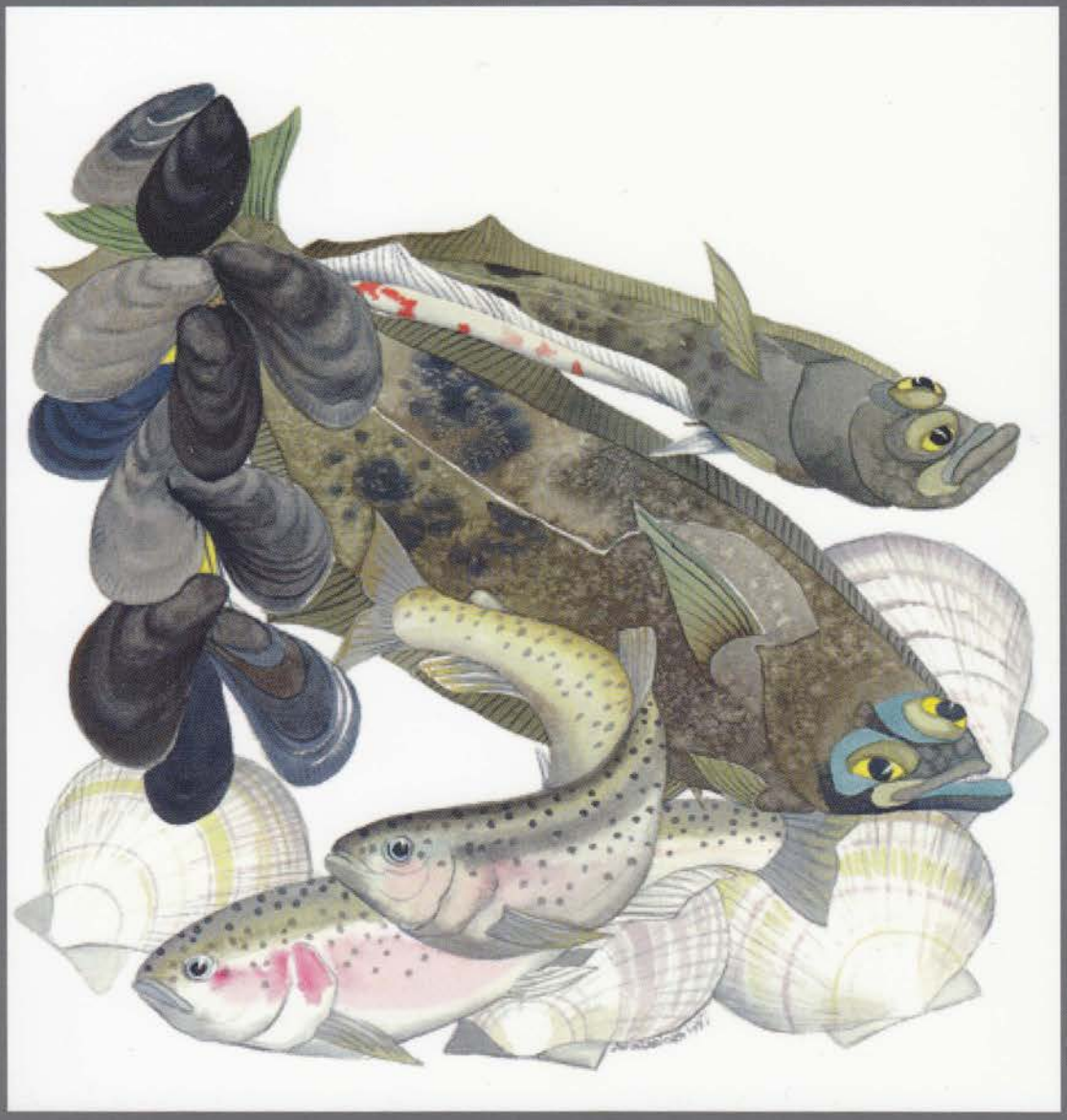
Bulletin

of the Aquaculture Association of Canada



Contributed papers
Aquaculture Canada '98

Edition 98-2
June, 1998


Aquaculture Canada '99
St. John's, Newfoundland

Bulletin
of the
Aquaculture Association of Canada

The *Bulletin* is available through subscription ($40. per year) or as a benefit of membership in the Aquaculture Association of Canada, a nonprofit charitable organization. For membership information contact: Aquaculture Association of Canada, P.O. Box 1987, St. Andrews, N.B., Canada EOG 2X0 [telephone 506 529-4766; fax 506 529-4609; e-mail aac@mar.dfo-mpo.gc.ca; web site http://www.ifmt.nf.ca/mi/aac]. Annual dues are $40 for students and seniors, $50 for individuals, and $85 for companies; 25 percent of dues is designated for *Bulletin* subscription.

The *Bulletin* is indexed in Aquatic Sciences and Fisheries Abstracts (ASFA) and the Zoological Record. Mailed under Canada Post Publications Mail Product Sales Agreement No. 525375. Change of address notices and undelivered copies should be mailed to AAC. Return postage guaranteed.

ISSN 0840-5417
Printed by McCurdy Printing Ltd., Sackville, N.B.

Special Thanks
to the
Atlantic Canada Opportunities Agency (ACOA)

AAC gratefully acknowledges the the financial support of ACOA (St. John's, NF) for funding the publication of this proceedings (the views expressed in this publication, however, are not necessarily those of ACOA.).

Cover: Original artwork by Diana Dabinett, commissioned by the AAC and used as the Aquaculture Canada logo. Diana Dabinett resides in Shoe Cove, Newfoundland. Photography on inside covers and in the meeting report by John Castell and Keith Rideout; photocollages by Chris Hendry.

Mailbox

Editor,

An article entitled *Drug resistance of atypical Areomonas salmonicida from Atlantic salmon and rainbow trout in Newfoundland* by L. Hawkins et al. in edition 97-2 of your journal includes information regarding the approval of oxytetracycline which requires further clarification. In the first sentence under Conclusion (page 41), it is stated that oxytetracycline is approved in Canada.

It would be appreciated if you would provide a correction as Terramycin-Aqua is the only form of oxytetracycline which is approved for treating salmonids in Canada. MIB#35A—1&2, from the Medicating Information Brochure issued by the Canadian Food Inspection Agency, is attached for your reference.

Trade memoranda are issued periodically by the Canadian Food Inspection Agency regarding any changes to the Compendium of Medicating Ingredient Brochures (CMIB).

Bruce I Groves, DVM
Animal Health
Pfizer Canada Inc.
195 Dufferin Avenue
London, Ontario

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the AAC Aquaculture Discussion Group

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Aquaculture Canada '98

The Honourable Gilbert Normand, Secretary of State for Fisheries and Oceans, Agriculture and Agri-foods speaking at the Opening Session of Aquaculture Canada '98.

Sunny warm weather greeted the five hundred and thirty-four registrants of the Aquaculture Canada '98 conference and trade show in St. John's, Newfoundland from May 31 to June 3. Delegates attended from across Canada, the United States, Europe, and Asia. The theme of the conference was "Research: An Investment in our Future" and many of the sessions and talks focused on this theme. The opening ceremony featured welcoming remarks from myself, as AAC President, Marc Kielley, Executive Director of the Newfoundland Aquaculture Industry Association (NAIA), and Marli MacNeil, President of the Canadian Aquaculture Industry Alliance (CAIA). The Secretary of State for Fisheries and Oceans, Agriculture and Agri-foods, the Honourable Gilbert Normand spoke at the opening session and the student BBQ, where he addressed the Federal Government's role in aquaculture, the imminent appointment of a new federal commissioner for aquaculture development, and the possibility of new federal programs for the aquaculture industry. Dr. Normand was also

L to R: Mr. Mark Kielley, Executive Director, Newfoundland Aquaculture Association, The Honourable Gilbert Normand, Secretary of State for Fisheries and Oceans, Dr. Jay Parsons, President of the Aquaculture Association of Canada, Mr. John Efford, Newfoundland Minister of Fisheries and Aquaculture, and Mr. Beaton Tulk, Newfoundland Minister of Development and Rural Renewal donning their Aquaculture Canada '98 aprons.

Aquaculture Canada '98

conference committee

Jay Parsons and Marc Kielley, co-chairs

Organization and Arrangements

Nigel Allen
Joe Brown
Brian Burke
Lynette Carey
Cyr Couturier
Sharon Ford
Marc Kielley
Tom McKeever
Brian Meaney
John Moores
Jay Parsons
Rod Penney
Paul Strickland

Program

Cyr Couturier (Contributed)
Marc Kielley (Technical, Plenary)
Jay Parsons (Plenary, Special, Technical)
Jerry Ward (Plenary)

Publicity and Promotion

Jay Parsons
Marc Kielley
Jan Woodford
Anne Lamar
Brain Burke

Student Activities

Keith Rideout
Rod Penney
Sean MacNeil
Chris Hendry

Back row (l to r): Jay Parsons, John Moores, Joe Brown, Nigel Allen, Paul Strickland, Cyr Couturier.
Front row (l to r): Lynette carey, Brian Meaney, Marc Kielley, Sharon Ford, Tom McKeever, Rod Penney.

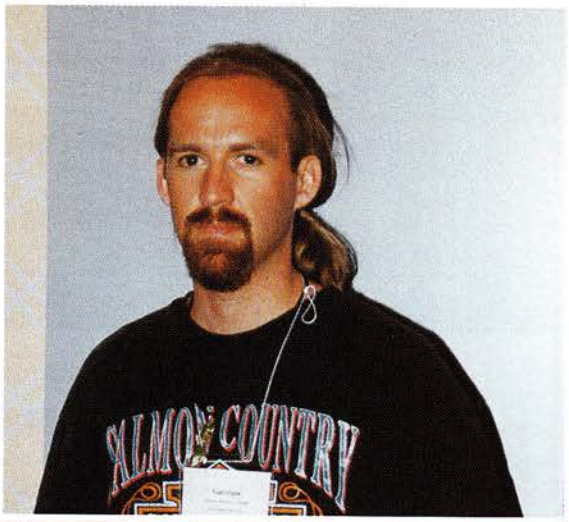
available the entire opening day and met with the Boards of Directors of AAC, NAIA, and CAIA. The Newfoundland Minister of Fisheries and Aquaculture, John Efford, and the Minister of Development and Rural Renewal, Beaton Tulk, also spoke at the opening session and announced a new funding agreement for the Newfoundland shellfish industry.

Aquaculture Canada '98 was co-hosted by the Newfoundland Aquaculture Industry Association (NAIA). This arrangement worked well and contributed to the overall success of the meeting. Executive Director Marc Kielley and the staff at NAIA (Lynette Carey and Brenda Smith) organized the Trade Show. The industry response was tremendous — the Main Ballroom of the Delta Hotel was quickly booked and then the hallway was filled to capacity. In all, forty-nine exhibitors were represented.

The program for the conference was comprehensive. There were contributed, technical, and special sessions organized for each of the three days. The plenary session organized by Jerry Ward, Assistant Deputy Minister, Newfoundland Department of Fisheries and Aquaculture, focused on markets and sales. For the first time, AAC organized a technical session for aquaculture suppliers as an opportunity for exhibitors in the trade show to present new technologies and their expertise to a wider audience. The session was chaired by John Gracey of Northern Aquaculture and attracted a large number of participants and many questions from the audience.

There were 10 Special Sessions held throughout the week: the Seaweed Aquaculture Session was chaired by Steve Moyse (NAIA), John Moores (DFO) organized and chaired the Newfoundland Species Update, Ed Trippel (DFO) organized and chaired the Broodstock Research and Techniques Session, John Castell (DFO) organised and chaired the Live Feeds Session, Michael Reith (NRC-IMB) organised and chaired the Applications of Molecular Biology Session, Cyr Couturier (MI-MUN) organised and chaired a Mussel Production Capacity Workshop, Thomas Landry (DFO) organised the Mussel Species Performance and Distribution Session, Brian Meaney (NF DFA) organised and chaired the Multi-Resource Use Session, Paul Strickland (ACOA) organised and chaired the Transportation and Insurance Session, Rob Armstrong organised and chaired a Fish Health Session, and Nigel Allen (CASD–MUN) organised and chaired an engineering debate on open ocean technology versus land-based technology.

Cyr Couturier organized the contributed paper sessions, which included oral and poster presentations on shellfish culture, scallop culture, sea urchin culture,



Best Student Oral Presentation — Tie for First Place

Upper photo: Todd Cook of AVC–UPEI received the Moore-Clark Prize for Best Student Oral Paper.

Lower photo: Kara Firth of AVC–UPEI, receiving the Connors Bros. Award for Best Student Oral Paper from Jay Parsons.

Sponsors of Aquaculture Canada 98

Atlantic Canada Opportunities Agency (ACOA)

Canada/Newfoundland Agreement on Economic Renewal

Newfoundland & Labrador Department of Fisheries and Aquaculture

Canadian Centre for Fisheries Innovation

Marine Institute of Memorial University

National Research Council of Canada —
Industrial Research Assistance Program

Fisheries and Oceans Canada

The following groups also provided support

Newfoundland & Labrador Department of Tourism, Culture and Recreation

National Research Council of Canada — Institute for Marine Biosciences

Heritage Aquaculture

Aqua Health Ltd.

Northern Aquaculture

Future SEA Farms Ltd.

Atlantic Fish Farming

Farm Credit Corporation

Contact Canada

Economic Development and Tourism, City of St. John's

ture, and finfish aquaculture, fish physiology, and early larval rearing.

Students from across Canada participated in the conference. There were 26 student poster and oral presentations and, once again, they were of a high quality. Dr. Andrew Boghen, Vice-President and Chairman of the Awards Committee, was very successful in obtaining industry support for student travel and best student presentation awards. The Connors Bros. Award for Best Student Oral Paper went to Kara Firth of AVC-UPEI. Kara shared the top honour with Todd Cook, also of AVC-UPEI, who received the Moore-Clark prize. A.D. (Joey) Johansen of the Department of Biochemistry, Memorial University, received honourable mention for her presentation. The Canadian Centre for Fisheries Innovation first prize for Best Student Poster went to Karen Whalen of the Ocean Sciences Centre, Memorial University. The second place prize in the student poster session, donated by the University of Guelph, went to Nicole Brun of the Université de Moncton.

Student travel bursaries were awarded to Nicole Brun, Corina Rice, Chris Hendry, Christine Ouellette, Sean Tibbetts, Eric Bataller, Kara Firth, Janice Lawrence, Todd Cook, and Cheryl Wartman.

Heritage Aquaculture sponsored a student luncheon featuring salmon burgers. Mr. Bill Robertson spoke briefly to the fifty-plus students in attendance encouraging them in their research. Many students provided assistance in running the audiovisual equipment. Chris Hendry ably coordinated their efforts.

The conference social events featured aquaculture products from across Canada. The hors d'oeuvres served at the opening reception included BC and PEI oysters, BC clams, tilapia, seafood crepes, mussels, whole scallops, and flambé scallop meats. The Marine Institute of Memorial University hosted the student BBQ, and the salmon teriyaki kebabs were very popular. Dr. Gilbert Normand was the guest speaker at the BBQ. The Banquet featured smoked mussels

Best Student Poster Paper

Karen Whalen, of the Ocean Sciences Centre, Memorial University, receiving the Canadian Centre for Fisheries Innovation Prize for Best Student Poster Paper from Dr. Joe Brown.

Honourable Mention

A.D. (Joey) Johansen (right), Department of Biochemistry, Memorial University of Newfoundland, receiving an AAC Certificate of Honourable Mention for her student oral paper from Dr. Willy Davidson.

Trade Show Exhibitors

Atlantic Canada Opportunities Agency
AIMS Ltd.
Air Liquide Canada
Alchem Industries
Atkinson & Bower
Atlantic Group Ltd.
Avid Canada
C & W Industrial
Canadian Aquaculture Industry Alliance
Canadian Aquaculture Institute
Canadian Technology Network
Canadian Centre for Fisheries Innovation
Cold Water Sea Products
Contact Canada — Canadian Aquaculture Directory
Controls & Equipment
Department of Fisheries and Aquaculture (St. John's, NF)
Department of Fisheries and Oceans (St. Andrews, NB)
Department of Fisheries and Oceans (St. John's, NF)
Drover Financial
Eastern Empire Corp.
Enviro Med Analytical
Fish Harvesters' Resource Centre
Fukui North America
Future Sea Farms Inc.
GMG Fish Services Ltd.
Go Deep International
Government of Canada Rural Exhibit
Honda One
IMP Group Ltd.
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National Research Council — IRAP
National Research Council — Institute of Marine Biosciences
Newfoundland Aqua Service Ltd.
Newfoundland Styro Inc.
Northern Aquaculture
Nova Scotia Agricultural College
Plastik Belt Newfoundland
Poly Steel Atlantic
RockWater Scuba / Adventures Inc.
Schering-Plough Animal Health
Sub Aqua Diving
TRC Hydraulics Inc.
TrisMar Research Inc.
Valox Ltd.
Waterworks Supplies — EMCO
Wedgwood Insurance
Wright Systems & Equipment

and salmon as an appetizer, and steelhead and Atlantic salmon for the main course. The after-dinner entertainment included guest speaker John Efford, Newfoundland Minister of Fisheries and Aquaculture, Sods N' Rhymes, a local comedy troupe, and the Ennis Sisters, also from Newfoundland.

