

Exposure Assessment involves estimating exposure of receptors to each of the chemicals of concern, typically by integrating

environmental characterization data (such as chemical-specific concentrations in media of concern (e.g., soil, sediment, air, water, and tissue)) into a series of multimedia environmental exposure models.

Toxicity Assessment involves a critical review and assessment of toxicity data to determine an appropriate exposure limit or toxicity reference value (i.e., concentration of a chemical not expected to be associated with adverse health effects) for each of the chemicals of concern. These types of limits are typically established by regulatory agencies, such as the US EPA, Health Canada, the World Health Organization, and provincial/state environmental departments.

Risk Characterization involves the estimation of potential risks associated with the estimated exposures and toxicity, through the comparison of the information collected in the above-mentioned phases.

Risk Management involves the development of risk management criteria, as well as the identification of potential mitigating measures (i.e., methods of pathway intervention, etc.), which can help eliminate or reduce any predicted risks to human health.

Collection and Validation of Data is implemented throughout the risk assessment process, with the goal of providing enough high-quality data to replace certain assumptions, and reduce uncertainty in the risk assessment.

Finally, ***Risk Communication*** is also used throughout to engage all stakeholders in the risk assessment process, so that the results of the risk assessment address all relevant concerns.

Risk Communication in the Aquaculture Sector

Within the aquaculture sector, several key stakeholders are important to involve in the risk communication process. *Industry* is represented by the fish farmers and fish feed producers. The risk communication role for this stakeholder group is presently accomplished by trade associations, such as the Canadian Aquaculture Industry Alliance (CAIA), the Fisheries Council of Canada (FCC), and Salmon of the Americas (SOTA). *Government* stakeholders are often represented by spokespersons from various departments that either regulate or promote the sector, such as Fisheries and Oceans Canada, Health Canada, Industry Canada, and Environment Canada. The *Public*, often the most important stakeholder, is typically represented by consumers, Non-Governmental Organizations, First Nations groups, and the media. Finally, the *Scientific Community*, including academics, government researchers, analytical laboratories, and scientific consultants, is also a key stakeholder within this sector.

As many risk issues in the aquaculture sector are related to food safety, there is generally a high level of stakeholder interest. Communication of environmental and health issues affecting the aquaculture sector has recently been dominated by the media. Unfortunately, members of the news media are not scientists and scientific information is often misrepresented or misinterpreted in the media. All stakeholders concerned with health risk issues in the aquaculture sector can benefit from having scientific information communicated to them by objective, knowledgeable, and experienced individuals with the ability to adequately characterize, interpret and communicate risk. Scientists are crucial stakeholders, as they are most familiar with the data generated and used in risk assessments (e.g., chemical concentrations, toxicity/exposure/risk information), and have ultimate responsibility for appropriately characterizing, evaluating and interpreting the information used. Traditionally, however, scientists have not had specific training and/or expertise in risk communication. Nevertheless, they have a crucial role to play in the risk communication process, and should enhance these skills in order to effectively fulfill this role.

The Role of the Scientist in the Risk Communication Process

While scientists have the qualifications, knowledge, and experience (e.g., education, accreditation, and research experience) to communicate risks related to the aquaculture industry, it is imperative that they also:

- maintain independence and objectivity in carrying out their function as risk assessors; in other words, they need to maintain their role as expert, not advocate, or clearly state their bias (state assumptions) and the resulting implications on the results (e.g., a scientist working for Environment Canada will

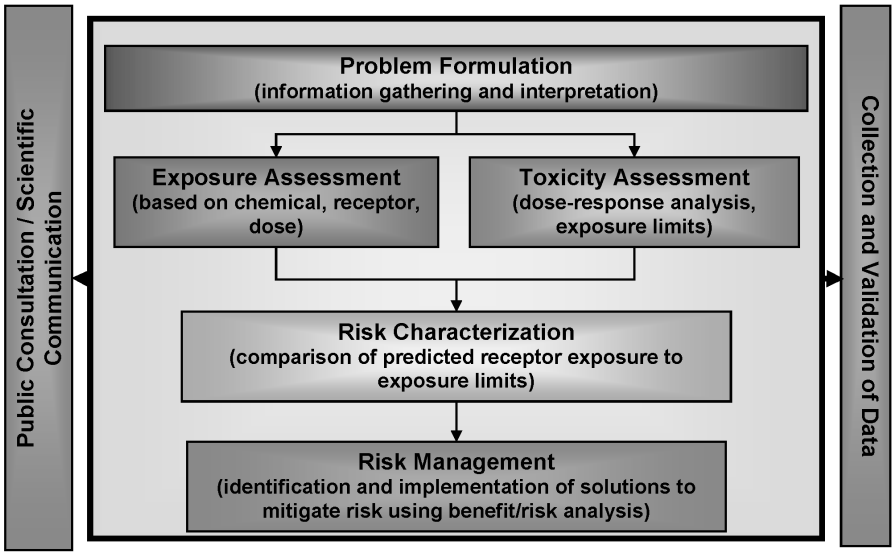

Figure 1.
The Risk Assessment Paradigm.

have a different bias than a university professor or an analytical chemist at a private laboratory);
- have the required current auxiliary expertise (e.g., understanding of analytical techniques, statistical methods, etc.);
- have familiarity with relevant and up-to-date published information;
- highlighting the implications of uncertainties on the characterization of risk; and, most importantly,
- in the context of risk communication, have a comprehensive understanding of the science of risk assessment.

Thus, by increasing the involvement of scientists in communicating health risk information, complex issues can be put into context and effectively communicated to all stakeholders. Some examples of how scientists can play a role in effective risk communication for the aquaculture sector are as follows:

1. identifying state-of-the-art analytical methods, and providing clear information on their strengths and limitations;
2. defining standards of data quantity and quality;
3. defining assumptions and uncertainties in the data;
4. highlighting the implications of uncertainties on the characterization of risk; and, most importantly; and.
5. providing context by integrating other scientific factors.