The enormous job of organizing registration, printing name badges, managing the registration database, etc., was ably handled by a very expectant Sharon Ford who delivered a baby boy, Christopher, on June 25. Assistance at the registration desk, was provided by Jennifer Dufour of the BC Salmon Farmers Association, by Lorelei Levy, Melissa Mooney, and Miranda Pryor from the Marine Institute, and others.

This was the Aquaculture Association of Canada's 15th annual meeting since 1984 and we are proud of our success as the longest-running annual aquaculture conference in Canada. I was particularly pleased that this year's conference and trade show was co-hosted by the Newfoundland Aquaculture Industry Association. Such "strategic alliances" are important in ensuring that the newest technologies and information are delivered to the widest audience possible. I trust such coordination and growth will continue next near in Victoria and into the future.

Since 1984, the success of AAC's annual meeting has depended on a dedicated group of volunteers — a team. This year was no different. From the organizing committee, to the student volunteers, to the people helping out during the meeting, my thanks to you all!

— Jay Parsons, President

Second Prize — Best Student Poster

Nicole Brun, Université de Moncton receiving second prize in the best student poster competition from Cyr Couturier, organizer of the contributed poster session. The prize was donated by the University of Guelph.

Aquaculture products were donated to Aquaculture Canada '98 social events by the following producers

Heritage Aquaculture
Great Maritime Scallop Trading Co. Ltd.
Thimble Bay Farms
Pearl Sea Products
Atlantic Ocean Farms
Northern Tilapia
Integrated Aquatics
San Mateo Shellfish Ltd.
Atlantic Shellfish Inc.
Stolt Sea Farm Inc.
Rainbow Seafood
Les Aliments Perrola Inc.

Marli McNeil (right), President of the Canadian Aquaculture Industry Alliance (CAIA) and Jay Parsons, President of the Aquaculture Association of Canada at the Opening Session.

L to R: Jennifer Dufour, BC Salmon Farmers Association, and Miranda Pryor and Melissa Mooney, graduate students at the Marine Institute, Memorial University, at the Aquaculture Canada '98 registration desk.

The following companies generously donated to the Student Endowment Fund

A/F Protein
APICS
Aquaculture Centre, University of Guelph
Canadian Centre for Fisheries Innovation
Corey Feed Mills Ltd.
Connors Bros.
Franken N.A.. Equipment
Moore-Clark
Stolt Sea Farm Inc.
Superior Systems AS
Syndel Laboratories
Valox

Advantages of industrial wastewater heat sources for aquaculture production

A. Dumas,[1] *L. E. Hansen,*[1,2] *and L. Bouchard*[1]

In many parts of Canada, cold water temperature is the limiting factor for aquaculture production even when the water supplies are of sufficient quantity and quality. Salmotherm Inc. is a pilot project that is using the heat taken from industrial wastewater effluent to raise freshwater temperatures to optimum levels for salmonid culture. Since 1995, Salmotherm Inc. has produced several metric tons of landlocked salmon (*Salmo salar ouananiche*) and brook trout (*Salvelinus fontinalis*). Landlocked salmon (mean initial weight = 90 g) and brook trout (fry) reached 800 g in 8 and 15 months, respectively. Over the same period of time in a nearby fish farm, landlocked salmon and brook trout weighed only 200 g and 110 g, respectively. With the potential of maintaining water temperatures of up to 25°C, Salmotherm Inc. is now experimenting with the rearing of walleye (*Stizostedion vitreum*) and yellow perch (*Perca flavescens*). Aquaculture production has now become possible in regions where it was previously not feasible and represents new opportunities for creating employment in many northern communities.

Introduction

Groundwater in Canada is generally cold (< 8°C) and surface water temperature vary greatly (0 to 25°C) depending on the season. These conditions are the main limiting factors of aquaculture production when water of sufficient quantity and quality is available.[3-5]

The use of electricity or fossil fuels to raise water temperatures only a few degrees can compromise the profitability of aquacultural production.[6] In order to solve this problem, a pilot project (Salmotherm Inc.) was started in 1995 using heated wastewater effluent from a pulp mill to warm the incoming river water for a fish farm. The pilot project is evaluating the biological, technical, and economical feasibility of using industrial waste heat for aquaculture production. Salmotherm Inc. raises salmonids, mainly landlocked salmon (*Salmo salar ouananiche*) and brook trout (*Salvelinus fontinalis*), although Arctic char (*Salvelinus alpinus*) are being raised in much smaller numbers.

The purpose of this paper is to present some of the results obtained during the three years of production and to demonstrate the advantages of using industrial wastewater heat for commercial aquaculture production.

Project Facilities

Heat exchangers transfer heat from the industrial effluent of the pulp mill Produits Forestiers Donohue Inc., St-Félicien, to the surface water used to rear the fish. The surface water comes from the Ashuapmushuan River, a non-polluted watercourse of the Canadian Shield. The water undergoes filtration, disinfection, heating, degassing, and oxygenation before it is distributed to the fish tanks.

Surface water is pumped at a flow rate of 1200 L/min, and distributed to six tanks of 6.4 m and two tanks of 3.0 m in diameter that are all self-cleaning. Each fish tank is supplied with three types of water: heated, cold, and oxygen supersaturated. In each rearing unit the three inlets are controlled and mixed in order to maintain a steady temperature and adequate levels of oxygen. All facilities are built within a greenhouse to minimise heat loss and to shelter the equipment from bad weather. The production unit was designed to produce 10 metric tons annually.

Results

During the winter, surface water temperature increased from 0 to 20°C during its passage through the heat exchangers. The industrial wastewater flow rates

required to achieve this can vary from 300 to 1300 L/min depending on the type of heat exchangers. Water temperatures in the fish tanks are easily maintained between 12 and 17°C.

Care must be taken to avoid drastic changes of water temperatures that can occur during an industrial shutdown. The Donohue pulp mill has two type of industrial waste water: the water used to cool the various chemical and physical procedures within the mill (cooling water) and the water coming from the different processes in the mill (processing water). Using processing water instead of cooling water became necessary for Salmotherm Inc. in order to maintain an adequate temperature during a shutdown. Processing water is retained in a large reservoir in which the temperature remains relatively steady for a few days. However, the cooling water is pumped by the mill only when the machines are operating. If heat exchangers use the cooling water to warm the fish farm influent, the temperature within the fish tanks will drop drastically during a shutdown, since no cooling water will be provided. The mean annual cooling water temperature was 45.3 ± 19.1°C; the processing water temperature remains more steady at 50.0 ± 7.0°C. However, the processing water contains high levels of suspended matter so precautions have to be taken to reduce the risk of plugging the heat exchangers.

Fish growth is obviously better in Salmotherm Inc. than in a standard fish farm using groundwater or surface water. For instance, fish from a domestic line of landlocked salmon weighing 90 g in October reached 800 g by the following June. The same line of fish, raised with groundwater in a fish farm in St-Félicien, weighed 200 g after the same period of growth (Fig. 1). Hence, fish production can be increased greatly when industrial waste heat is used to warm fish farm water supplies. Similar results were also observed with brook trout. It took 15 months to bring regular fry to a size of 800 g (Fig. 2). Brook trout normally weigh approximately 110 g after 15 months in a fish farm using groundwater (water temperature = 5 to 9°C).[7]

Last winter, juvenile walleye (*Stizostedion vitreum*) grew from 11 g (98 mm) to 26 g (142 mm) in 49 days at a mean water temperature of 20.9 ± 2.0°C. That correponds to a growth rate of 0.89 mm/d. Summerfelt and Summerfelt[8] reported a growth rate of 0.90 mm/d at 24°C with the same species (initial length = 87 mm).

Thermal Waste Utilization: Some Advantages In Aquaculture

Besides the possibility of warming fish farm influents at a lower cost, the use of thermal waste provides the opportunity of maintaining optimal temperatures for growth of a given species all year round. Faster growth means greater annual production, lower live fish inventories and, consequently, lower capital costs. Also, fish sales, and therefore incomes, can occur all year round if fish stocks are managed appropriately. Consequently, the financing period can be shortened, and the profitability increased. Very often, fish sales are done on a seasonal basis in regular cold-water aquaculture. Because the production activities in a heated water supply are spread more uniformly over the year, workers can be employed more consistently than in a regular farm having the same facilities and using groundwater or surface water. Similarly, equipment use is maximised, meaning further savings.

Furthermore, industries that discharge thermal effluents (e.g., pulp and paper, aluminium smelters, thermoelectric plants, etc.) are rela-

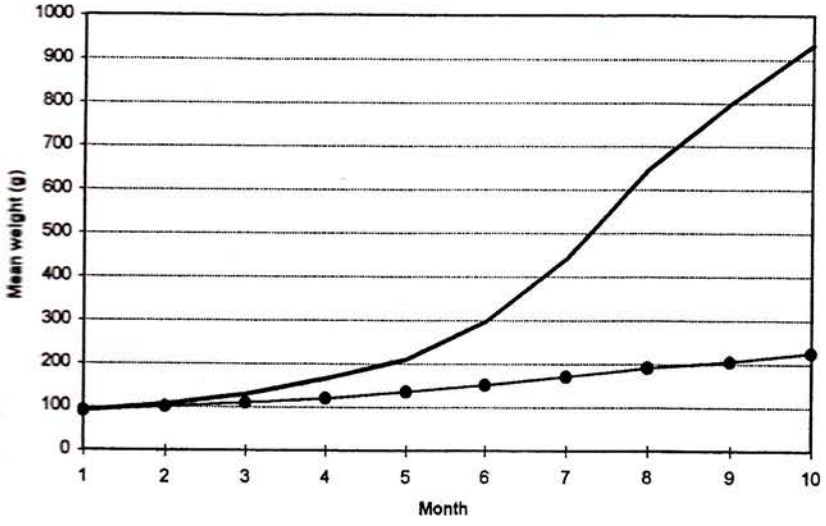

Figure 1. Growth of landlocked salmon (*Salmo salar ouananiche*) in Salmotherm Inc. (SALMO— plain line) and in a standard fish farm (STANDARD — line with points) in Québec.

tively numerous in Canada (more than 200 sites). Many additional benefits will ensue from the development of a partnership between these industries and aquaculture. While many of these benefits are difficult to evaluate in monetary terms, their benefits in terms of environmental impact and better commercial and social image are important. The industry can reduce negative environmental impact through water re-use optimisation and wise energy use. The social implication of job creation by the association with an aquaculture facility is another important consideration for these industries. Aquaculture allows those industries with suitable sites to strengthen their environmental management plans and advance toward the goal of sustainable development.

Conclusion

The use of thermal waste to warm fish farm influents is not only technically feasible, but also advantageous. By maintaining an adequate water temperature, fish growth and annual production increase, revenue is generated throughout the year, and employment remains more steady. Moreover, the factory discharging thermal effluents gains a better image and reputation by developing a partnership with the aquaculture industry.

Salmotherm Inc. is now experimenting in the raising of walleye (*Stizostedion vitreum*) and yellow perch (*Perca flavescens*), since the water temperature can be maintained up to 25°C in the facility. The development of the culture of these fishes will open up many sites for fish farming where summer water temperatures are too high for salmonids.

In Canada, the use of thermal waste heat for aquaculture production is now possible and represents a new opportunity to produce fish and create jobs.

We gratefully acknowledge Produits Forestiers Donohue Inc. St-Félicien for their encouragement and support in this project. The CEGEP de Saint-Félicien, its personnel, and the 1996, 1997 and 1998 graduating classes in aquaculture, contributed greatly to the success of the experiment. We thank the Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec, the Bureau Fédéral de Développement Régional du Québec, Hydro-Québec, the Société Québécoise de Développement de la Main d'Oeuvre, and the Centre d'Innovation et de Développement Expérimental du Lac Saint-Jean Nord for their financial support.

Notes and References

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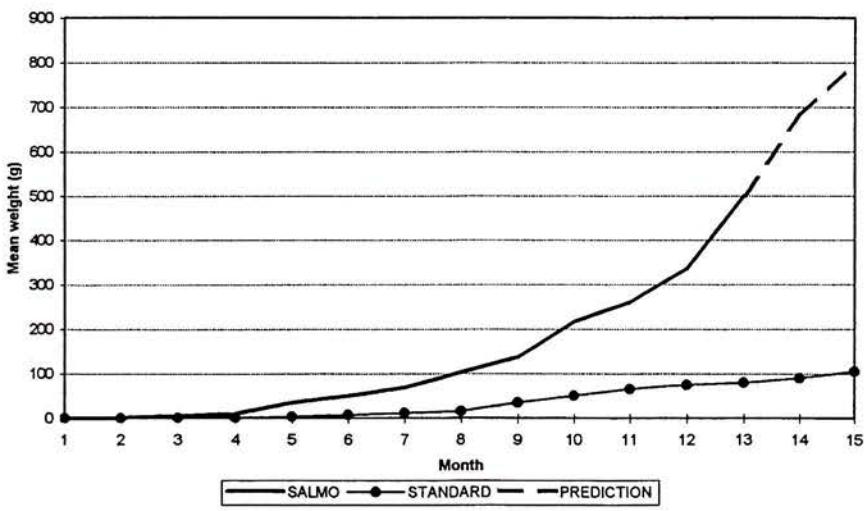

Figure 2. Growth of brook trout (*Salvelinus fontinalis*) in Salmotherm Inc. (SALMO) and in a standard fish farm (STANDARD) in Québec.

Preliminary observations on the larviculture of witch flounder (*Glyptocephalus cynoglossus*)

J. H. Rabe,[1] *J. A. Brown,*[1] *D. A. Bidwell,*[2] *and W. H. Howell*[2]

Little is known about the life history of cold-water marine finfish despite the fact that these species are of major commercial importance. Witch flounder (grey sole) once formed an important component of Newfoundland's flatfish resource and its high market value and consumer acceptability make it an excellent candidate species for aquaculture. In 1997 we began a feasibility study on the potential of this species for aquaculture. Larvae grew well on a diet of cultured enriched rotifers and *Artemia*. Larval length and age at metamorphosis were 65 mm and 120 days, respectively. Survival was high (70%) during the extended larval stage. Weaning to an artificial diet was not complete until day 160. Metamorphosed juveniles displayed a low occurrence of pigmentation (20%) and eye migration abnormalities. Protocols for maintaining wild broodstock are being developed. Our preliminary observations allow us to conclude that the potential for commercial aquaculture of witch flounder is high, as larvae exhibit high survival and good growth in culture systems.

Introduction

Witch flounder (*Glyptocephalus cynoglossus*) is a small, right-sided pleuronectid inhabiting the relatively deep waters of the North Atlantic.[3] This species forms an important component of Newfoundland's flatfish resource, although catches are markedly reduced compared to previous years.[4] Witch flounder is commercially known as grey sole and often approaches the price of Atlantic halibut on New England markets. Given the need to diversify the range of cultured species, and the high market value of witch flounder, we are currently investigating the potential of this species for aquaculture.

The life history of witch flounder poses some challenges to the fish culturist due to its deep-water habitat, slow growth rates[3] and long larval period.[5] Our preliminary research focused on the larval period, which is often the most difficult stage in the life cycle of fish. Literature reports suggest that the larval period of this species can last up to 6 months[6] and that the larvae metamorphose at a large size.[5] Because of the interesting biological characteristics of witch flounder and its high market price, our research can benefit aquaculture as well as enhance the understanding of the life histories of marine fish.

Materials and Methods

Rearing Protocol

Witch flounder were stripped at sea in the Gulf of Maine in early September 1997, and the fertilized eggs were brought to the Ocean Sciences Centre for incubation. Unfortunately, few eggs (2 mL) were collected. Eggs were incubated at 12°C and hatched in approximately 10 days. Larvae were fed rotifers enriched with *Isochrysis* twice daily. After 30 days, *Artemia* enriched with Algamac (Bio-Marine) or Selco (INVE) were added. Larvae were moved to a 3 000-L flat- bottomed tank on day 40. *Artemia* were added to the tank four times daily. Rotifers were also added four times a day, but the gut color indicated that the diet consisted mainly of *Artemia.* Microalgae-enriched water was used until day 100 after weaning had begun. It was not until day 70 that larvae were observed to ingest an artificial diet and weaning was not complete until day 160. A wide variation in the size of the larvae was observed, which likely caused the long weaning period. Larger larvae were easily weaned while smaller larvae continued to rely on *Artemia*. The average rearing temperature was 10°C and ranged from 8.4° to 13.2°C.

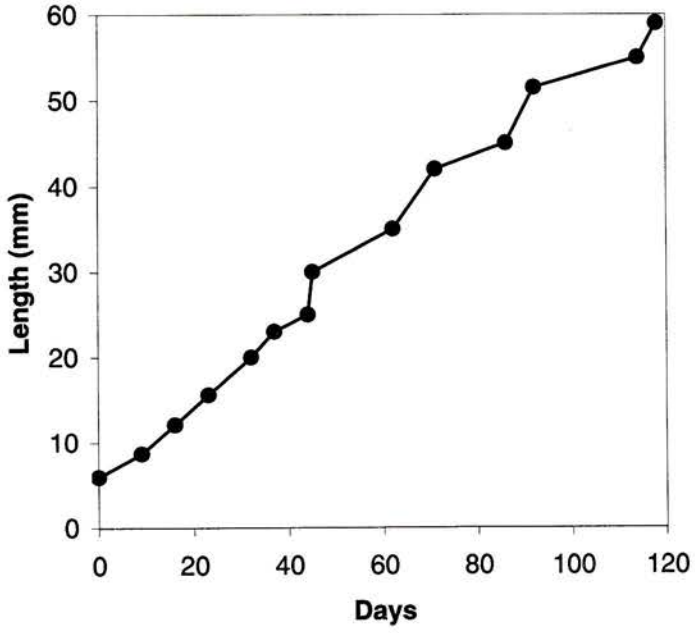

Figure 1. Growth in length of witch flounder larvae from hatching to 50% settlement.

Results and Discussion

Survival

The survival rates of witch flounder in culture were high. Because of the long larval period, we divided the larval period into four stages: incubation, yolk-sac, start-feeding, and metamorphosis (defined as the time from when the first settled individual was observed to the time when 50% had settled). Heavy mortalities (50%) occurred during egg incubation, which can be attributed to losses during egg transportation. During all other stages, the survival was high (90%). Our results showed no evidence of a critical period of increased mortality during start-feeding or metamorphosis.

In summary, from hatching to 50% settlement at day 120, we produced approximately 750 fish from 2 mL of eggs.

Growth

Witch flounder larvae grew well during the extended larval period (Fig. 1). However, a wide variation in size was observed. Unfortunately this is not reflected in Figure 1 as sample sizes were kept small (1 to 4 fish) due to the small number of animals. A large variation in age at settlement also occurred: the first settled individual was observed at week 9 while some individuals still had not settled by week 32. Witch flounder were observed to metamorphose and settle at the large size of 45 to 65 mm. These growth rates are comparable to larval growth rates of other species which have a shorter larval period and metamorphose at a smaller size.

The high survival and good growth rates of witch flounder larvae is encouraging for the development of a new species for aquaculture. Future research will be directed at refining larval rearing techniques including the effects of prey density, light intensity, and microalgae-enriched water on growth and survival of witch flounder. We will also develop juvenile on-growing and broodstock management protocols. Our preliminary results are very positive and allow us to conclude that the potential for commercial culture of witch flounder is high.

The authors thank the Canadian Centre for Fisheries Innovation and Fishery Products International for financial support. We also thank the staff at the Ocean Sciences Centre, Maritime Mariculture International and Sea Forest Plantation for assistance.

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Effect of dietary n-3 HUFA on growth and body composition of juvenile yellowtail flounder (*Pleuronectes ferrugineus*)

K. S. Whalen,[1] *J. A. Brown,*[1] *C. C. Parrish,*[1]
S. P. Lall[2] *and J. S. Goddard*[3]

In order to develop grower diets for marine flatfish for aquaculture in Newfoundland, the effect of n-3 highly unsaturated fatty acids (HUFA) on the growth of juvenile yellowtail flounder (*Pleuronectes ferrugineus*) was studied. Most cold-water fish require high levels of n-3 HUFA in their diets for biological functions, including membrane fluidity. Juvenile yellowtail flounder were fed standard ICES diets containing varying levels of n-3 HUFA ranging between 0.4 and 2.5%, and a commercial diet. Juvenile fish (0+) weighing 1.92 ± 0.50 g were fed for 12 weeks in triplicate treatments. Experimental diets, fish liver, and muscle were analysed for proximate and fatty acid composition. Although no mortalities were observed, fish fed the lower n-3 HUFA levels showed significantly lower growth than those fed the commercial diet and 2.5% HUFA diet. Significant trends were also found in the biochemical compositions of the fish in the various treatments. Fish fed the 0.4% n-3 HUFA diet had the highest levels of lipid in the liver and the lowest levels in the muscle; however, hepatosomatic indices showed no significant differences among treatments. Triacylglycerol levels were highest in the livers and lowest in the muscle of the fish fed the diet lowest in n-3 HUFA and lipid transport may have been impaired in these fish, indicating an essential fatty acid deficiency. Fatty acid composition in both liver and muscle was affected by diet. Fish fed the 0.4% diet preferentially conserved the n-3 HUFA in the polar lipid fraction. Results suggest that juvenile yellowtail flounder require approximately 2.5% n-3 HUFA in their diets on a dry weight basis.

Introduction

Yellowtail flounder, a small right-eyed flounder from the family Pleuronectidae,[4] is a desirable commercial species because of its high fillet-to-body ratio. However, stock numbers on the Grand Banks off Newfoundland have been steadily decreasing since the 1970s.[5] Research into the culture of yellowtail flounder (*Pleuronectes ferrugineus*) has successfully produced increasing numbers of juveniles yearly, but production is limited by the lack of knowledge on the nutritional requirements of this species.

Lipids provide energy and essential fatty acids (EFAs) in the diet and are therefore of particular interest to aquaculturists. EFAs are required by the fish for normal physiological functioning and include three highly unsaturated fatty acids, DHA, EPA and AA.[6] Most studies have been done on warmer water fish, and these have shown requirements of 0.5 to 2.0% n-3 HUFA in dry diets.[7] Cold-water fish require higher levels of n-3 HUFA to maintain fluid membranes[8] and it is unknown whether commercial diets satisfy this requirement. It is also unknown whether fish fed these diets will develop fatty acid deficiencies, which include high mortality, high lipid levels in the liver, "shock syndrome", fin erosion and poor growth.[6]

This study looked at the effects of varying levels of n-3 HUFA on growth and body composition of juvenile yellowtail flounder and compared these to perfromance of fish fed a commercial diet.

Materials and Methods

One hundred and eighty 0+ yellowtail flounder with an average weight of 1.92 ± 0.5 g were reared at the Ocean Sciences Centre in Logy Bay, Newfoundland. Fish were randomly assigned to 12 tanks. Triplicate tanks of fish were fed on of three ICES standard-formulated diets, with differing levels of n-3 HUFA (0.4%, 1% and 2.5%) and a commercial diet (Kyowa Hakko Kogyo Co. Ltd., supplier). All diets had a total lipid content of approximately 10 to 12%, protein levels of 55 to 58%, and were isocaloric at 5Kcal/g. Fish were fed three times daily every second day at 4% body weight. Fish were weighed and measured biweekly and were sampled initially and at the end of the experiment for proximate analysis, lipid analysis, and hepatosomatic indices. For lipid analysis, only the muscle and livers were examined. Water temperature was maintained at 7.3 ± 0.01°C during the12 weeks of the experiment.

Statistical analyses were performed using Minitab [9] with significance at 0.05. Differences between growth were assessed using ANCOVA with time as the covariant. Biochemical differences were determined using ANOVA and Tukey's tests were used to examine specific differences.

Results and Discussion

Over the course of the experiment, there were no mortalities and no significant differences between whole-body carcass proximate analysis or hepatosomatic indices. However, growth differences (both wet weight and standard length) became obvious at 8 weeks into the experiment. Fish fed both the 0.4% and 1% n-3 HUFA diets grew significantly slower ($P<0.05$) than fish fed the 2.5% n-3 HUFA diet or the commercial diet.

Lipid analyses showed that fish fed the lower n-3 HUFA diets had a higher amount of lipid in the liver and lower amounts in the muscle than fish fed diets containing higher levels of n-3 HUFA, although the difference was not significant. When the lipd classes were examined, fish fed the 0.4% and 1% n-3 HUFA diets had significantly ($P<0.05$) higher levels of triacylglycerol in their livers. It is likely that these fish had difficulty transporting lipids out of the livers and into the muscle, causing an accumulation of depot lipid, as has been seen in other EFA-deficient animals.[10]

Differences were also found in fatty acid levels of the tissues of fish fed the different diets. Increasing the dietary n-3 HUFA level from 0.4 to 1% did not greatly increase levels of n-3 HUFA in the tissues of the fish, but an increase to 2.5% HUFA significantly elevated levels of EFAs in the neutral and polar faction of the liver and the neutral lipid of the muscle. However, there were no differences observed in the polar lipid of the muscle in fish fed different diets ($P<0.05$). Although fish fed the 0.4 and 1% HUFA were beginning to show some signs of deficiency at the end of the experiment, they may preferentially conserve the n-3 HUFA in the membrane portion of the muscle. This is important to cold-water juvenile fish and yellowtail flounder seem to be able to retain n-3 HUFA for fairly long periods of time.

In summary, juvenile yellowtail flounder require 2.5% n-3 HUFA in their diet for optimal growth at temperatures below 10°C. Fish fed lower levels of n-3 HUFA may develop EFA deficiency signs, including poor growth and fatty livers.

The authors wish to thank the Canadian Centre for Fisheries Innovation and Fishery Products International for funding of this project as well as the technical staff at the Ocean Sciences Centre, especially Danny Boyce, Sue Budge, Laura Halfyard, Sharon Kennedy and Jeanette Wells.

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Designing rearing environments for on-growing of juvenile yellowtail flounder (*Pleuronectes ferrugineus*)

D. L. Boyce,[1] *C. F. Purchase,*[1,2]
V. Puvanendran,[1,2] *and J. A. Brown* [1]

Research on new aquaculture species requires focus on several areas, including broodstock management, egg incubation, larval rearing, on-growing of juveniles, and marketing. On-growing of juveniles may involve examination of endogenous factors, determination of optimal environments, feeding requirements, and disease prevention. Research into yellowtail flounder aquaculture has been conducted at Memorial University of Newfoundland's Ocean Sciences Centre for several years. One of the main areas of focus has been the environmental factors affecting growth and survival of juveniles. Some of the most important of these are water quality, temperature, lighting conditions, ration, and stocking densities. Presented here are summarized results of several experiments, current growth rates of hatchery-reared yellowtail flounder, and areas for future research.

Introduction

The yellowtail flounder (*Pleuronectes ferrugineus*, formerly *Limanda ferruginea* Storer) is a relatively small pleuronectid that occurs along the east coast of North America.[3] Research has commenced to evaluate its potential as a cold-water aquaculture species along the east coast of Canada.

The development of a new aquaculture species requires research into several areas, including broodstock management, egg incubation, larval rearing, juvenile on-growing, and marketing. Research into on-growing of juveniles focuses on endogenous factors, such as performance differences between the sexes, disease prevention, and the determination of optimal rearing environments.

Environmental factors play a large role in determining the growth of fishes.[4] Some of the most important are lighting conditions, stocking densities, ration, temperature, and water quality. Presented here are summarized results of several experiments examining the effect of environmental factors on growth and survival of juvenile (0-group) yellowtail flounder.

Materials and Methods

Yellowtail flounder oocytes and sperm were obtained from captive broodstock held at Memorial University of Newfoundland's Ocean Sciences Centre in Logy Bay. Incubation and larval rearing followed standard Ocean Sciences Centre protocols. Juveniles were maintained on an artificial diet and 18 h light:6 h dark (18L:6D) photoperiod prior to the start of experiments.

Growth and survival were compared in: experiment one — four photoperiods (12L:12D, 18L:6D, 24L:0D, and ambient);[5] experiment two — three stocking densities (30%, 60%, and 120% bottom cover); and experiment three — four feed rations (1%, 1.5%, 2%, and 3% body weight per day). In all experiments, random samples were taken every three or four weeks for growth measurements. For each sampled fish, wet weight (0.01 g) and standard length (0.1 cm) were measured.

Results and Discussion

No substantial differences in the growth of juvenile yellowtail flounder reared under the different photoperiods,[5] stocking densities, or feed rations were observed. Survival was high (> 95%) in all experiments.

It seems 0-group juvenile yellowtail flounder can be reared under a short photoperiod (≤12L:12D) without adverse affects on growth.[5] Photoperiods of shorter duration, and the effect of photoperiod on maturation, are still to be examined. Stocking densities of 120%

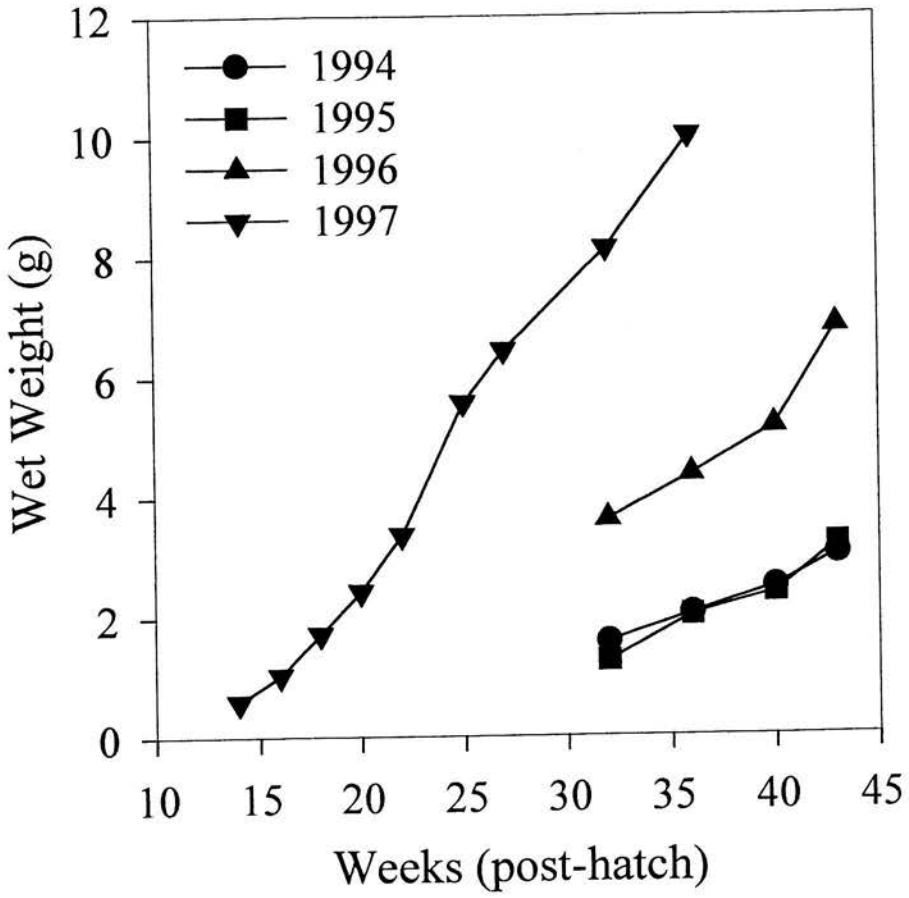

Figure 1. Average wet weight (g) at age of yellowtail flounder reared at the Ocean Sciences Centre between 1993 and 1997.

bottom coverage showed no negative effects on growth. A feed ration of 1% body weight per day did not negatively affect growth in juvenile yellowtail flounder. Therefore, a ration of this amount or lower (yet to be determined) could be used to obtain the highest gain in weight per amount of food given.

A combination of improvements in feeding strategies, tank size, and temperature control have led to improved growth rates of hatchery-reared yellowtail flounder over the past four years (Fig. 1). Further research examining environmental factors affecting growth and survival on juvenile yellowtail flounder is still needed before rearing protocols can be developed. Some areas of consideration are light intensity, light quality (spectrum), temperature, salinity, ammonia levels, and dissolved oxygen concentration.

The authors thank Fishery Products International and the Canadian Centre for Fisheries Innovation for funding this research, and the staff at the Ocean Sciences Centre for their help in conducting these experiments.

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Frequency of feeding in juvenile yellowtail flounder (*Pleuronectes ferrugineus*): possible regimes for grow-out

K. S. Whalen,[1] *J. A. Brown,*[1] *C. C. Parrish,*[1]
S. P. Lall[2] *and J. S. Goddard*[3]

Interest in the culture of small flounder has increased in recent years. Research at the Ocean Sciences Centre has focused on egg production and larval rearing of yellowtail flounder (*Pleuronectes ferrugineus*) with large numbers of juveniles produced yearly. Protocols for grow-out of juveniles have not yet been developed and little is known of the nutritient requirements and feeding of this specie. Experiments were designed to determine the best feeding regimes for optimal growth and food conversion for 0+ fish. Feeding frequencies of four times daily, twice daily, once daily, and twice every other day were used. The fish were fed to satiation by hand and measured biweekly for a period of 10 weeks, at which time the average weight of the fish had doubled. There were no significant differences in growth between treatments at the end of the experiment, indicating that fish fed at the lower frequencies were hyperphagic and displayed compensatory growth. Meal size was higher in fish fed less frequently; however, the total consumption was higher in fish fed at higher frequencies. Behavioural observations also showed that ingestion rate was higher and capture success lower in fish fed less frequently. Results suggest that to obtain high specific growth rates and low food conversion ratios in juvenile yellowtail flounder, feeding twice daily is optimal, but total growth is not affected by restricted feeding regimes.

Introduction

Feeding frequency is an important factor in optimal growth and food conversion efficiency in fish. In addition, the correct feeding regime minimizes food wastage, improves water quality, and thereby decreases costs. The number of times a day fish are fed is influenced by size, age, species, environmental factors and food quality.[4]

In catfish, groups of fish fed twice daily grew faster and used food more efficiently than fish fed 24 times a day[5] whereas optimum feeding frequency for juvenile spotted grouper is between four to six times daily.[6] Cold-water marine fish, however, usually have a large storage capacity and return of appetite takes longer than in other species. For example, Jobling[7] found that plaice fed once every other day developed hyperphagic guts and displayed compensatory growth, which is faster than normal growth.

Yellowtail flounder (*Pleuronectes ferrugineus*), a small right-eyed pleuronectid, is a promising candidate for aquaculture in this province but little is known of its feeding habits at the juvenile stage. It is therefore important that feeding frequency be examined for the efficient development of grow-out protocols.