The challenge for scientists is to communicate effectively to stakeholders with a less in-depth understanding of the science related to the risk assessment.

The case study presented below, in which Cantox Environmental Inc. (CEI) provided scientific advice to industry stakeholders, demonstrates the important role that scientists can play as risk communicators to aquaculture sector stakeholders.

Case Study: Analysis and Reporting of PCB Concentrations in Farmed Salmon

Recent reports in the primary scientific literature[7,8] and the internet and media (e.g., EWG[9] and numerous newspaper articles), related to levels of polychlorinated biphenyls (PCBs) in farmed and wild salmon, have had a significant impact on public opinion and consumer behaviour, thereby influencing the sales of farmed salmon in North America and Europe. There has been substantial controversy regarding the potential implications of the data reported on human health (e.g., Letters to the Editor, *Science*[10]). Significant variability exists in the way that these analyses are conducted by analytical laboratories, how the data are reported to industry and the general public, and how data are subsequently used to determine the potential for human heath risk.

While working on this important issue, our role involved the preparation of a comprehensive review and evaluation of all methods used for analysis of PCBs in fish tissue. We discovered in this comprehensive review, that the analysis and reporting of PCB concentrations (e.g., Aroclors vs. congeners vs. total PCBs vs. total 12 Dioxin-like PCBs vs. total other congeners) was extremely variable among laboratories. Another significant issue identified in our review was consistency in the use of various methods and reporting units. Specifically, there was a lack of comparability (i.e., 'apples' vs. 'oranges') among laboratories, clients, other scientists, and regulatory agencies. As a result, CEI's role was to clearly define the most up-to-date and appropriate analytical methods, reporting units and format to use, so as to increase comparability and reduce uncertainty with respect to evaluations of PCB fish tissue data.

Subsequent to a critical review of PCB concentrations in farmed and wild salmon tissue, CEI defined data quantity/quality standards by answering fundamental questions related to what constitutes a proper 'weight of evidence'; for example, how many fish need to be analyzed in order to assure that the sampled fish represent the majority of fish in the food system? The issue of data reliability was also explored in this context, for example:

- was the lab conducting the analysis accredited according to an international quality standard (e.g., ISO Guide 17025[11]);
- what level of resolution (i.e., method detection limits relevant to regulatory guidelines) could be achieved by the laboratory;
- how much experience/expertise did the laboratory have in conducting these analyses; and,
- did the laboratory conform to fundamental quality assurance/quality control (QA/QC) elements?

Our role, in this context, was to evaluate the QA/QC elements of the data, and clearly identify threshold criteria for these elements.

Defining assumptions and uncertainties in the data in relation to risk, involved asking questions such as: which parts of the fish were analyzed? In particular, it was highly significant whether the belly flap and/or skin (which contain the highest lipid concentrations) were removed prior to analysis. This required an understanding of the chemical characteristics of PCBs (e.g., that they are highly fat-soluble), and some understanding of consumer behaviour in the context of risk (e.g., some people eat the skin, but most rarely eat the belly flap).

Highlighting the implications of uncertainties on the characterization of risk involved carefully reviewing how analytical data were presented and reported (e.g., expression of data uncertainty), and the consideration of potential modifying factors (e.g., processing, consumer behaviour such as trimming, cooking, the health benefits of eating salmon, etc.). In addition, CEI considered the appropriateness of applying various guidelines, such as the US EPA (2000) Fish Advisory Guidelines[12], whose hyperconservative assumptions were applied for the protection of anglers and subsistence fishers.

Finally, one of the most important roles for the scientist in the risk communication process is providing context for the issue by integrating other relevant scientific factors. With respect to the case study, we addressed the following types of questions:

- What do the tissue concentrations of PCBs in fish mean in practical terms? When considering how much consumers typically eat, are these concentrations significant from a health perspective;
- What are the PCB concentrations found in other foods commonly consumed; and,
- What are the risks of not eating salmon?

Integrating these factors into the interpretation and communication of risk was a critical aspect of CEI's work as it put the potential risks into context, and addressed known public concerns related to fish consumption.

Summary and Conclusions

Risk communication is a critical and ongoing activity in risk assessments. Input from all stakeholders is important to ensure that all key concerns are addressed and properly balanced in both the assessment and communication of risks. Scientists have a crucial role to play in the risk communication process in the aquaculture sector, as scientists are often the most familiar with the data generated and used in risk assessments. The recent issue related to the analysis and reporting of elevated levels of PCBs in farmed salmon has highlighted the necessity of including and engaging scientists in the process of communicating risk. Of the many roles that scientists can play in this process, one of the most important is providing context by integrating other scientific factors.

If the tasks outlined as part of the role of the scientist in risk communication are regarded as essential parts of an objective process, it becomes clear that scientific involvement is also essential. Industry, government and the public make decisions on how they will manage risk based on how scientific data and risk are communicated to them. It is critical that risk management efforts are directed to where they can have the greatest impact on reducing risk; this can only be achieved by basing risk management decisions on reliably communicated science.

Acknowledgements

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Canada and Viet Nam: Two Views of Marine Aquaculture and Its Importance to Our Coastal Communities and Economies

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Canada and Viet Nam have many common physical and economic characteristics which drive the development of their marine aquaculture industries. These include extensive coastlines, over-exploited wild fisheries, the need to ensure food security, poverty alleviation, and job creation particularly for economically depressed rural communities, and the need for material sources for export. Canada's aquaculture industry is based on environmental and economic sustainability, with commercial production for 2002 being about 177 000 t and valued at over CDN$636 million. Viet Nam's total national aquaculture production for 2003 was about 1 110 000 t worth US$2.2 billion. Culture productivity rates for Canada and Viet Nam have been approximately 16-17% for the past few years. Canada's marine production is primarily based on salmonids and molluscs, but other marine species are being developed (e.g., cod, wolffish). Cultured shrimp accounts for 30-40% of the total aquaculture exports for Viet Nam, however, marine cage culture for various finfish and shellfish species (e.g., grouper, lobster) are being developed. Issues discussed include trends in marine aquaculture development for Canada and Viet Nam, sustainable aquaculture development – particularly in relation to environmental and social aspects, sea bed resource management, and the need to diversify aquaculture production with the promotion of native species. The trend of organic culture will also be discussed as feedback from Viet Nam's hosting of the first organic aquaculture and sea farming conference in June 2004.