Materials and Methods

One hundred and eighty 0+ juveniles (6.8 ± 0.19 g) were reared at the Ocean Sciences Centre in Logy Bay, Newfoundland, and were randomly placed in twelve 13-liter containers. Four feeding schedules were assigned in triplicate: four times daily (9:00 am, 12:00 noon, 3:00 pm and 6:00 pm), twice daily (9:00 am and 3:00 pm), once daily (9:00 am) and twice every other day (9:00 am and 3:00 pm). Fish were hand-fed a

commercial diet (Kyowa Hakko Kogyo Co. Ltd., supplier), containing 55% protein, 10% lipid, 17% ash and with an energy content of 5 kcal/g according to the supplier's specifications. Fish were fed to satiation and tanks were siphoned daily before feeding. Food was weighed before and after every meal. Fish were measured and weighed biweekly and ambient water temperatures were used for the 10-week duration of the experiment (5.2 to 10.7°C). Behavioural observations began about 6 weeks into the experiment and consisted of observing 5 fish at each feeding for a period of one minute and recording attempts at eating and ingesting pellets.

Statistical analyses were performed using Minitab [8] with significance at 0.05. Wet weight and standard length over time were analysed using an ANCOVA with time as the covariant; other differences were tested using ANOVA.

Results and Discussion

After a 10-week period, fish had almost doubled in weight and there were no significant differences in wet weight ($P = 0.97$) or standard length ($P = 0.90$) between treatments. Fish fed twice daily were only slightly larger than the other treatments at the end of the experiment. Meal size was significantly larger ($P<0.001$) in fish fed the two lowest feeding frequencies but overall consumption was higher ($P=0.02$) in fish fed at the highest frequencies. Larger meal size (hyperphagia) but lower total food consumption in fish fed at the lower frequencies implied that lack of growth differences were possibly due to compensatory growth; this is, fish were able to "catch up" with the fish on other feeding regimes.

Relatively good growth rates and food conversion efficiencies were obtained in fish from all treatments which suggests that fish were not negatively affected by the restricted feeding regimes. Specific growth rate was higher ($P = 0.03$) in fish fed at the highest frequencies and the average food conversion ratio was also higher ($P = 0.013$) in fish fed four times daily. Fish fed twice daily grew faster and had a lower food conversion ratio than the other treatments.

Behavioural observations indicate that fish fed four times a day had significantly higher capture success ($P=0.03$) and a lower ingestion rate ($P<0.001$) than other groups.

Conclusions

Feeding four times daily did not result in increased growth or lower food conversion ratios. Because there were no significant differences in the size of the fish at the end of the experiment, feeding juvenile yellowtail flounder once daily or twice every other day is sufficient for grow-out. These are also the least labour-intensive and cost-effective feeding regimes.

However, the highest specific growth rate and lowest food conversion ratios were obtained by fish fed twice daily. Therefore it is recommended that 0+ juvenile flounder be fed twice daily.

The authors wish to thank the Canadian Centre for Fisheries Innovation and Fishery Products International for funding on this project as well as the staff and support at the Ocean Sciences Centre. Thanks also to Grant Dwyer for assistance in preparing the poster for presentation at Aquaculture Canada 98.

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Effects of temperature and salinity on fertilization of halibut (*Hippoglossus hippoglossus* L.) eggs

D. J. Martin-Robichaud and M. A. Rommens [1]

The objective of this study was to determine whether the temperature and salinity of the water used during artificial fertilization of halibut eggs affect fertilization success and egg viability. Halibut eggs from 5 egg batches were fertilized in water of various temperature and salinity combinations (2, 4, 6 and 8°C; 27, 30, 33 and 36 ppt). There were significant differences in egg viability among batches of eggs, but temperature and salinity did not affect viability. Fertilization success was significantly different among batches and was also affected by temperature. Temperatures of 6 and 8°C resulted in the highest fertilization success, although the difference was not significant for all batches. Salinity did not significantly affect egg viability or fertilization success.

Introduction

Halibut (*Hippoglossus hippoglossus*) is a marine fish being considered as an alternative species for aquaculture in Canada. A dependable supply of seed stock is crucial for commercialization to be a success. Broodstock fish are generally limited in number so that maximum yield of fertilized eggs from those that are spawned is required. Halibut eggs are fertilized artificially after collecting gametes from the broodstock. The eggs and milt are mixed together in water to activate the milt and allow hardening of the eggs. Local sea water, which may vary in temperature and salinity during the course of the spawning season and with the location of the facility, is often used for this process. The purpose of this experiment was to determine whether the temperature and salinity of the water used for fertilization affect the viability and fertilization rate of halibut eggs.

Materials and Methods

Milt and eggs were stripped from halibut broodstock housed at the Biological Station, Fisheries and Oceans Canada, St. Andrews, N.B. The eggs were collected at the predicted time of ovulation which was determined from monitoring the female's ovulatory cycle.

Milt (0.2 mL) and eggs (20 mL) were gently mixed together in a randomly selected beaker containing 100 mL of sea water. The sea water was at one of 16 different combinations of temperature and salinity. Intended temperatures were 2, 4, 6 and 8°C. Temperatures were obtained by immersing the beakers containing the fertilization water in water baths. Actual average temperatures with standard deviations were 2.2 ± 0.6°C, 3.7 ± 0.5°C, 5.9 ± 0.5°C and 7.8 ± 0.5°C. Tested salinities were 27, 30, 33 and 36 ppt, achieved by mixing ambient sea water with distilled water or a brine solution made from Instant Ocean™. Each temperature was combined with each salinity to give a total of 16 treatments in duplicate. Fertilization trials were conducted with egg batches from five females.

Eggs and milt remained in the fertilization water in the temperature baths for a minimum of 15 min. The fertilization water was then decanted from the eggs and the eggs were poured into 1-L beakers with fresh 33 ppt sea water. The eggs were incubated in darkness at 5°C for at least 16 hours. After incubation, the water was decanted, the eggs were mixed well, and a subsample of eggs were fixed in Stockard's solution[2] until the proportions of viable and fertilized eggs were determined.

For determination of viability and fertilization estimates, eggs were examined using a dissecting microscope (16x). At least 200 eggs were counted and classified as either fertilized, unfertilized or dead. Fertilized eggs were defined by the presence of dividing cells. Unfertilized eggs were either completely

clear without inclusions or clear with only the germinal disk evident. Dead eggs were characterized by the presence of opaque spots of coagulated yolk. The proportion of viable eggs was calculated by dividing the number of fertilized eggs plus the number of unfertilized eggs by the total number of eggs. The proportion of fertilized eggs was calculated by dividing the number of fertilized eggs by the total number of eggs counted.

Statistical analysis was done using SPSS-X (vers. 4). Proportions of fertilized and viable eggs were transformed with an arcsin transformation prior to analysis.[3] Multi-factor analyses of variance (ANOVAs) were run on transformed proportions of viable and fertilized eggs. When factors were not deemed significant they were excluded and the ANOVAs recalculated. Where significant effects were indicated by the ANOVA, multiple range testing was executed using Tukey's honest significant difference method. A P-value of less than 0.05 was required for the results to be considered significant.

Results

Viability was not significantly affected by either temperature or salinity. Batch was the only factor that significantly contributed to variability ($P<0.001$) in egg viability. Viability rates ranged from 89 to 100% for all batches (Fig. 1).

Fertilization rates ranged between 72 and 98% for the batches tested. The greatest difference in fertilization rates occurred between batches ($P<0.001$) (Fig. 1). Temperature also had a significant effect on fertilization success ($P<0.001$). However, a posteriori testing that included all batches together obscured the effect of temperature so that multiple range testing for temperature was conducted separately for each batch. In general, fertilization rates were best at 6 and 8°C although the differences were only significant in three batches (Fig. 1). Salinity had an insignificant effect overall ($P>0.05$).

Egg viability and fertilization success were positively correlated ($r^2=0.61$, $0.001<P<0.01$).

Discussion

Viable eggs include the proportion of eggs that are alive at fertilization but do not become activated by sperm to begin cell division. In this experiment, viability was unaffected by temperature and salinity but

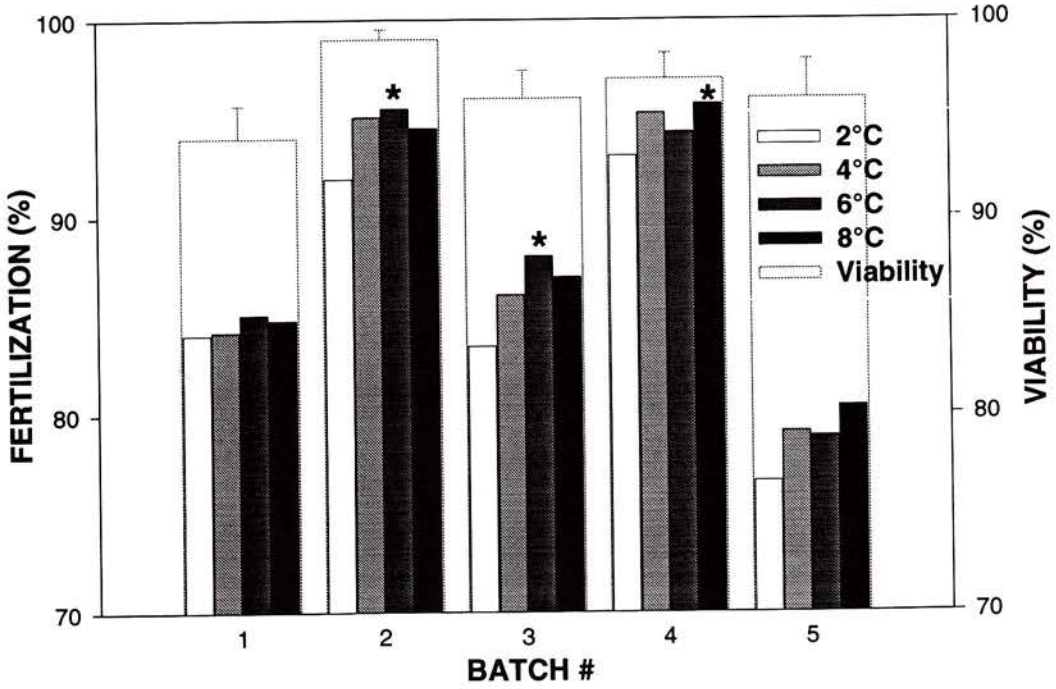

Figure 1. Viability of five batches of eggs and percent fertilization of eggs fertilized at seawater temperatures of 2, 4, 6, or 8°C. Bars on the viability means represent one standard deviation from the mean. Asterisks over groups of eggs fertilizated at different temperatures indicate there is significant difference in fertilization success in that group of eggs compared with others in the batch (Tukey's HSD, $P < 0.05$).

varied between batches. The differences between batches may be due to the numerous factors that affect egg quality in fish. These range from health and nutrition of individual females and males to timing of gamete stripping in relation to ovulation. Numerous reviews are available on this topic.[4,5] As expected, fertilization success was positively correlated with viability.

The effect of temperature on fertilization success was statistically significant but not practically significant. The greatest difference in fertilization rates between temperatures was only 5% and was not consistent between batches. No common trend was apparent for salinity. More obvious and consistent trends in fertilization success would be necessary to change the protocols being used for fertilization of halibut eggs. Currently, eggs are fertilized in water that is from the same source as the water used for holding the broodstock. This seawater, which is in the range of salinities and temperatures tested, is an appropriate medium for ensuring high fertilization rates.

We acknowledge financial support from the Canada/New Brunswick Cooperative Agreement for Aquaculture Development of Non-Traditional Species.

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Marker assisted selection for Arctic char (*Salvelinus alpinus*) broodstock development

A. D. Johansen,[1,3] G. Wilton,[2] and W. S. Davidson[1]

Arctic char (*Salvelinus alpinus*) is regarded as an excellent candidate for aquaculture. This species grows well at low water temperatures and high stocking densities, and secures a higher price than other commercially-grown salmonids. However, the size variability exhibited by this species inhibits large scale production. The fish used in this study were the Fraser River strain, originally collected from the Fraser River, Labrador, in 1984 under the supervision of Brian Glebe of the Huntsman Marine Science Centre, St. Andrews, New Brunswick. The fish were held at Daniel's Harbour Hatchery where 15 families were produced in the autumn of 1995. The fish were grown under identical conditions for 14 months at which point they were weighed. A 10-fold difference in weight between the largest and smallest was found. DNA was extracted from tissue taken from the largest 5% of the fish and the smallest 5%. This DNA was screened using 74 primer pairs designed for amplification of microsatellite loci in other salmonid species to determine if there is a microsatellite locus which is related to growth rate. Of these primers, 33 resulted in a monomorphic product, 10 resulted in a polymorphic product, 14 resulted in no product, and 17 are still being analyzed. Considering the small number of fish from which the Fraser River strain originated, this result is not unexpected. Of the 10 variable loci, a significant difference between small and large fish was detected at four of the microsatellite loci.

Introduction

In order for the culture of Arctic char to be economically viable undesirable characteristics such as the size variability exhibited must be minimised. Studies into the growth rate of Arctic char have indicated that some individuals may never grow to harvest size and these "runts" may be genetically programmed for slow growth.[4,5]

Microsatellites are areas of repetitive DNA with repeat units 1 to 4 base pairs (bp) in length and an average length of 20 to 300 bp.[6] A microsatellite may be linked to a genetic locus affecting growth. If an allele is found to be positively correlated with growth it is probably linked to such a gene and therefore by selecting this allele there is an indirect selection for increased growth. This study had two objectives: 1) to test primers designed to amplify microsatellites in other salmonid species to see if they also amplify a homologous site in the Arctic char genome and 2) to determine if there is a correlation between certain alleles and growth rate.

Materials and Methods

The fish used in this study originated from the Fraser River, Labrador. The fish were held at Daniel's Harbour Hatchery where 15 families were produced in the autumn of 1995 by crossing each of 5 males with 3 females. The fish were held under identical conditions for 14 months at which point they were weighed. The largest 5% had part of their tails removed and the smallest 5% were killed and stored frozen. DNA was extracted according to Taggart et al.[7] The samples were then screened using 74 primers designed for amplification of microsatellite loci in other salmonid species. The following PCR conditions were used: Initial denaturation for 3 minutes at 94°C followed by 35 cycles of 45 sec at 94°C, 45 sec at the primer specific annealing temperature and 30 sec at 72°C. The resulting products were separated using electrophoresis on a 6% polyacrylamide sequencing gel run at a constant power of 42 watts. The gels were then dried and visualised using autoradiography. The results were analysed using a G-test to determine if there was a significant difference in genotypic distribution and

Table 1. P values (α=0.05) for genotypic and allelic frequencies at polymorphic microsatellite loci in small and large Arctic char.

Microsatellite Locus	P-Value Genotypic Frequency	P-Value Allelic Frequency
SFO 18	0.1072	0.0839
µF 43	0.5074	0.6132
One 18	0.2491	0.3232
as 1.22	0.0562	0.0999
SSLee T47	0.5219	0.5592
Ogo 4	0.0344	0.0167
SSOSL 456	0.0459	0.0083
SFO 23	0.0086	0.0348
µ5.27	0.0366	0.0021

a probability test (Fisher exact test) to determine if there was a significant difference in allelic distribution. The critical P-value (α) was set at 0.05.

Results

Of the 10 polymorphic loci, 4 resulted in a significant difference between large and small fish. The P-values for 9 of these loci are given in Table 1. The tenth primer, SSLEE I84, amplifies two loci which means it is possible for each individual to have from 2 to 4 alleles and for this reason it was not possible to analyse the results at this locus.

Discussion

Forty-three of the microsatellite primers that were tested resulted in a product; of these, 75% resulted in a monomorphic product. These results were not unexpected as the Fraser River Arctic char broodstock originated from a very small number of individuals which has resulted in a large amount of inbreeding over the years. Four of the ten polymorphic loci show a significant difference between small and large fish. There are two ways that these differences may occur; one of the populations (small or large) may have an allele that the other population does not or one of the populations may have a genotype which occurs at a much higher frequency than the other population. Of the four loci, three of them had alleles which occurred in the small fish but not in the large fish. OGO 4, SSOSL 456 and µ5.27 all had one allele that occurred in the small fish and not in the large. SFO 23 had two alleles that occurred in the small and not in the large; as well, fish which were homozygous for allele 7 occurred at a much greater frequency in the large fish as compared to the small.

The next step is to determine if these differences are real or simply an artefact by testing these primers with more individuals. Six unrelated families were made by crossing 6 females with 6 males in the autumn of 1996. The fish were subjected to the same conditions as the 1995 year-class of fish and DNA was extracted from the top and bottom 5%. These individuals will be screened with the same primers to determine if these loci are actually correlated with growth. If these primers again result in a significant difference between small and large then in the fall of 1998 the potential broodstock will be screened at these loci and crosses will be set up using parents with known genotypes.

We would like to thank Daniel's Harbour Hatchery and MUN Department of Biochemistry for technical support and ACERA, CCFI and NSERC strategic grant for financial support. ADJ is the recipient of a graduate fellowship from the Aquaculture Programme at Memorial University

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Effects of temperature change on stress parameters in Atlantic salmon (*Salmo salar*) smolt

John F. Burka, Heather A. Briand, and Cheryl A. Wartman [1]

There is a general assumption that salmon are stressed in aquaculture situations, but no strong evidence for this exists. Information on the effects of environmental stressors, particularly temperature, on fish are limited. The present study was designed to compare hormonal responses in saltwater-acclimated Atlantic salmon smolt subjected to a gradual (1C°/day for 5 days) or acute (5C°/day) temperature change from their ambient conditions to determine whether a stress response was elicited. Plasma concentrations of electrolytes, glucose, cortisol, and T_4 did not change in response to temperature alterations, either gradual or acute. Any changes that occurred (i.e., increased cortisol and glucose concentrations) were likely the result of handling stress. Thus, it appears unlikely that a 5C° temperature change within the "optimal" range would cause stress to Atlantic salmon smolt.

Introduction

Common practices in salmonid aquaculture can subject the animals to stress. Smolt are moved from freshwater lakes to saltwater pens without adaptation to either salinity or temperature; the basic fact of being in a pen prevents fish from moving to "preferred" conditions, especially when temperatures rise in the summer or decrease in winter to those outside the optimal growth range for the strain of salmon being farmed.[2] It has become common to assume that salmon are stressed in aquaculture situations, but no strong evidence for this exists.[3] The present study was designed to compare hormonal responses in saltwater-acclimated Atlantic salmon smolt subjected to a gradual (1C°/day for 5 days) or acute (5C°/day) temperature change from their ambient conditions to determine whether a stress response was elicited.

Methods and Materials

Fish

Salmon smolts were obtained from the Cardigan Fish Hatchery, Prince Edward Island, and acclimated to artificial sea water in the Aquatic Animals Facility at the Atlantic Veterinary College.

Experimental design

At least 2 weeks prior to the experiment, 160 fish were randomly divided into 4 equal sized (1.5 m diameter) tanks. Fish in each tank were tagged and divided into 6 groups of 6 fish each. Three to four days prior to day 0, one group of 6 fish were removed by dip net from each of the 4 tanks. This group of fish was designated "Day 0" for analysis purposes. The fish were anaesthetized using metomidate (5 mg/L), weighed, measured for fork length, and bled via the caudal vein. The plasma was assayed for Na^+, K^+, Cl^-, glucose, cortisol, and T_4.

Protocols for temperature changes were designed as follows: In two tanks (designated as "Acute Treatment"), there was no temperature change until day 4 (Fig. 1). On day 4 the temperature was increased (Experiment 1) or decreased (Experiment 2) 5C° over the next 24 h. In the other two tanks (designated as "Gradual Treatment"), the temperature change began on day 0 and was increased or decreased by 1C° per day (Fig. 1). Fish were sampled at the same time each day from all sets of tanks, as noted above.

Data analysis

1. *Comparison of Day 0 Gradual and Acute Treatments:* All variables were compared using an independent samples *t*-test.
2. *Examination of the Trend from Day 0 to Day 4 and Comparison of Day 0 to Day 5 for Gradual and Acute Treatments:* All variables were examined using generalized linear models. Significant test group effects (gradual vs. acute) were compared using an independent samples *t*-test. Significant time effects were compared using appropriate multiple-range tests.

The level of confidence for all statistical tests was accepted as 5%.

Results

The fish in each tank followed a normal distribution curve in length and weight which did not differ between tanks. There were also no differences between test tanks and between control tanks, thus indicating no tank effect. Therefore results from paired control and test tanks, respectively, could be combined for analytical purposes.

Experiment 1:Temperature increase from 10°C to 15°C. Plasma concentrations of Na^+, K^+, Cl^-, and glucose were similar at day 0 for both the gradual and acute groups of fish and were within the "normal" reference range values for Atlantic salmon in seawater (standards developed by Diagnostic Services, Atlantic Veterinary College) (Table 1A).

Cortisol concentrations at day 0 (Gradual: 168.42 ± 98.30 nmol/L; acute: 172.92 ± 60.14) were somewhat above concentrations considered "normal" for resting salmonids (115 nmol/L)[4] and remained elevated throughout the experimental period, which may indicate that the animals were experiencing some acute stress. In contrast, T_4 concentrations were similar in the two groups in both experiments and comparable to T_4 concentrations obtained by other laboratories in Atlantic salmon smolts acclimated to seawater.[5]

The acute temperature change between days 4 and 5 did not appear to have any effect on plasma cortisol, glucose, or electrolyte concentrations. There was a statistically significant decrease in T_4 concentrations between days 4 and 5 (3.65 ± 1.77 nmol/L to 1.44 ± 0.98 nmol/L) in response to the 5°C temperature increase.

Experiment 2: Temperature decrease from 10°C to 5°C. Plasma concentrations of Na^+, Cl^-, and glucose were similar for both the gradual and acute groups of fish and were within the "normal" reference range values for Atlantic salmon in sea water (Table 1B). They stayed within this range throughout the experiment. Interestingly, K^+ levels on day 0 were high: 6.21 ± 3.50 mmol/L for the gradual group and 3.69 ± 1.94 mmol/L for the acute group ("normal" values: 0.60 to 2.90 mmol/L). These values dropped into the normal range by day 1 (acute) and day 2 (gradual) and remained "normal" for the remainder of the experiment.

Cortisol levels were reasonably low throughout the experiment. It was noted that the cortisol concentrations from the gradual group from day 0 to day 4 (i.e., 1C°/day temperature change) were significantly higher than those from the acute group (i.e., no temperature change during this period). The T_4 concentrations were all within "normal" range and there were no significant changes between the groups.

The acute temperature change from 10°C to 5°C between days 4 and 5 did not appear to have any effect on the parameters examined.

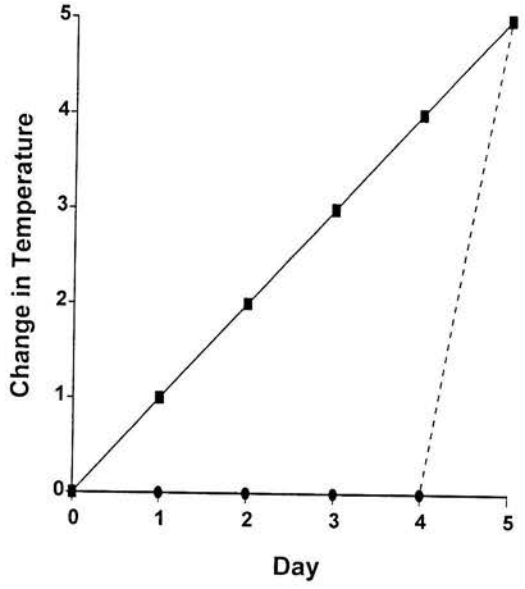

Figure 1. Temperature changes of the "Gradual" and "Acute" groups of fish. "Gradual" group (■): temperature change began on day 0 and increased or decreased by 1C°/day. "Acute" group (●): temperature retained at 10°C until day 4, at which point temperature increased or decreased by 5C° over the next 24 h.

Discussion

Various studies have indicated that temperature changes affect various enzyme and receptor mediated reactions *in vitro* which, in turn, influence physiological responsiveness *in vivo*.[6,7] Thus, physiological processes, particularly metabolic rates, are dependent on the ambient temperature. However, studies on the role of temperature and temperature change in inducing stress in fish have not been conclusive. Experiments have also been carried out to determine the thermal tolerance for Atlantic salmon by subjecting fish to a temperature gradient. [8] These authors studied stress behaviourally and avoided any handling stress. The water temperature was gradually changed within the tanks, as in our study. They noted that the critical thermal maximum for salmon parr was not affected by acclimation temperature,[8] which suggests that the fish were not

Table 1. Plasma concentrations of cortisol, T_4, Na^+, K^+, Cl^-, and glucose in response to increasing (A) or decreasing (B) temperatures.

A. Increasing temperature from 10°C to 15°C

	Gradual Temperature Change		
	Day 0	**Day 4**	**Day 5**
Cortisol (nmol/L)	168.42 ± 98.30	139.58 ± 108.43	147.22 ± 74.91
T_4 (nmol/L)	5.61 ± 2.06	2.24 ± 0.95	2.91 ± 1.97
Na^+ (mmol/L)	161.42 ± 3.42	165.08 ± 2.39	171.72 ± 5.04
K^+ (mmol/L)	2.33 ± 0.54	1.69 ± 1.10	1.43 ± 0.74
Cl^- (mmol/L)	132.08 ± 2.61	135.58 ± 3.45	135.56 ± 6.92
Glucose (mmol/L)	4.20 ± 0.88	5.31 ± 1.15	6.19 ± 1.21
	Acute Temperature Change		
	Day 0	**Day 4**	**Day 5**
Cortisol (nmol/L)	172.92 ± 60.14	203.92 ± 122.68	160.17 ± 85.14
T_4 (nmol/L)	5.68 ± 2.71	3.65 ± 1.77	1.44 ± 0.98
Na^+ (mmol/L)	164.75 ± 4.18	162.08 ± 2.84	167.50 ± 3.94
K^+ (mmol/L)	1.97 ± 0.91	1.48 ± 0.95	1.39 ± 0.54
Cl^- (mmol/L)	133.00 ± 5.15	134.92 ± 3.40	134.61 ± 4.29
Glucose (mmol/L)	4.71 ± 0.78	5.38 ± 0.86	5.99 ± 1.20

B. Decreasing temperature from 10° to 5°C

	Gradual Temperature Change		
	Day 0	**Day 4**	**Day 5**
Cortisol (nmol/L)	36.00 ± 18.60	66.83 ± 51.33	29.08 ± 31.40
T_4 (nmol/L)	4.09 ± 2.12	8.05 ± 2.69	6.82 ± 3.65
Na^+ (mmol/L)	152.55 ± 5.57	162.08 ± 4.60	156.50 ± 6.75
K^+ (mmol/L)	6.21 ± 3.50	1.90 ± 0.43	2.61 ± 0.26
Cl^- (mmol/L)	131.00 ± 3.84	139.17 ± 6.21	133.58 ± 5.33
Glucose (mmol/L)	3.92 ± 0.64	4.87 ± 1.10	4.52 ± 0.82
	Acute Temperature Change		
	Day 0	**Day 4**	**Day 5**
Cortisol (nmol/L)	25.08 ± 6.52	20.36 ± 10.93	41.58 ± 28.21
T_4 (nmol/L)	3.40 ± 1.61	4.81 ± 1.79	4.72 ± 4.05
Na^+ (mmol/L)	156.25 ± 4.49	160.17 ± 3.16	154.83 ± 6.07
K^+ (mmol/L)	3.69 ± 1.94	2.78 ± 0.43	2.63 ± 0.43
Cl^- (mmol/L)	132.75 ± 6.11	135.92 ± 2.43	125.25 ± 5.61
Glucose (mmol/L)	4.04 ± 0.66	4.07 ± 0.92	4.97 ± 0.92

under initial stress due to the acclimation temperature.

Plasma concentrations of electrolytes, glucose, cortisol, and T_4 did not change in response to temperature alterations, either gradual or acute. Any changes that occurred (i.e., particularly increased cortisol concentrations) were likely the result of handling stress. The increased plasma potassium concentrations in the day 0 fish for both groups (acute and gradual) of fish where the temperature was to be decreased was somewhat surprising. No physical trauma that could have damaged cells was observed in these fish. However, plasma potassium concentrations were within "normal" reference range for subsequent days, precluding any need for further investigation.

Ideally, it would have been interesting to include an experiment where a sudden change in temperature occurred. However, it was not possible to induce an abrupt change in temperature in our facility without moving fish from one tank to another, which would have induced handling stress. Also, the likelihood of sudden temperature changes in aquaculture situations is low, because water has a high heat capacity. Thus, it appears unlikely that a 5C° temperature change within the "optimal" range, even within a 24-h period, would cause stress to Atlantic salmon smolt.

This research was supported by a grant from NSERC.

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Assuring sustainable salmonid aquaculture in Bay d'Espoir, Newfoundland

Michael F. Tlusty,[1] *M. Robin Anderson*[2] *and Vern A. Pepper*[2]

Net-pen culture under ice cover is a critical component of aquaculture in Newfoundland. However, winter salmonid culture provides several environmental challenges not inherent to summer husbandry. First, overwinter areas tend to be more subject to accumulation of waste products. Second, fish digestion slows, and this may result in increased organic material being deposited on the bottom. These principles will impact future development of aquaculture in Bay d'Espoir. Environmental monitoring of winter sites for the previous 1.5 years revealed little effect of aquaculture on water quality, but a more pronounced benthic effect. More research is needed on overwinter sites to fully understand how the particular site characteristics and increased organic loading impact the environment.

Introduction

The culture and physiology of any species is intimately linked to the environment. Much of the finfish research presented at this conference investigated how the physical (e.g. feed regimes, temperature, photoperiod) or social (crowding) environment influences finfish culture. However, it is equally important to consider the impact of fish physiology and culture practices on the environment. Minimizing aquaculture's impact on the environment is one of the most important keys to assuring the long-term sustainability of this industry.[3]

Bay d'Espoir is a critical location for aquaculture in Newfoundland as approximately 90% of the value of aquaculture product for the province is comprised of the Atlantic salmon (*Salmo salar*) and steelhead (*Oncorhynchus mykiss*) produced there. It is a 250 km^2 estuarine fjord characterized by 11 basins (range of 30 to 200 m deep), divided by 12 sills (range of 10 to 25 m), and a 1 to 3 m deep freshwater surface layer. At about 47°50″ N, and subject to the influence of the Labrador current, this fjord freezes over for roughly four months of the year. The ice cover makes it necessary to place the net cages in protected areas to avoid destruction by ice pan movement. Compared to summer areas, the winter locations tend to be characterized by slower moving water, longer flushing times, and most often are located over naturally accumulating bottoms. This combination of characteristics makes these areas prone to accumulation of aquaculture wastes. In addition, the environmental impact of overwinter aquaculture will be different than that of summer culture since the digestive ability of salmonids at low temperatures decreases. While feeding rate is decreased, a decrease in the digestive efficiency will yield feces with a higher content of organic matter. The long-term environmental impact of deposition of this type of feces remains to be studied.

The total area available for winter culture in Bay d'Espoir is limited to three main areas: Voyce Cove (250,000 m^2), Roti Bay (2,660,000 m^2), and Northwest Cove (500,000 m^2). Voyce Cove has been the most used winter site (in terms of effort and length of time), Roti Bay is intermediate, and Northwest Cove has been used by one farm continually for the past 3 years. This limited area for over wintering cages may constrain future development of aquaculture in Bay d'Espoir. Thus, in January 1997, we initiated a study of the assimilative capacity of overwinter sites in Bay d'Espoir. This research had 3 main components: measurement of water movement through each basin, estimates of water quality parameters, and an assessment of any benthic effects. Because of limited space, this report will focus on the latter two research areas.

Methods and Results

The water quality work consisted of vertically profiling the water column at 30 stations. Discrete samples were taken 1, 3, and 5 m from the surface and from 1 m off the bottom. This sampling collects water from each of the main layers of the water column (the freshwater upper, the transition, the tidal, and the

lower basin water layer). The 30 stations were located so that the quality of water could be compared preceding aquaculture activity in the estuary, in the different basins (each with a different level of aquaculture effort), and with respect to local variation around the cage sites. For each discrete sample, we conducted a maximum of 20 assays for water quality characteristics (chlorophyll-*a*, oxygen [dissolved , saturation, 5d demand], carbon [particulate], nitrogen [ammonium, nitrite, nitrate, particulate], orthophospate, pH, redox potential, salinity, secchi depth, sulfide, tannin, turbidity, and temperature).

Since January of 1997 we conducted over 12,000 assays on more than 2,000 water samples. We did not observe any increased nutrification as a result of aquaculture. All aquaculture sites had similar water quality levels compared to control sites. The only locations that had depressed water quality levels, but still adequate for salmonid production, were areas which had decreased water flow or flushing times (Northwest Cove and the inner basin of Roti Bay). Given no significant aquaculture impact on water quality, we will not discuss the water quality parameters in this report.

The benthic sampling reflected the same effort directed at sampling the water column. We collected sediment cores at 25 stations. Since the bottom beneath the winter cage sites was typically deeper than 25 m, samples were collected by a surface deployment of an Ekman grab sampler. This limited the depth of samples we could collect to a maximum of 12 cm. Through the top of the sampler, we would take a 3-cm diameter piston core, and measure % solids, organic mater (% loss on ignition [500°C for 12 h] of dry matter), and pore water gradients in cm increments for the length of the core. In this discussion, we will focus on only analysis of organic matter in the top 1 cm.

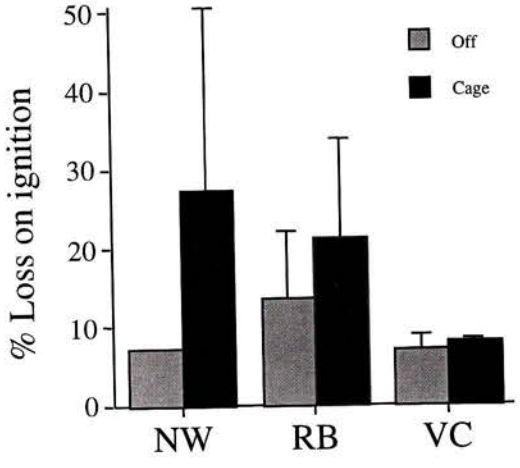

Figure 1. The average % loss on ignition from the top 1 cm of benthic material directly under (cage) and 50 m (off) from winter cage sites in Bay d'Espoir. Error bars are 95% confidence intervals.