Le Canada et le Vietnam partagent de nombreuses caractéristiques physiques et économiques qui propulsent le développement de leurs industries maricoles : vaste littoral, surpêche sauvage, sécurité alimentaire, réduction de la pauvreté et création d'emplois, en particulier dans les collectivités rurales économiquement défavorisées, et besoin de matières premières aux fins d'exportation. Au Canada, l'industrie de l'aquaculture prend assise sur la durabilité environnementale et économique; en 2002, la production aquacole commerciale canadienne se chiffrait à quelque 177 000 t, d'une valeur de plus de 636 millions de dollars canadiens. En 2003, la production aquacole totale du Vietnam a atteint environ 1 110 000 t, d'une valeur de 2,2 milliards de dollars américains. La productivité aquacole du Canada et du Vietnam au cours des dernières années se situait approximativement entre 16 et 17 %. La production maricole du Canada repose essentiellement sur les salmonidés et les mollusques, quoique l'élevage d'autres espèces marines est en voie de développement (p. ex. morue, loup). Les crevettes d'élevage constituent de 30 à 40 % du total des exportations vietnamiennes de produits aquacoles, quoique l'élevage en cage de diverses espèces de poissons et de crustacés (p. ex. mérou, homard) est en voie de développement dans ce pays. Les enjeux qui seront abordés lors de cette présentation incluent les tendances dans le développement de la mariculture au Canada et au Vietnam, le développement durable de l'aquaculture, en particulier du point de vue environnemental et social, la gestion des ressources des fonds marins et le besoin de diversifier la production aquacole par la promotion de l'élevage d'espèces indigènes. La tendance vers l'aquaculture organique sera aussi abordée en regard de l'accueil, par le Vietnam, de la première conférence sur l'aquaculture et la mariculture organiques, tenue en juin 2004.

Introduction

Canada and Viet Nam have many common physical and economic characteristics driving the development of their marine aquaculture industries, including extensive coastlines, over-exploited wild fisheries, the need to ensure food security, poverty alleviation and job creation particularly for economically depressed rural communities and the need for material sources for export. Canada's aquaculture industry is based on environmental and economical sustainability, with commercial production for 2002 being about 177 000 t and valued at over $639 million CAN[(1)]. The value is approximately two billion dollars to the Canadian economy with the inclusion of the service sector and value addition. Viet Nam's total national aquaculture production for 2003 was about 1 110 000 t worth $2.2 billion USD[(2)].

As illustrated in Figure 1, comparison of Canada and Viet Nam shows tremendous differences in land and water masses

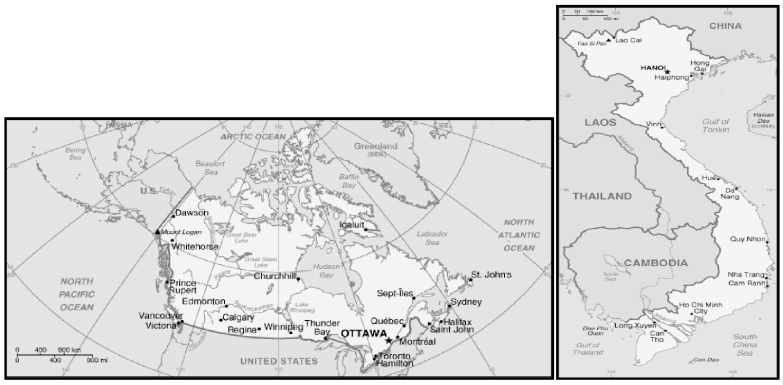
available, with approximately 1/60 coastline in Viet Nam but three times the population and over six times greater aquaculture production than Canada. Culture productivity rates for Canada and Viet Nam have been approximately 16-17% for the past few years. Canada's marine production is primarily based on salmonids and molluscs, but other marine species are being developed (e.g., cod, wolffish). Cultured shrimp accounts for 30-40% of the total aquaculture exports for Viet Nam, however, marine cage culture for various finfish and shellfish species (e.g., grouper, lobster) are being developed.

This paper will discuss trends in marine aquaculture development for Canada and Viet Nam, sustainable aquaculture development particularly in relation to environmental and social aspects, sea bed resource management and the need to diversify aquaculture production with the promotion of native species. The trend of organic culture will also be discussed as feedback from Viet Nam's hosting of the first organic aquaculture conference.

Job Creation and Social Community Issues

Canada

Local impacts include job creation, new dollars in communities, retention of young people in rural areas, and secondary service industry creation. Job creation by the aquaculture industry accounted for approximately 14 000 jobs in Canada[1] and 1.5 million in Viet Nam[2]. In Canada, for example, the New Brunswick salmon farming industry has a farm gate value of greater than $200 million, with value-added worth >$300 million, a 5-year growth rate of 83%, and providing over 1700 jobs, which is 1 in 4 jobs in the area[4]. In Newfoundland, the Sunrise Fish Farm is a family-operated blue mussel farm operating in the Notre Dame Bay region[5]. Technology employed at the farm is the continuous socking method which is similar to New Zealand green mussel culture. The farm employs approximately 15 people, mainly from one community where unemployment is ex-