The overwinter sites in Bay d'Espoir are typically over naturally accumulating bottoms, and our results confirm this. The percent loss on ignition (% LOI) averages between 10 and 15% for reference sites > 50 m from cages (Fig. 1). This is similar to that of the Kiel Fjord in the Western Baltic.[4] The effect of aquaculture on the benthic environment differed in each location. The apparent impact of aquaculture was inversely proportional to the amount of effort and length of time each area had been utilized. Much of the reason aquaculture had the most pronounced effect in Northwest cove was because this area was not fallowed during the previous two winters. Even though this is the most impacted area we examined, it would still be categorized as a "low impact" site when compared to those in New Brunswick.[5]

The other main point Figure 1 demonstrates is that the amount of benthic organic material is highly variable. Much of this variation is a result of where the net pens are located in the basin. For example, in Roti Bay, we sampled the benthos from under 4 farm sites, and 6 control sites. The lowest amount of organic material was observed at a control site (4%), with the second lowest under one farm (5%). The greatest amount of organic material was found under one of the cage sites (37%), with the second greatest loading in the back basin where there has never been any aquaculture activity (26%). Thus the amount of background natural organic matter will greatly influence the relative amount under the net pens. However, these single values can be misleading. Any small bottom depression can accumulate material and inflate the organic matter estimate. This occurred during repeat sampling of a farm where one sample had a value of 74% LOI. Conversely, 4 of 10 bottom grabs from this farm struck rock, and could not be sampled for organic material.

The amount of organic material reaching the benthos can be a combination of feed, feces, other material (plankton, algae, human derivatives), and resuspended benthic material. In terms of addressing the formation of bottom material, we can secure pure feed or feces, sediment trap material, and finally material from the bottom to see what the net result is (including bacterial metabolic processes). The organic matter content of feed is approximately 90%, that of feces 56%,[6] and the benthic material should be approximately 10% as discussed above. We have not found literature values for amount of organic matter in sediment trap samples, but it should be an average of all inputs.

In our analysis of this process, we measured the amount of organic matter from feed, feces (extracted from the last 5 cm of intestine of lethally-sampled fish), material from sediment traps (1m off the bottom, n=4 traps), and the top 1-cm layer of the benthos from locations in Voyce Cove. The surface water temperature was 6°C, while the temperature at 5 m was 3°C. Our feed was 90% organic matter. To our surprise, the fecal organic matter averaged 87% (n=11, Fig. 2). It appeared that when digestive efficiency decreased in cold temperatures, the fish could not process the feed as effectively as during warmer conditions, and expelled feces with a higher organic matter content (partly due to an incomplete processing of lipids[6]). The organic matter from the sediment traps ranged from 12 to 71% with no difference if the trap was under or > 50 m from a cage. We did not screen the sediment material and this range in values is likely to be influenced by the inclusion of feed or feces. Finally, the organic matter from the benthic samples ranged from 2 to 13% and did not differ if collected beneath or >50 m from a cage site.

Discussion

This research leads to several key questions particularly regarding benthic processes. Primary is the process of benthic material formation and the loss of organic material during this process. What should normal organic matter values be for sediment trap samples, how fast does the organic matter decay, and how does this correlate to organic matter lying on the bottom? The second question which this research raises is what degree of impact does the reduction in dietary efficiency have on the environment? If fish in the winter utilize less ingested feed, they will void fewer metabolites into the water, while excreting more organic material in the feces. Will a mass balance study show an increased rate of carbon deposition on the bottom? This aspect of salmonid physiology makes it less surprising that environmental monitoring of overwinter sites in Bay d'Espoir has shown few effects of aquaculture on the water column, but some impact on the bottom.

While the industry's year 2005 production goal of 9,000 tonnes is attainable based on a water column model using 1.5 years of environmental monitoring, the industry must proceed with caution. The benthic effects we observed may be explained by the fact that fish were reared in areas with low flushing rates or because the areas were not properly managed (overfeeding, digestive inefficiency, and not fallowed). We need to understand the effect of flushing time and fallowing to properly manage the benthic environment of overwinter sites. In addition, the variability of the benthic environment makes it critical that rigorous sampling programs are implemented and followed as a routine aspect of aquaculture activities. The future effort of this project will be directed at evaluating appropriate models of assimilative capacity. We anticipate that the unique winter conditions experienced in Bay d'Espoir may require a data analysis approach custom tailored to this area.

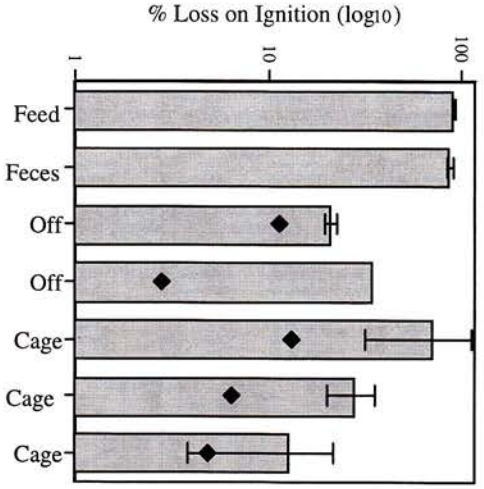

Figure 2. The amount of organic matter (average % loss on ignition at 500°C for 24 h) for feed, feces, samples from sediment traps both 50 m and directly underneath cage sites, and from the top 1 cm layer of the benthos directly under the sediment traps (black diamonds). Error bars are 95% confidence intervals.

This work was funded through the Aquaculture Component of the Economic Renewal Agreement. We thank R Anderson (DFO) who set the foundation over which this research is conducted. C Diamond, R Mercer, and L Snook provided valuable assistance in the laboratory and field. Dr L Hawkins and P Osbourne of the Department of Fisheries and Aquaculture provided access to fish intestines for fecal analysis. P James provided useful comments on a draft of this manuscript.

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Effect of temperature on incubation time and development of Atlantic cod (*Gadus morhua*) eggs and larvae

Miranda Pryor[1] *and J. A. Brown*[2]

Incubation time of Atlantic cod (*Gadus morhua*) eggs was shorter at high (7.0-8.0°C) and medium (3.0-4.0°C) temperatures than at low temperature (0.0-1.0°C). Developmental time was 40% longer at the low temperature. There was no significant difference between dry weights of eggs incubated at these temperatures. There were significant differences in egg diameters between the treatments; diameter increased proportionately with temperature. Larval development was slowest at 0.0-1.0°C; yolksac absorption took nearly twice as long as in the other treatments. Larval growth was significantly different between treatments. Total length was greatest at the lowest temperature both at 50% hatch and at the end of the yolksac stage. Yolksac width at 50% hatch was significantly greater at 3.0-4.0°C. Larval dry weights at 50% hatch and the end of the yolksac stage were not significantly different among the treatments. It appears that while incubation time decreases with increasing temperature, the larvae are smaller which may indicate they are of poorer quality and less able to survive mass rearing conditions. Further study is needed to determine the viability of larvae reared at these temperatures so that proper rearing conditions can be determined.

Introduction

The Atlantic cod has long been a major commercial species for the fishing industry of Newfoundland, but with the recent collapse in fish stocks new avenues for producing cod are needed. Atlantic cod is a promising species for aquaculture with potential for natural stock enhancement procedures as well. However, major constraints limit commercialization of this species, primarily related to mass rearing of fry.[3]

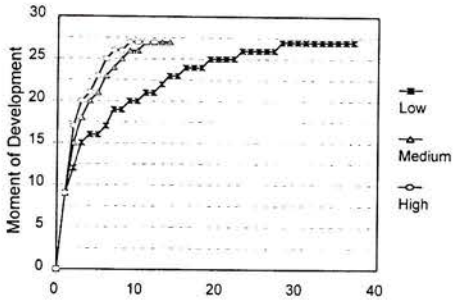
Temperature is an influential factor in marine species,[3] influencing physiological processes by affecting the rate at which biochemical reactions occur and are catalyzed.[4] In yellowtail flounder (*Limanda ferruginea*),[4] and Atlantic halibut (*Hippoglossus hippoglossus*) larvae,[5] increasing temperature generally decreases incubation time and increases mortality rates.[6] The understanding of temperature effects on cod would be of benefit to the aquaculture industry as it might result in a decrease in the time required for larval development. In addition, hatchery workers would have the ability to accelerate or slow development to better use or extend the hatching season for this fish. Therefore, this study was designed to examine the effect of temperature on incubation time and development of cod eggs and larvae.

Materials and Methods

Eggs (3000 mL) were collected from broodstock at the Ocean Sciences Centre on January 29, 1997. The eggs were measured to determine egg diameter (mm) (n = 25), and dry weight (± 0.0001 mg) (n = 50).

Three temperature regimes were used: low (0.0-1.0 °C), medium (3.0-4.0°C) and high (7.0-8.0°C). Two trays (0.3x0.2x0.2 m) were placed in each tank, at each temperature. A monolayer of eggs (300-400 mL) was placed in each tray. Five eggs/tray/day were sampled and stage and moment were recorded.[7] Egg dry weights (n=20) and diameters (n= 25) were recorded at 35 degree-days, halfway to hatch.[3] However, eggs at 0.0-1.0°C hatched before 35 degree-days was reached. Egg dry weights (n=20) and diameters (n=25) were obtained a third time when the eggs in each tray had reached moment 27, the last stage of development prior to hatching. To monitor larval growth, dry weights (± 0.0001 mg) (n=50), total length (mm) (n=30) and yolksac width (mm) (n=30) were measured at 50% hatch for each temperature (Fig. 1). Five larvae/tray/day were sampled to monitor yolksac absorption with larval dry weights (± 0.0001 mg) (n = 50), and total length (mm) (n = 30) taken at the end of the yolksac stage.

(A)



(B)

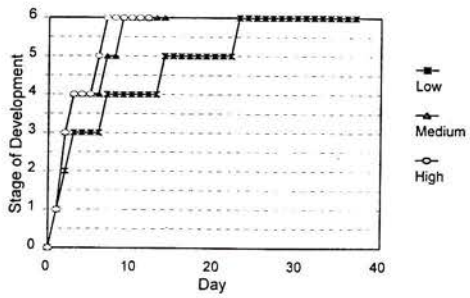


Figure 1. The 27 moment (A) and 6 stage (B) system of development, measured over time, in Atlantic cod eggs under low (0.0-1.0°C), medium (3.0-4.0°C), and high (7.0-8.0°C) temperature treatments.

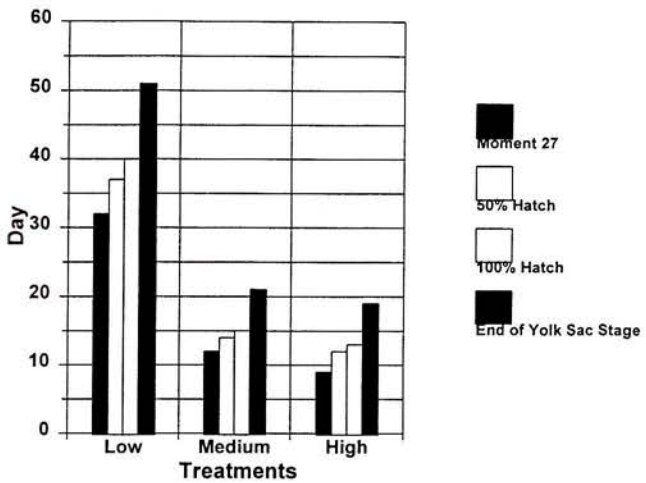


Figure 2. Incubation times (days) for low (0.0-1.0°C), medium (3.0-4.0°C), and high (7.0-8.0°C) temperature treatments to reach moment 27, 50% hatch, 100% hatch, and the end of the yolksac stage in Atlantic cod eggs and larvae.

Results

Egg Development. Little difference was observed between incubation times at the high and medium temperatures. Eggs at the lowest temperature took 43% longer (28 d) to pass from stage 3 (gastrulation) to stage 4 (formation of pre-organs), or 39% longer to reach moment 27, and 9 d, or 3 times longer, to pass from moment 27 to 50% hatching than the other treatments.

To reach 50% hatch, the medium treatment took 58.8 degree-days. The high treatment reached 50% hatch at 96.9 degree-days. Due to problems calculating degree days using zero or negative numbers, temperatures for the low treatment were averaged to give an incubation time to 50% hatch of 37 days at 0.44 ± 0.39°C.

The dry weight of the eggs after fertilization was 0.1222 ± 0.0047 mg. A 1-way ANOVA found no significant difference between dry weight at the three temperatures over time ($F=1.03$, $P = 0.364$, $df = 2$).

Egg diameter after fertilization was 1.46 ± 0.05 mm. Unlike dry weight, a 1-way ANOVA found that egg diameter was significantly different between the temperature treatments over time ($F=15.70$, $P< 0.001$, $df=2$).

Larval Growth and Development. Larvae at the high and medium treatment had the shortest incubation periods (19 and 21 d); both took 6 days for yolksac absorption (Fig. 2). The low treatment, instead of taking 1 d to pass from 50% to 100% hatching, hatched over a 3-d period. Hatching at the coldest temperature began on day 26 and 50% hatching was reached on day 37. Yolksac absorption took 11 d, nearly twice as long as the other treatments (Fig. 2).

In the medium treatment, yolksac width at 50% hatch was significantly different from the other treatments ($F=23.52$, $P<0.001$, $df=2$) (Fig. 3).

Larval lengths increased proportionately for each treatment from 50% hatch to the end of the yolksac stage (Fig. 3). Total larval length was significantly different over time between the treatments ($F=29.90$, $P < 0.001$, $df = 2$). Dry weights at 50% hatch and at the end of the yolksac stage (Fig. 3) were not significantly different ($F=.54$, $P = 0.223$, $df = 2$).

Discussion

Egg Development. In Atlantic cod eggs, incubation temperature affects incubation time[6] and has an impact on larval growth and development. Eggs incubated at 0.0-1.0°C took 40% longer to reach 50% hatch than those at 3.0-4.0°C and 7.0-8.0°C. As stated in Pauly and Pullin,[8] eggs are "driven" by the prevailing temperature to the point of hatch-

ing as it is subject to the laws of thermodynamics and is completely poikilothermic. Cod eggs are no exception; eggs at higher temperatures develop faster, yet development time does not appear to affect growth of the eggs.

In this study, egg diameter was significantly different among the temperature treatments and increased with temperature. There was no significant difference among egg dry weights. One explanation is that larvae with a shorter incubation period (high temperature) absorb less of the egg components prior to hatching. This would also help explain why dry weights remained constant among temperature treatments—eggs at the lowest temperature required a long incubation period, and had a small diameter, but the larvae had more time to develop properly as they were not "driven" to hatch.

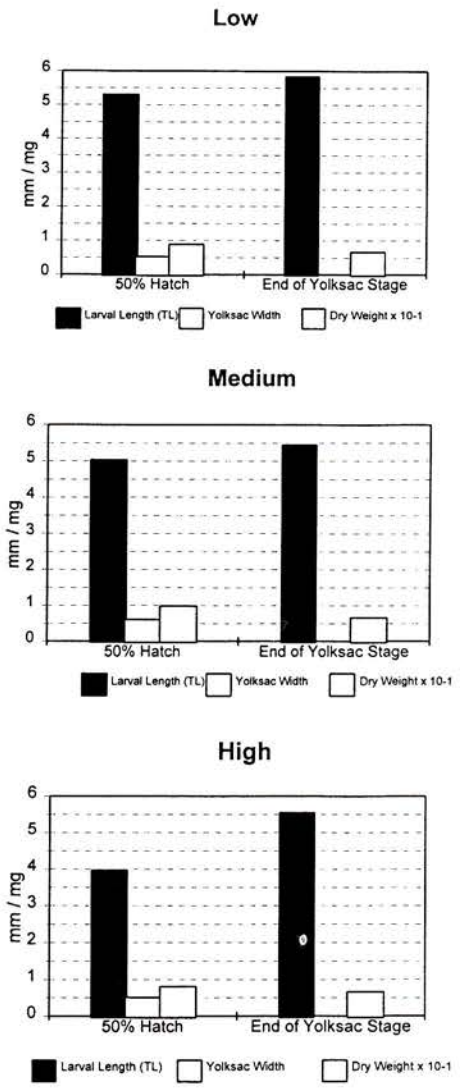


Figure 3. Larval lengths (TL) (mm), yolksac widths (mm) and dry weights (mg) at 50% hatch and larval lengths (TL) (mm) and dry weights (mg) at the end of the yolksac stage for Atlantic cod larvae at the three temperature treatments: low (0.0-1.0°C), medium (3.0-4.0 °C), and high (7.0-8.0°C).

Larval Growth and Development. Development of the larvae was distinctly different between the treatments. Time to yolksac absorption defines the period during which the larva relies on endogenous energy reserves and thus determines the time available for larvae to find suitable food reserves.(6) Larvae at the low temperature took 11 d to absorb their yolksac. This may mean that these larvae were better adjusted to their environment and more successful at food capture in the long run.

The largest diameter eggs occurred at the highest temperature prior to hatching, but these resulted in the smallest larvae, again illustrating the idea that shorter incubation times result in incomplete development. The largest larvae at the lowest temperature may have already absorbed some of their yolksac material prior to hatching and displayed better growth due to a much longer incubation period. This may be advantageous to growers as Pepin(6) found that bigger larvae were less susceptible to variations in environmental factors.

Conclusions

When temperatures are manipulated, cod egg and larval growth are affected and the appropriate incubation temperature varies with the intended use of the cod. For example, temperature may be adjusted to provide a specific size of larvae or hatchery operators can use temperature manipulaton to extend the hatching season. Production of high quality cod eggs is not a constraint due to the high fecundity of cod which spawn naturally in captivity.(3) Rather, problems exist when attempting to satisfy the environmental and feeding requirements of the larvae to overcome heavy mortality during start-feeding. Therefore, if temperature regimes could be identified that would yield larger, healthier larvae then perhaps startfeeding would not be as great a problem.

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Growth, survival, lipid and amino acid composition in striped wolffish, *Anarhichas lupus*, fed commercial marine starter diets

L. C. Halfyard,[1] D. Drover,[2] C. C. Parrish[3] and K. Jauncey[4]

The formulation of marine starter diets to coincide with the live *Artemia*-feeding stage poses nutritional and economical problems. In this study three commercial diets were examined as potential first-feeding diets for striped wolffish, *Anarhichas lupus*. Proximate composition, fatty acids, lipid classes, amino acids and other dietary factors were examined as criteria for selection of a commercial diet for wolffish. Diets fed to the fish over a 60-day period were comparable in terms of DHA:EPA ratios (1:1); however, diet 1 had the lowest phospholipid and taurine content while diets 2 and 3 had higher levels. After 60 days, fish fed diets 2 and 3 were significantly larger in wet weight and length, had better survival rates (diet 1 = 41%, Diet 2 = 49%, diet 3 = 57%) and showed significant differences in threonine, taurine, EPA and DHA.

Introduction

Striped wolffish, *Anarhichas lupus*, is a cold-water marine species with potential for culture. It has a ready market as an excellent white-fleshed fish and is well-developed at hatching, allowing exogenous feeding to begin immediately. However, the formulation of small particle artificial diets which are equivalent in size to live *Artemia* (300-500 µm) is associated with inherent difficulties of particle density, stability, leaching, production costs, and the mechanics of microparticle formation. This study compared three commercially available marine starter diets, as a preliminary test of the suitability of these diets for first-feeding wolffish.

Methods

Eggs were collected from the wild and incubated in a 6°C upwelling Heath tray egg incubation system. Newly hatched fish were stocked in 9 shallow-water green raceways (0.61 m x 0.25 m x 0.05 m). Rearing parameters included: 1.5 L/min water flow, 6 ± 1.5°C water temperature, 33 ± 1‰ salinity, 40 to 80 lux light intensity, and 70 fish/L stocking density. Feeding conditions consisted of simultaneously offering SuperSelco enriched *Artemia* (1000/L) and a dry artificial diet (diet 1, 2, or 3 to excess) during the first 30 days, weaning to day 40 and providing a dry diet from day 40 to 60; 5 to 7 feedings per day were provided and each diet was tested in triplicate. Diets were analyzed for proximate composition, amino acids and lipid profiles. Fish were sampled at 20-day intervals from day 0 to 60: 20 fish per raceway for growth (weight, length), l3 samples of 15 fish per raceway for lipid analysis, and 1 sample of 5 fish per raceway for amino acid analysis. Daily tank cleaning and removal of mortalities allowed a cumulative calculation of survival rates.

Fatty acids were determined using a 2:1 chloroform and methanol extraction, a C17:0 internal standard, derivatization to fatty acid methyl esters with H_2SO_4 and measurement using a GC with a 30 m DB225 (J&W Scientific) column. Lipid classes were determined using an internal standard of 3-hexadecanone and a Mark V Iatroscan. Amino acids were determined using an AEC internal standard, deproteinization, the buffer lithium method and a Beckman 121MB amino acid analyzer, using a Benson D-X8, 25 cation xchange resin and a single column. The amounts of fatty acid and amino acid were calculated based on the organic matter portion of the diets.

Results

At the end of the 60-day trial, growth (wet weight, length) and survival rates were significantly better ($P<0.05$) for fish fed diets 2 and 3. Specific growth

rates were 1.96, 2.46 and 2.23 for diets 1, 2, and 3, respectively.

Proximate composition of the 3 commercial diets showed significant differences ($P<0.05$) in crude protein (diet 1 = 53.1%, diet 2 = 59.6% , diet 3 = 64.5%), crude lipid (diet 1 = 13.1%, diet 2 = 15.8%, diet 3 = 16.5%), and gross energy (diet 1 = 4.8 Kcal/g, diet 2 = 5.1 Kcal/g, diet 3 = 5.1 Kcal/g). Diet 1 contained the lowest phospholipid (PL) and taurine levels, diets 2 and 3 had comparable PL content and diet 3 had the highest taurine levels (Fig. 2).

Total free amino acids (FAA) were not significantly different in either the diets or the fish after 60 days of feeding. However, high levels of threonine were present in diet 1 and in the fish at day 60. Taurine was significantly higher for diet 3.

Analysis of acyl lipids (fatty acid containing lipids) showed that diets 2 and 3 had higher levels than diet 1 but this difference was not evident in the fish at day 60. There was a net increase from day 0 to day 60, but no significant difference was noted between diets 1, 2 or 3 (Fig. 2). Analysis of polyunsaturated fatty acids (PUFA) showed that the three diets were comparable for docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) ratios (1:1), the actual amounts of EPA and total fatty acids. However, after 60 days of feeding, the amounts of EPA and DHA were significantly higher in diet 1 fish.

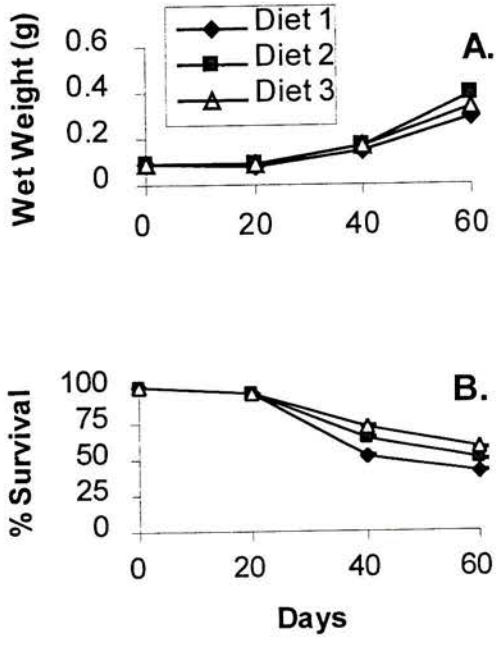

Figure 1. A) Growth and B) survival rates of striped wolffish, for the first 60 days after hatching, fed three commercial marine starter diets. Data are mean ± SE for three samples.

Discussion

Proximate analyses (protein, lipid, ash and carbohydrate) for the three commercial diets tested fell within the ranges recommended for marine finfish.[5] Growth and survival rates were comparable to those of other studies using dry diets for start feeding of wolffish.[6,7] However, subsequent studies extending the feeding time resulted in total mortality by day 80 for diet 1 (unpubl. data).

Previous studies have determined the 10 essential and non-essential amino acids recommended in microparticulate diets for rearing cold-water fish. The three commercial diets tested contained comparable amounts of total FAA and no differences were evident in the fish by day 60. However, essential amino acids as a proportion of the total FAA were significantly higher in diet 1. This supports the suggestion that some control of amino acid catabolism may occur and that the fish may be able to conserve essential amino acids at the expense of non-essential amino acids.[8] One essential amino acid, threonine, was present in significantly higher quantities in diet 1 and in the fish at day 60. It also increased from hatch to day 60 for diet 1 but decreased for diets 2 and 3, suggesting that fish fed diet 1 were conserving this FAA.

Taurine, a non-essential amino acid, showed a similar trend between diet and fish for diet 3. In one study of newly hatched halibut larvae, most of the FAAs decreased steeply and eventually levelled off or declined slowly, but taurine deviated from this pattern by showing no major changes.[9] In this study taurine levels sharply decreased from hatching to day 60 as noted for most FAAs in halibut. However, higher levels of taurine than of any other amino acids were present in diet 3 and this trend was maintained in the fish through to day 60. Fish fed this diet had better growth and survival rates suggesting that taurine may be an important amino acid.

The amount of acyl lipid was lowest in diet 1 but by the end of the 60-day feeding trial there were no significant differences in the fish fed either diet, suggesting again that diet 1 fish were better able to accumulate fatty acid-containing lipids. Several studies have suggested that for the promotion of growth in larval fish PL are superior to triacylglycerols (TAG) and that fish appear to contain a single bile-activated lipase.[10] The percentages of TAG in diets 1, 2, 3 were 70%, 55%, 64% and for PL values were 16%, 32%, 27%, respectively. Although the growth rates were significantly better for fish fed diets 2 and 3, which contained higher levels of PL, by day 60 diet 1 fish had accumulated equal amounts of TAG and PL as the fish fed the other two diets.

The ratio of DHA:EPA, which studies have shown to be ideally about 2:1, was about 1:1 for all 3 diets. But

by the end of the 60-day feeding trial, fish fed diet 1 had higher amounts per unit wet weight and per fish than fish fed diets 2 or 3. Both the acyl lipid and EPA values suggest that the fish fed diet 1 were conserving these fatty acid components. Research has also shown that the DHA:AA (arachidonic acid) ratio and the dietary levels of AA may play an important role in changes in prostaglandin production, which could affect growth and general health of the fish.[11] In this study there were no significant differences in AA levels in the diets, as with DHA and EPA, but differences were evident in the fish at the end of the trial.

Summary

The fish fed Diet 1 appeared to be conserving certain amino acids and fatty acids in the face of some dietary deficiency which tended to lower growth and survival. Low PL and DHA:EPA levels may be limiting factors in this diet. Also, the mineral and vitamin contents of these diets were not analyzed and any deficiency could affect skeletal growth and survival.

We thank the Canadian Centre for Fisheries Innovation (CCFI), the Wesleyville Hatchery (Cape Freels Development Association and ACOA funding), the Marine Institute and Memorial University for supporting this work.

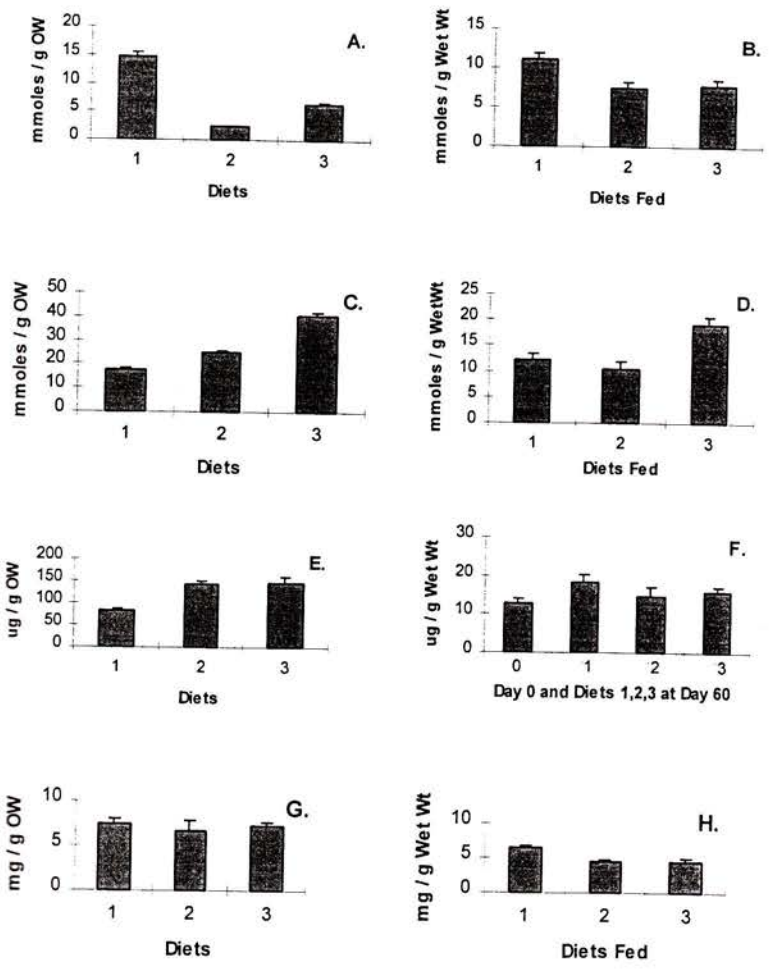

Figure 2. A) Threonine in diets (P<0.05, 2<3<1), and B) in wolffish at day 60 (P<0.05, 1<2<3). C) Taurine in diets (P<0.05, 1<2<3) and D) in wolffish at day 60 (P<0.05, 2=1<3). E) Acyl lipids in diets (P<0.05, 1<2=3) and F) in wolffish at day 0 and 60 (no significant difference at day 60). G. EPA in diets (no significant difference) and H) in wolffish at day 60 (P<0.05, 2=3<1). Diet values are based on organic weight (OW). Data are mean ± SE for three samples.

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Good sperm motility increases egg fertilization rates in ocean pout (*Macrozoarces americanus*)

Z. Wang and L. W. Crim[1]

The ocean pout, *Macrozoarces americanus*, is a northwest Atlantic groundfish with positive cold water aquaculture potential. In this species, eggs are fertilized internally, a rare reproductive strategy in teleosts. To investigate the correlation between ocean pout sperm motility and egg fertilization rate, we studied sperm of different motility (percentage of motile sperm) and egg fertilization capacity. During the spawning season in August, fresh eggs stripped from an ovulated female were mixed with milt of differing sperm motility for a duration of five hours. Following the initial incubation period, the eggs were transferred into incubators supplied with flowing ambient seawater. Egg fertilization rates were determined under a binocular microscope at day four or five. Four separate trials were conducted with different females when they ovulated. The results show that egg fertilization capacity of high motility sperm (motile sperm ≥ 75%) significantly exceeds that of low motility sperm (motile sperm ≤ 25%) ($P < 0.0001$). This study suggests that sperm quality is important and can be assessed by sperm motility in ocean pout.

Introduction

The ocean pout, *Macrozoarces americanus*, is a northwest Atlantic groundfish with positive cold-water aquaculture potential. In this species, interestingly, eggs are fertilized internally, a rare reproductive strategy in teleosts. Ocean pout is also a good model species for studying the reproductive mechanisms of wolffish (*Anarhichas lupus*), another potential new species for cold-water aquaculture in eastern Canada. This is because ocean pout and wolffish share similar reproductive biologies,[2,3] and the latter is more difficult and expensive to handle under experimental conditions. Our previous studies at the Ocean Sciences Centre in St. John's, Newfoundland, have provided information about the spawning season of male ocean pout, the correct timing for collecting milt with good sperm motility, and a basic knowledge of sperm physiology and biochemistry. However, whether sperm quality can be assessed by sperm motility in ocean pout is still unknown. To answer this question, this study investigated the correlation between ocean pout sperm motility (percentage of motile cells) and egg fertilization rate.

Materials and Methods

Adult ocean pout (2 to 4 kg) were collected from Newfoundland waters by SCUBA divers during the spawning season in 1993 and 1994. Fish were maintained at the Ocean Sciences Centre throughout the year in indoor round (2 x 2 x 0.4 m) fiberglass tanks supplied with flowing ambient seawater which ranged from -1.9 to 18.6°C seasonally. The animals were provided with a simulated natural photoperiod and fed chopped capelin twice a week.

During the spawning season in 1995, four separate artificial insemination trials were conducted on August 22, 25, 26, and 30 when each individual female ovulated. Freshly ovulated eggs were stripped from females into a beaker held in crushed ice and then allocated into 3 replicate aliquots (80 or 100 mL eggs) in separate 200 mL beakers for each trial (Table 1). Milt of differing sperm motility was diluted 1:3 (v/v) in an ocean pout physiological ionic medium[4] and added to and mixed with the eggs in covered beakers. During the initial 5-hour incubation period, the eggs were gently stirred every half-hour during the first 2 hours. Next, the eggs were transferred into incubators supplied with flowing ambient seawater which ranged from 8 to 12°C. Egg fertilization rates were deter-

Table 1. Artificial insemination trials with different sperm motility in ocean pout. Sperm concentration is calculated by the equation Y = 1.46 + 1.48X, where X is spermatocrit and Y is sperm concentration (10^8/mL).

Trial #		Control (no milt)			Motile cell ≤ 25%			Motile cell ≥ 75%		
		1	2	3	1	2	3	1	2	3
1	egg (mL)	80	80	80	80	80	80	80	80	80
	milt (mL)	0	0	0	1.5	1.5	1.5	1.5	1.5	1.5
	sperm no. x 10^6/egg	0	0	0	3.19	3.19	3.19	2.14	2.14	2.14
	fertilization rate (%)	0	0	0	77.2	64.5	69.2	88.9	83.1	87.5
2	egg (mL)	80	80	80	80	80	80	80	80	80
	milt (mL)	0	0	0	2.0	2.0	2.0	2.0	2.0	2.0
	sperm no. x 10^6/egg	0	0	0	3.32	3.32	3.32	2.68	2.68	2.68
	fertilization rate (%)	0	0	0	15.6	7.3	5.0	21.5	27.1	21.3
3	egg (mL)	100	100	100	100	100	100	100	100	100
	milt (mL)	0	0	0	1.0	1.0	1.0	1.5	1.5	1.5
	sperm no. x 10^6/egg	0	0	0	1.59	1.59	1.59	2.10	2.10	2.10
	fertilization rate (%)	0	0	0	0	16.5	–	35.4	49.7	42.1

mined microscopically at day four or five, according to Yao and Crim.[5] The effect of sperm motility on egg fertilization rates was statistically analysed using two-way ANOVA which accounts for the sperm motility effect as well as between-trial variability.