		Canada	Viet Nam	Comparison
Population (million)		32.5	82.7	… 3×
Coastline (km)		202,080	3,444	… 1/60
Aquaculture Production		177,000t (2002)	1,110,000t (2003)	… 6.3×
Value	Can$	639 M	2.76 B	… 4.3×
	US$	511 M	2.2 B	
Areas (km²)				
total:		9,984,670	329,560	
land:		9,093,507	325,360	
water:		891,163	4,200	

Canada (2002)	
Species	**Prod (t)**
Salmon	132,021
Trout	7,080
Steelhead	2,034
Other	1,521
Mussels	20,540
Oysters	11,520
Clams	1,400
Scallops	106
Other	474
Total	176,696
Value Can$	639 M

Viet Nam (2003)	
Species	**Prod (t)**
Tilapia	
Indian Major Carp	750,000
Catfish	
Shrimp & Prawns	220,000
Grouper	
Seabass	
Crabs	
Lobsters	130,000
Bivalve molluscs	
Gastropod molluscs	
Clams	
Seaweeds	
Total	1,100,000
Value Can$	2.76 B

Figure 1.
Comparison of Canada and Viet Nam land / water areas and major species / production levels[1-3].

tremely high and there has been an out migration of young people. The farm has allowed several young families to remain and build homes, and it is estimated that each new dollar generated by the mussel farm circulates approximately three times in the local economy.

However, the Canadian government and communities often give mixed signals concerning the perspectives for aquaculture development. Well-financed advocacy groups, such as the Suzuki Foundation, have been lobbying the public and consumers to discourage the culture and purchase of farmed salmon. Ottawa[6], CAIA, the Salmon of the Americas, and other organizations/agencies are attempting to provide more valid scientific data and information to the consumers, particularly in relation to the health benefits of omega-3 fats and the environmentally sustainable fish farming practices. But the Canadian government sectors are often viewed as providing a restrictive regulatory environment and funds to counteract the negative media are limited.

Viet Nam

Fisheries and aquaculture contribute about 4% to the GDP of Viet Nam, of which aquaculture is 60% of revenue generated and 40% of the volume. In Viet Nam it is estimated that 1.7 million hectares of water surface are potentially available for aquaculture and culture based capture fisheries including: 120 000 ha for small ponds, 340 000 ha for larger water bodies, 580 000 ha for rice fish area, and 660 000 ha for tidal areas.

Sustainable aquaculture in Viet Nam is based on the diversification of species, experimental use of non-fish meal sources (e.g., green mussel silage), promotion of Better Management Practices and Good Aquaculture Practices under the FAO CCRF/A, development of extension service delivery, aquatic animal health advisory board establishment and disease surveillance systems via digitalized SMS messages from regional aquaculture associations to a national hub, and the initiation of a registration/licensing system for all production units for international tracking and tracing requirements. Viet Nam aquaculture concerns include local pollution from cages and shrimp ponds, exotic species introductions, mangrove swamp and wetland loss, disease outbreaks following unplanned aquaculture growth, and use of trash fish for feeds. Constraints to industry development are quality seed supply, planning processes for industry development, and adequate extension services since 77% of aquaculture households have less than 0.1 ha of pond area and 34% of farmers are in the Red River and Mekong delta regions.

The Viet Nam-Canada Project is a Canadian International Development Agency (CIDA)-funded project (collaborating Canadian institutions include SIAST, the Marine Institute, St. Hyacinth University, and Malaspina College/University) based at the new Tra Vinh Community College (TVCC) located in Tra Vinh province in the Mekong Delta region. Tra Vinh is one of the poorest Viet Nam provinces, with approximately one third of its population being of Khmer ancestry. The government has targeted this region for aquaculture development of shrimp farming, with funding and training supporting these initiatives. In less than three years TVCC's new aquaculture programs have a student intake of 250-300+ per year, plus short industry courses are being offered by the Departments of Fisheries and Extension to foster industry development. Young educated and trained people are remaining and returning to Tra Vinh town and the province. Efforts to recruit and train minority populations in the region for aquaculture and integrated aquaculture/agriculture activities hope to alleviate some of the social and economic restrictions in this region.

Organic Aquaculture Farming

In June 2004 the first Organic Aquaculture and Sea Farming Conference was hosted in Ho Chi Minh City, Viet Nam, by FAO, INFOFISH, and the Viet Nam Association of Seafood Exporters and Producers. Participants from about 35 countries discussed certification standards, perspectives from different countries, negative and positive implications, and examples of organic farming practices.

Private standards and certification organizations include IFOAM, Naturland, BioSuisse, BioGrow, Erute, KRAU, DEBIO, SOIL Association, SIPPO, IMO, and others. Some of Naturland standards include the defining of culture systems to

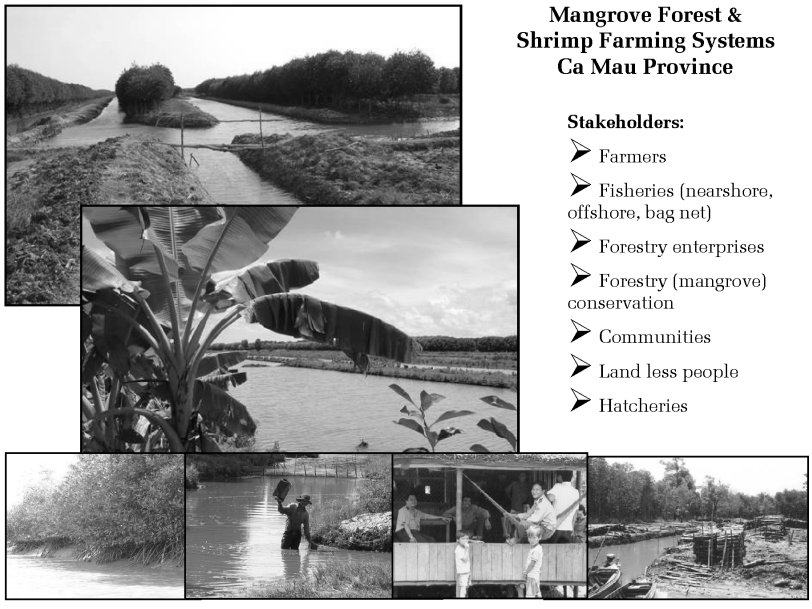


Figure 2.
Mangrove forest and shrimp farming systems in Ca Mau Province in the southern Mekong Delta region of Viet Nam have different stakeholders with input into various activities. Organic fish farming concepts are being developed to improve sustainability and economic returns (SIPPO-supported project). (Photos by Christensen)

species behavioural needs, setting limits for stocking densities, sustainability of fishmeal sources, limiting phosphorus and fishmeal content in diets, limiting energy consumption, demanding intensive monitoring of environmental impact, and requiring protection of the surrounding ecosystems. Some concerns in relation to certification were expressed for recirculation systems, use of aeration, wild fry sources, ingredients in feeds, ligation of shrimp eyestalks, cleaning agents used in farms and processing facilities, and the cost of monitoring/inspections for certification.

Positive perspectives for organic aquaculture farming include the possible transfer of IFOAM (International Federation for Organic Agriculture Movement) principles, growth opportunities for the organic market, good consumer acceptance, and perceptions expressed as confidence in the products, national and international labeling providing traceability and standards, and organic standards inherently having sustainable effluent and environmental practices. Negative perspectives were prices that would require a premium due to lower and more costly production levels, economic return rates (figures of about 20% return may not be enough), consumer issues that have been highlighted by negative media, and the lack of international accepted standards and certification.

A lot of aquaculture is already organic but is not certified. Examples of certified products are the New Zealand green-lipped mussel, an Equador shrimp hatchery (Naturland 2002), and the Switzerland (SIPPO) project in Ca Mau, Viet Nam (Fig. 2). Switzerland hopes to import only organic food products into the country within the next decade, starting with seafood products. Figure 2 illustrates the mangrove forest and shrimp farming systems stakeholders involved, whereby both Viet Nam and Swiss communities benefit through organic and sustainable production techniques.

Conclusion

Canada has the water, land, and people resources to develop its aquaculture industry which is crucial to the social and economic basis of many rural communities. One AIMS report[7] quoted the Minister of Fisheries and Oceans as stating that "aquaculture is here to stay and here to grow and is a legitimate user of our ocean resource." But as suggested by Sharratt[8], "time is running out and Canada is missing out on a chance to generate huge amounts of wealth that will go to other nations who are prepared to respond to the growing market demands." Viet Nam is one of these nations who is aggressively pursuing the development of its aquaculture sectors, with shrimp farming leading the way but species diversification plans accounting for regional differences and technology appropriate for small farm operations.

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Organic and Beyond the Box: Some Perspectives on Niche Marketing Aquaculture

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This paper explores some of the alternative routes to market which have, and might yet be, adopted by producers in response to expanded aquaculture production. The paper draws from a number of research projects on some of the key species within the European market over recent years. A common thread has been to move away from commodity price-based competition towards generating higher unit values in markets by differentiating the product across a range of attributes. Organic status has been one of the more high-profile strategies adopted, fuelled in part by consumers' responses to an array of food scares, environmental concerns, and animal welfare. Evidence suggests that the organic route, and related green paths, have led to some success. However these are not a panacea for all producers and other approaches have also been tried. Product differentiation through labelling, certification, and quality schemes has been prominent and is increasingly prevalent in a number of niche and mass markets. As the number of new species-based aquaculture product launches expands, it seems likely that further attempts to differentiate will be made. Nonetheless, such measures are not without cost and there are wider sectoral strategic considerations which might increasingly influence marketing policy and practice.

Nous examinons quelques-uns des parcours de rechange jusqu'à la mise en marché des produits aquacoles que les producteurs ont adoptés ou pourraient utiliser en réponse à la plus forte production aquacole. Pour ce faire, nous avons utilisé les résultats de plusieurs projets de recherche portant sur certaines des principales espèces commercialisées en Europe au cours des dernières années. Le commun dénominateur a été d'abandonner la concurrence axée sur le prix du produit en faveur d'obtenir un prix unitaire plus élevé sur les marchés par le biais de la différenciation du produit à l'échelon d'une gamme de caractéristiques. La désignation de produit biologique est l'une des stratégies adoptées les plus médiatisées, alimentée en partie par la réaction des consommateurs à une gamme de psychoses alimentaires, de préoccupations d'environnement et de campagnes de défense des droits des animaux. Les informations disponibles donnent à penser que la voie biologique, et les voies vertes connexes, ont eu des résultats fructueux. Mais ces voies ne sont pas une panacée pour tous les producteurs; d'autres approches ont aussi été mises à l'essai. La différenciation des produits par le biais de l'étiquetage, de l'homologation et d'assurance de la qualité se fait régulièrement et prend de plus en plus d'ampleur dans un certain nombre de marchés à créneaux et de marchés de masse. À mesure que le nombre de nouveaux produits aquacoles augmentera, il semble probable que l'on tentera de les différencier davantage. Mais cela engage des coûts. Des considérations sectorielles stratégiques de plus grande envergure peuvent aussi avoir une incidence de plus en plus marquée sur la politique de marketing et les pratiques commerciales.

Introduction

Aquaculture is now widely recognised as an increasingly important component in the emergent global food industry attempting to reconcile the various competing demands for different foods with a rapidly changing supply potential. Continued sustainable growth is conditional upon satisfaction of a number of criteria typically found in any food supply chain. Of critical concern is the delivery of assurances of product health and food safety; consumers have come to expect no less and are much less willing to embrace uncertainty and levels of risk that they perceive to be high in their food consumption decisions.

Risk reduction and the promotion of consumer confidence can be promoted through the adoption of apposite processes which may invoke HACCP systems, due diligence, and traceability so that reassurance can be provided[1,2]. Food consumers feel reassured by incorporation of such practices and consequently these processes have become commonplace demands for organisational buyers, such as supermarkets and others, within the supply chains.

Satisfaction of such demands for more sophisticated processes of course incurs costs in their establishment, implementation, and monitoring. Whilst these need not necessarily prove to be an insurmountable barrier to entry, they do tend to favour larger-volume producers who may enjoy the benefits of scale economies. The emergence of scale economies is not restricted to improved product and process safety measures but has combined with other cost phenomena, ranging from R&D through production to marketing, to favour industrial concentration[3].

Niche Drivers

The growth of larger aquaculture firms has followed a number of different routes which have incorporated mergers and takeovers resulting in transnational global players. Like many other sectors of the food industry, aquaculture has witnessed a concen-

tration of ownership and profits, often controlled at locations some distance from the point of production. In some cases this has effectively dislocated the national linkage of the production site and its owner, a relationship which raises some interesting questions about national-based product associations and linkages.

The emergent patterns of production location have been determined by spatial variation in production costs and in many cases these have become now trenchant relative product-cost differential positions. Consequently, the sector has become less footloose, but this has created more pressure to realise yet further cost savings. With an overriding emphasis upon cost-based differentials the industry product output tends to become standardised, assumes greater homogeneity, and collectively moves towards a commodity market.

Expanding production volumes of similar, undifferentiated product has contributed to conditions of market saturation with attendant falls in prices and profitability[4]. Poorer financial performance would appear to have focussed the mind of many participants more on yet further cost-reduction strategies rather than attempts to differentiate output through new product development and more proactive consideration of potential market wants.

It might be argued that the marketing function has been only loosely interpreted with a direction more rooted in selling and some ad hoc promotional activity rather than any more involved consideration of the constituent parts of the function. In particular, there would appear to have been only limited explicit attempt to identify more precisely exactly what different consumer groups might want in aquaculture products. In the absence of any such data or detailed understanding, the task of creating values in products sought by consumers is at best reduced to dependence on imitation of what has already been delivered from traditional captured fish supplies.

In an aquaculture environment dominated by cost minimisation there has been only limited finance made available for incursions into promoting product to communicate differentiating attributes[5]. Worse than this, much of the limited communications spent has been given over to retrospective defensive messages fired in response to adverse media stories. None of this has done much to endear the product to the prospective consumer nor have matters been made better by the failure of some producers to deliver good quality products to the market on a consistent and dependable basis.

The Organic Route: Green Pastures and Consumers?

Concerns about the seemingly inexorable tendency to produce more homogeneous standardised product have inspired some producers to consider organic production. By working to stipulated specifications and practices which are inspected, verified and certified producers aim to satisfy the demands of an increasingly important group of consumers[6,7]

Organic consumers typically reflect a wider concern and awareness with food issues and the processes involved in it reaching their tables. The provenance of the food, including the processes and treatments undertaken are of particular concern and typically reflect rejection of GMOs, most chemical treatments and unethical practices. Such groups are normally found to have above average education and income levels and are willing to pay price premiums to consume their desired attributes[8].

The organic market has shown consistent growth for some years and this reflects a response to a number of push factors. Many consumers have become increasingly sceptical and distrustful of the claims made for foods. A significant factor in this has been the response to various media campaigns which have sought to exploit the newsworthiness of peoples' concerns with what they eat. In some cases, notably farmed Atlantic salmon, this has led to an ongoing dialogue from a variety of perspectives and in some cases predicated upon data presented with questionable analytical rigour[9]. More generally this has fuelled scepticism of claims and facts provided about food products by government, scientists and other traditional sources of authorative information.

When applied to fish, organic consumer expectations typically reveal a set of product goals which will include a range of criteria. Production should be environmentally friendly and this will most likely mean a more sustainable and less intensive production system. Fish products should be natural, unadulterated, safe and without the addition of artificial chemicals or pesticides or be GMOs. Husbandry should reflect appropriate consideration for fish welfare and all of these concerns should be communicable and verifiable through brand credibility and identity.

Whilst not dissimilar from broader organic food consumer expectations in many respects, the delivery of an organic fish product does highlight a number of specific challenges. Perhaps the most trenchant is the fact that fish production is seemingly below the traditional level of consciousness associated with terrestrial and domesticated animals: many consumers regard fish as such because it is out of sight, below water[10]. The novelty of this brand concept is however masked by the proliferation of international organic brands which combine to present the consumer with a confusing array of standards through a diverse mixture of logos and images at the point of purchase.

In a market increasingly international in origin and service, effective brand imagery needs to be made clearer and less prone to ambiguous interpretation of standards. Where consumer confusion reigns, perception will tend to agglomerate at the level of the lowest common denominator (LCD), expecting and accepting only the lowest of all admitted members of the organic club. Of course in the fullness of time this leads to dissatisfaction, subsequent uncertainty, and lack of trust in products so certified. Beyond this lie the prospects of additional consumer confusion and post-launch backlash against the entire product category, possibly including that of higher standards too. Indeed cannibalisation might result: because if organic brands are held out to be so superior it takes only a small extension of reflection to wonder about the inferiority of the rest. But critically, the rest commonly constitutes the majority of the volume produced. Attainment of a price premium for the elite may well thus be at the risk of a price reduction for the majority.

Despite some doubts and confusion within the market, organic attributes have appealed to an increasing number of consumers as is evident from purchase data. For example within the UK, consumer panel data to the year ended August 2004 shows a range of increases in the organic market: expenditure +53%; volume +40%; average price +9%; penetration +18%; and the average weight purchased +19%[11]. These represent substantial growth and performance in excess of levels found in other expanding sectors of the food market such as premium and healthy product ranges. Attainment of these growth rates owes something to the decision by supermarkets to place greater emphasis upon the organic product thereby improving accessibility to a wider range of prospective consumers.

Reaching a wider consumer base can present a significant challenge for organic producers as, being typically smaller scale, they commonly have only limited budgets for traditional modes of communications such as advertising. This can place them in a potentially vulnerable position when broader countermanding media messages are aired; smaller producers simply tend not to have the financial power to put their alternative perspective across, and gaining editorial space for corrective messages is never easy. Collective messages through organic associations and greater reliance upon word of mouth and more direct market channels such as farmers' markets represent more common pathways.

However certified organic status comes at the cost of a levy on all products sold and many smaller scale producers may question the marginal benefit of such status, notwithstanding the premiums mentioned above. In these more marginal cases which may embrace those electing not to join the organic club and those unable to meet entry conditions, the presence of an organic standard may become a barrier to trade within sections of the market. Alternative brand propositions may be appropriate in these circumstances and might include positioning on a shade of green rather than adoption of a more absolute 'hard' green organic or 'non'-green non-organic status. Such intermediate points could carry the benefits of differentiating output from the standard and conventional yet not aligning to the more extreme outlier market positions. Whilst such a diversified approach might engage more producers, it may still raise questions of their ultimate dependence upon larger retail outlets to communicate with the market through their established brand imagery.

Alternative Niche Propositions: Branding

Having identified some of the benefits and costs attached to the organic route, some mention should also be made of other alternatives to product differentiation. Organic status is only one of a number of an increasing number of options thrown up through the expansion of product ranges launched on both existing and new species. In addition to the attributes and nuances that may be placed upon products by individual producers, more generic points of differentiation may be established through the creation or adoption of a quality mark or standard. This has been done on a national country of origin basis within various farmed and capture fisheries and can provide a useful platform from which to build further propositions to the market.

Generic brands inevitably run the LCD risk identified earlier: consumers will tend to associate the brand values with only the lowest exponent of its worth. Anomalous and individual rogue purveyors of the brand standard may thus undermine the entire group's values and damage the efforts expended in their creation. As with any cartel there will always be some temptation for individuals to seek the benefits (higher market prices) of belonging to the club but to maximise gains through cutting costs in delivering product standards. Whilst such tendencies may be curtailed through inspection and enforcement practices, keeping monitoring costs to realistic levels always means that aberrations may periodically occur.

The use of brands and quality marks should also take account of the varied perceptions as to what constitutes 'quality'. Individual market segments and buyers will probably have their own opinion on what the most relevant factors are and their relative importance; e.g., flesh colour, texture, fat content, freshness, etc. Notwithstanding such variation and opportunities to alter the specific package of attributes marketed, the generic brand can offer a robust platform upon which to build. One good example of this has been the establishment of the Tartan Quality Mark (TQM) used by Scottish Quality Salmon (SQS) to promote its members' product. This differentiation from other farmed salmon was extended in the 1990s with the award of the prestigious French quality mark, Label Rouge[12].

Salmon became the first fish to receive this distinction which applies to al foods, and indeed was the first time that it had been awarded to a producer outside of France. The potential value of such differentiation was evident in a study of SQS which found a price premium of up to 20% depending on size and grade which equated to an additional £16million in 2000[13]. In addition other non-price benefits were identified and these too could have important impacts on the brand's perception and performance. More recently, further steps to differentiate the Scottish product have been taken with the September 2004 award of PGI status from the EU which emphasises the linkage of the product to a discrete geographical area[14].

Conclusions - Future Sustainable Directions?

The foregoing discussion has highlighted a number of different ways in which products might be prepared more specifically for targeted niche markets. The approaches outlined cannot be fully comprehensive within the constraints of this paper. However it should be clear that a diversity of options exists and that there are a number of directions from which further variants might be made.

In keeping with the organic route, the expanding green considerations of the food consumer would appear to hold obvious potential. Environmental criteria, animal welfare considerations, ethical treatments and other factors are all liable to feature more prominently in future. National identities too have been noted as a viable mechanism, although some scepticism might be expected as the process of ownership concentration naturally promotes transnational partnerships based upon a plural production base. Clearly all of these trends have the capacity to confuse as well as clarify discrete positions for the consumer, who ultimately is liable to be making a comparatively low-unit value, and low-involvement decision: to buy fish for a meal or not. Given the potential profusion of messages it is thus all the more important that these are clearly reinforced by the satisfactory delivery of consumers' expectations for the product. Assuming this is done, there should be reasonable prospect of niche markets co-existing and competing for a wider range of consumers in the future.

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Calendar

7th International Marine Biotechnology Conference, Delta Hotel and Conference Center, St. John's, Newfoundland and Labrador, Canada, 7-12 June, 2005, IMBC 2005 will offer an international forum for the world's leading scientists working at the cutting edge of marine biotechnology. Participants will be provided the opportunity to exchange information, ideas and innovations with researchers working in the many interdisciplinary fields aligned with marine biotechnology. For more information, contact Marie Lanouette, Conference Manager, Tel: 613-993-0414; Fax: 613-993-7250; e-mail: *imbc2005@nrc-cnrc.gc.ca*; website: *www.imbc2005.org*.

Aquaculture Canada 2005 / NAIA's Cold Harvest Tradeshow, Delta St. John's Hotel and Conference Centre, St. John's, NL, Canada, 3-6 July 2005. A comprehensive technical, industry-oriented program is being prepared and developed. Special symposia and workshops will highlight achievements by various sectors of the Canadian aquaculture industry. In addition, the trade show and an extensive social program will provide opportunity for valuable networking and knowledge exchange. Aquaculture CanadaOM 2005 will be co-hosted by the Aquaculture Association of Canada (AAC), the Newfoundland Aquaculture Industry Association (NAIA), and the Department of Fisheries and Aquaculture of the Government of Newfoundland and Labrador (NL DFA). For more information, contact the Chrissy McGregor, Conference Secretariat, Tel: 506-529-4766; Fax: 506-529-4609; e-mail: *aac@mar.dfo-mpo.gc.ca*; website: *www.aquacultureassociation.ca/ac05*.

Aquaculture Europe 2005, Norwegian University of Science and Technology, Trondheim, Norway, 5-9 August 2005. Themed "Lessons from the Past to Optimise the Future", this European and international meeting will bring together participants from as many as 40 countries to address the key issues and discuss some of the most advanced scientific results and technological tools through plenary sessions reviewing key themes, and parallel sessions consisting of selected oral presentations and poster sessions reflecting those themes and key issues for the future development of aquaculture. The conference also includes two special forums - one on Innovation in Aquaculture; the second on the 6th and 7th Framework Programmes of the European Union. For more information, contact the Conference Secretariat at Tel: +32 59 32 38 59; Fax: +32 59 32 10 05; e-mail: *ae2005@aquaculture.cc*; website: *www.easonline.org*.

Aqua Nor 2005, Trondheim Spektrum, Trondheim, Norway, 9-12 August 2005. The Aqua Nor trade shows are held biennially in Trondheim and recent events have attracted around 20,000 visitors from close to 50 nations. 300 exhibitors representing more than 600 manu-facturers and suppliers from all over the world present current developments in the fields of aquaculture technology, fish feed, fish health, quality assurance, research, training, funding, insurance, fish farmers network, grading, processing, packaging and distribution. For more information, contact the Nor-Fishing Foundation, Tel +47 73 56 86 40; Fax +47 73 56 86 41; e-mail: *mailbox@nor-fishing.no*; website: *www.no-fishing.no*.

Larvi 2005, 4th Fish & Shellfish Larviculture Symposium, Ghent University, Belgium, 5-8 September, 2005. The aim of larvi 2005 is to bring researchers and professionals together to evaluate recent progress, identify problem areas and stimulate future cooperation in research and industrial production of freshwater as well as marine fish and shellfish larvae. For more information, contact the Conference Secretariat at The Laboratory of Aquaculture & Artemia Reference Center, Ghent University, Tel: +32-9-2643754; Fax: +32-9-2644193; e-mail: *larvi@UGent.be*; website: *www.UGent.be/aquaculture*.

Cod Farming in Nordic Countries, Nordica Hotel, Reykjavik, Iceland, 6-8 September 2005. This conference aims at giving an overview of cod farming activities and research in the Nordic countries and define future challenges. For more information, contact the Congress Secretariat, Ragnheiður Stefánsdóttir, Tel: + 354 554 1400; Fax: + 354 554 1472; e-mail: *ragnheidur@iii.is*; website: *www.fiskeldi.is/codfarm/information.html*.

Sixth Symposium on Diseases in Asian Aquaculture, Colombo Plaza Hotel, Colombo, Sri Lanka, 25-28 October 2005. The Fish Health Section (FHS) of the Asian Fisheries Society is proud to announce the 6th Symposium on Diseases in Asian Aquaculture (DAA VI). The theme of DAA VI, "Aquatic Animal Health: Facing New Challenges" is an appropriate discussion topic in support of present day efforts in increasing aquatic food availability through sustainable and responsible aquatic production. A workshop, a training course, an expert consultation, and the 7th Triennial General Meeting (TGM-7) of FHS are being planned in conjunction with DAA VI. For more information, contact Dr. Melba B. Reantaso, Tel: + 39 06 570 54843; Fax: +39 06 570 53020; e-mail: *Melba.Reantaso@fao.org*; website: *www.daasix.org*.

Aquaculture America 2006, Riviera Hotel & Casino, Las Vegas, Nevada, 13-16 February 2006. The US Aquaculture Society will put together an extensive technical program featuring special sessions, contributed papers and workshops on all of the species and issues facing aquaculturists around the country. In addition, Aquaculture America 2006 will have the largest aquaculture trade show in the Western Hemisphere and one of the largest anywhere in the world with nearly 200 booths! This is your opportunity to inspect the latest in products and services for the aquaculture industry. It is the place to visit current suppliers and make new contacts. For more information, contact the Conference Manager, Tel: +1-760-432-4270; Fax: +1-760-432-4275; e-mail: *worldaqua@aol.com*; website: *www.was.org*.

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To access old messages — check the AQUA-L archives at: *http://www.ifmt.nf.ca/aqua-l.archive*.

To find out who is on the AQUA-L mailing list — Send a message to *majordomo@killick.ifmt.nf.ca*. In the message body, type **who aqua-l**.

To find out who is on the AQUA-L-DIGEST mailing list — Send a message to *majordomo@killick.ifmt.nf.ca*. In the message body, type **who aqua-l-digest**.

Aquaculture Canada^{OM} 2005 Silent Auction

This year, to continue the tradition, there will be a silent auction at the Student BBQ, all the proceeds of which will benefit the AAC Student Endowment Fund (SEF), an account used to fund AAC student travel awards as well as prizes for presentation awards. In addition to supporting AAC students, this is also an opportunity for increased exposure for a company or service. Last year's auction raised over $1800 for the SEF, and this year, we hope to pass this milestone!

Items that have been donated to previous auctions have included books, articles of clothing, artwork, gift certificates, etc., although any item would be much appreciated. All donors are acknowledged on the conference website, at the silent auction, and in the published conference proceedings.

I sincerely thank you for your consideration of this worthy cause, and hope to see you in St. John's in July. Please check out the AC05 website (*www.aquacultureassociation.ca/ac05*) for more conference information.

Chris Hendry
President-Elect, AAC

Please forward any donations to the following address:

AC05 Silent Auction
c/o Chris Hendry
58 Hardy Avenue
Grand Falls-Windsor, NL
A2A 2K2 Canada