Results and Discussion

This study of sperm motility and egg fertilizing capacity showed that sperm with high motility (motile sperm ≥ 75%) was associated with increased egg fertilization rates (Fig. 1). Statistical results from the ANOVA model indicated that the egg fertilizing capacity of sperm with high motility (motile sperm ≥ 75%) significantly exceeds that of low motility sperm (motile sperm ≤ 25%) ($P < 0.0001$).

Sperm quality is an important factor in the success of artificial fertilization in fish. Sperm quality of fish can be measured by sperm concentration, sperm motility, or according to some biochemical components of seminal plasma.[6,7] This study suggests that sperm quality in ocean pout can be determined from sperm motility (percentage of motile sperm), which provides valuable information in the manipulation of ocean pout gametes and their optimum use for artificial insemination. This study may also be beneficial to future work on domestication of the wolffish in eastern Canada.

Egg quality and the timing of egg stripping also influences fertility.[8] In trial #2 and #3 of this study, the eggs were more difficult to strip, which indicates that ovulation might have been incomplete.[9] This might lower the fertilization rate in these two trials independent of whether the sperm used to fertilize the eggs had low or high motility.

Besides sperm motility and egg quality, the sperm to egg ratio is also important in a fertilization test. To obtain optimum egg fertilization rates, about 1 to 2 x 10^5 sperm per egg are required in rainbow trout and wolffish, given milt of good quality.[10,11] However, when the motility of the sperm is low, fertilization rates can be increased by increasing the sperm to egg ratio.[8] In trial #1 of this study, low motility sperm yielded a high fertilization rate which might be due to

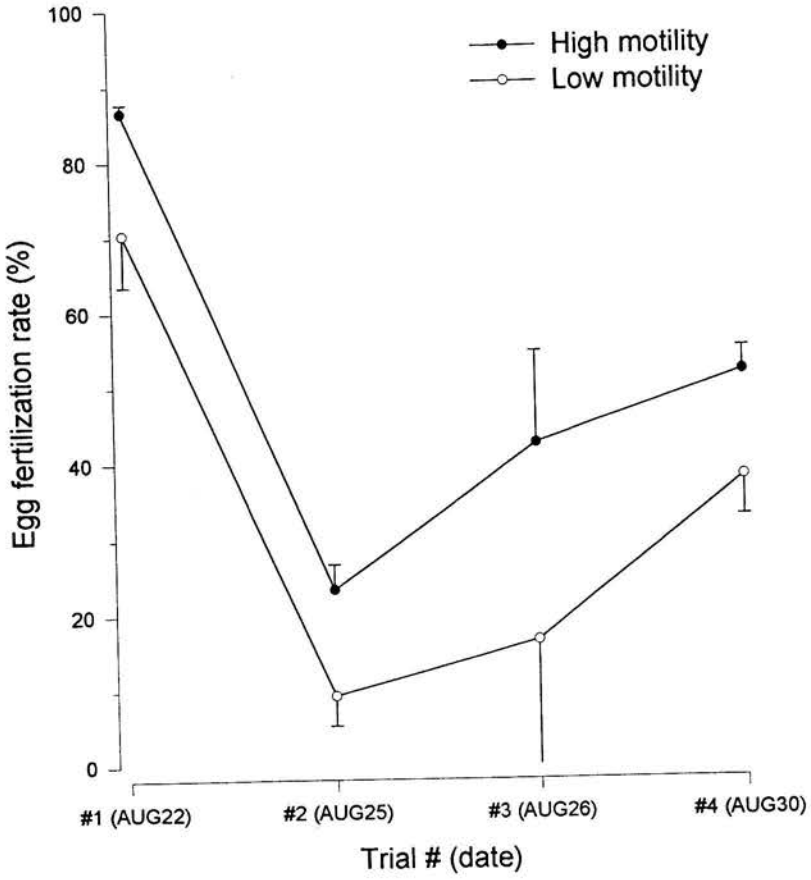

Figure 1. Effect of sperm motility on egg fertilization rate (high motility: motile sperm ≥ 75%; low motility: motile sperm ≤ 25%).

a high sperm to egg ratio (Table 1, Fig. 1). Further investigation is needed to determine the minimum number of sperm for maximum fertilization rates in ocean pout eggs.

The authors are grateful for the collection of wild ocean pout in good condition by SCUBA divers of the OSC Field Services Unit and the laboratory technical assistance provide by Ms. C. Wilson. This research was supported by NSERC grant A9729 to L.W. Crim. OSC Contribution No. 265.

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Grow-out cod farming in southern Labrador

J. S. Wroblewski,[1] *W. L. Bailey,*[1] *and J. Russell*[2]

We conducted the first cod farming experiment in coastal Labrador. Our goal was to demonstrate that "grow-out" of northern Atlantic cod (*Gadus morhua*) is technically feasible in southern Labrador. Farming cod from Gilbert Bay for 83 days resulted in a 40% net increase in biomass with a food conversion ratio of 4.9:1. The proportion of fish lost due to escapement and cannibalism was 19%. The average weight gain of individual cod was 71%, a growth rate similar to cod farmed along the northeast coast of Newfoundland. Inshore cod from Labrador are smaller in size-at-age than inshore cod of northeast Newfoundland, likely due to a shorter growing season. By holding Gilbert Bay cod in a net pen within the seasonal thermocline, the growth rate of farmed fish was increased.

Introduction

In the cod fishery of the future, many fishers will be both hunters and farmers. The traditional cod trap fishery can be combined with cod farming practices.[3,4] There is currently a moratorium on fishing the northern Atlantic cod stock. When the resource recovers to the point that fishing can resume, a portion of the trap catch could be farmed in net pens for weeks, months, or even over the winter.[5] Farmed cod have a higher value per unit weight than cod caught in the wild.[6] The experimental farming of cod on the island of Newfoundland began in 1986.[7] To our knowledge, no experiment previous to our own has been attempted in Labrador. We demonstrate the technical feasibility of farming cod in Labrador using inshore cod raised in a net pen constructed and maintained by a local fish harvester.

Materials and Methods

On 1 August 1997 eighty-eight cod (42 to 72 cm in length, 0.8 to 4.2 kg whole weight) were caught by hook and line in Gilbert Bay and transported by boat to a net pen (4.5 m x 4.5 m x 4.5 m deep, 2.5 cm mesh with a roof covering of the same mesh) located in Fox Cove near Williams Harbour. The length and weight of each fish was recorded before placement in the pen. These fish were fed cut herring *(Clupea harengus harengus* L.) every 2 to 3 days from 8 August to 29 October. Herring were caught locally using gill nets. The amount of herring fed to the fish on each occasion was recorded. After 8 days of starvation, the fish remaining in the pen were again measured for length and weight on 7 November.

Temperature and salinity at the cod farm site in Fox Cove were measured using a Seabird Electronics Inc. Seacat SBE 19-03. Temperature at the bottom of the net pen was recorded using a Vemco Ltd. Sealog-TD.

Results

Of the 88 cod placed in the net pen on 1 August, 71 were still present on 7 November (loss of 19%). Some fish may have died after capture. Escapement from the pen was possible, but not observed. Of the fish that were 42 to 49 cm in length when they were placed into the pen on 1 August, all but one were missing in November. This suggests mortality due to cannibalism.

The total weight of cod placed into the pen on 1 August was 146 kg. The total weight of the fish in the pen on 7 November was 205 kg, a net increase in biomass of 40%. The total amount of herring fed to the cod was 287 kg. The feed conversion ratio (amount of feed divided by the net increase in biomass) was 4.9:1.

The average weight gain of individual Gilbert Bay cod (n=53) was 71% (S.D. 18%) over this 83 day period. The average cod increased in weight from 1.7 kg (S.D. 0.7 kg) to 2.9 kg (S.D. 1.2 kg). The average cod grew in length from 53 cm (S.D. 7 cm) to 59 cm

(S.D. 7 cm). This growth was independent of the initial size (weight or length) of the fish (Figs. 1 and 2). Body condition factor (whole weight/length^3 x 100) increased from an average of 1.09 (S.D. 0.09) to 1.36 (S.D. 0.16).

Seawater temperatures at the bottom of the net pen (5 m depth) were 7 to 9°C during August, 7 to 10°C in September, 4 to 6°C in October. Salinity ranged from 26 to 29 ppt.

Discussion

The 40% net increase in biomass achieved by farming Gilbert Bay cod for 83 days is similar to results obtained at cod grow-out operations in Newfoundland during the summer and fall of 1997.[6] Three operations in Trinity Bay experienced net increases in cod biomass of 38%, 117% and 128% over 98 days, 112 days and 112 days respectively. A cod farm on the northern peninsula obtained a net increase of 48% over 63 days.

The feed conversion ratio of 4.9:1 calculated for farmed Gilbert Bay cod lies within the range of ratios (3.7:1 to 8.9:1) computed from grow-out operations in Newfoundland during 1997.[6] The average weight gain of 71% for individual Gilbert Bay cod exceeds the average weight gain of 65% reported[8] for Conception Bay cod held in outside tanks at the Ocean Sciences Centre at Logy Bay, Newfoundland, and fed capelin to satiation three times a week between 11 August and 27 October 1980.

Our results demonstrate that northern Atlantic cod farmed at sites along the coast of southern Labrador can grow at the same rate as farmed cod on the island of Newfoundland. Temperatures in Gilbert Bay reach similar values during the summer as in Trinity Bay, but these warm temperatures do not persist as long.[9] Wild cod in Gilbert Bay are smaller-at-age than inshore cod of Trinity Bay,[9] likely because Labrador

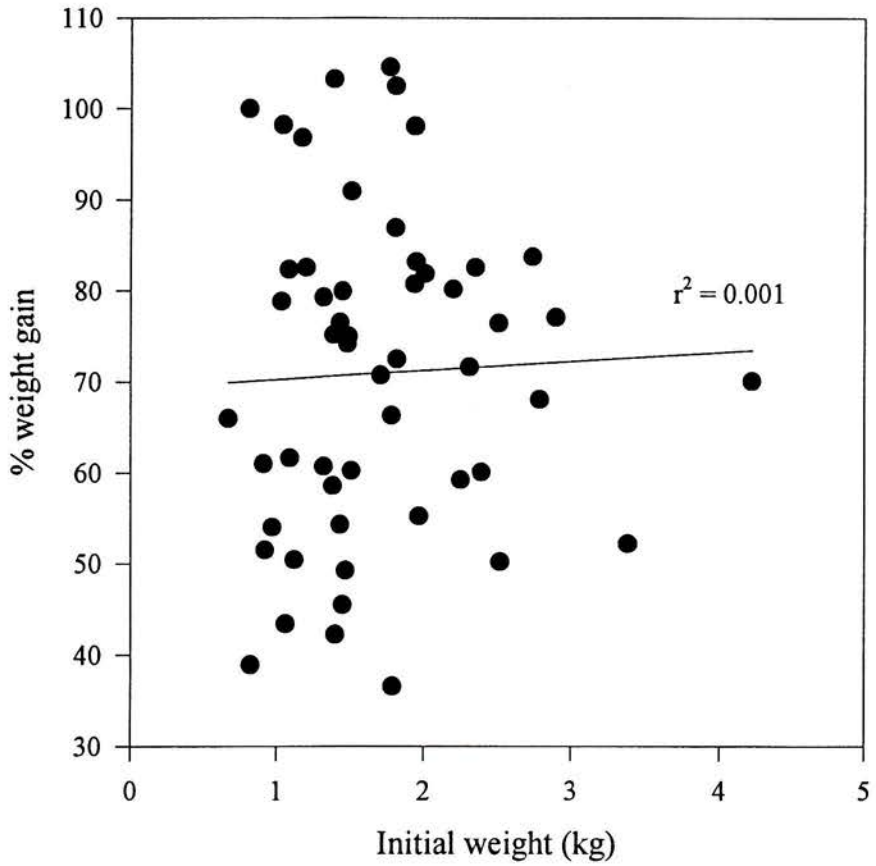

Figure 1. Weight gain plotted against initial weight for Gilbert Bay cod (n=53) farmed from 8 August to 29 October 1997 near Williams Harbour, Labrador. The proportion of the variance in the data explained by the regression model (r^2) is 0.1%.

cod spend longer winter periods in waters of subzero temperature. Holding Gilbert Bay cod in a net pen within the warm surface layer increased the growth rate of these farmed fish over that of wild cod in the bay.

Recommendations

Now that it has been demonstrated that northern Atlantic cod can be farmed along coastal Labrador, the economic infrastructure of grow-out cod farming needs to be established in fishing communities. The harvesting, processing and marketing of farmed fish from Labrador should be included in plans to develop the cod aquaculture industry in Newfoundland.[6]

This research was supported by the Canada-Newfoundland Comprehensive Labrador Cooperation Agreement, the Canadian Centre for Fisheries Innovation, and the Government of Newfoundland, Department of Fisheries and Aquaculture.

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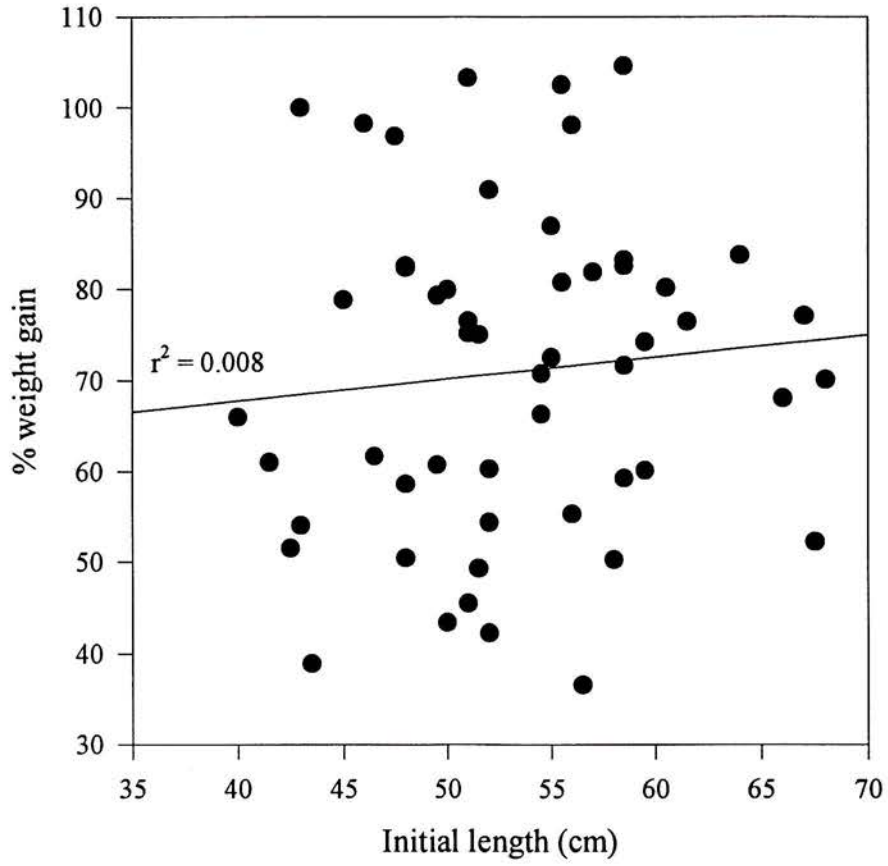

Figure 2. Weight gain plotted against initial length for Gilbert Bay cod (n=53). The variance explained by the regression model is 0.8%.

Optimum protein level in a practical fishmeal–based diet for juvenile American eel *(Anguilla rostrata)*

S. M. Tibbetts,[1] *S. P. Lall*[2] *and D. M. Anderson*[1]

American eels were used in an experiment to determine the level of dietary crude protein required to optimize growth performance. Elvers, initial mean weight 8.11 ± 0.07 g, were fed to satiation for 84 days on diets based on herring meal containing 35, 39, 43, 47 and 51% crude protein (as-fed basis). Data was collected to determine the relationship between dietary protein level and growth performance, nutrient digestibility, and nutrient retention. Highest mean weight gain (P=0.0070) and specific growth rate (P=0.0087) were obtained when protein was 47% and 51%, with values of 14.2 ± 0.66 g and 13.1 ± 0.61 g for mean weight gain, respectively, and values of 1.20 ± 0.04 %/day and 1.14 ± 0.04 %/day for specific growth rate, respectively. Optimum feed conversion ratio (P=0.0078) of 1.17 ± 0.05 g feed/g gain was achieved when feeding 47% protein and was significantly lower than that of the 51% protein diet. Digestibilities of crude protein and energy were similar among the 39, 43, 47 and 51% crude protein diets, with mean crude protein digestibility of 90.7 ± 0.54% and mean energy digestibility of 90.3 ± 0.60%. Digestibility of the 35% crude protein diet was significantly lower with values of 84.9 ± 0.97% for protein (P=0.0207) and 85.2 ± 0.98% for energy (P=0.0342). Highest carcass protein gain (P=0.0013) of 2.2 ± 0.11 g/fish was achieved when feeding 47% and 51% protein, while highest carcass lipid gain (P=0.0013) of 1.8 ± 0.07 g/fish was achieved when feeding 47% protein. Based on all measured criteria, the optimum level of dietary protein for juvenile American eel is estimated to be 47% or 22 g digestible protein/megajoule digestible energy.

Introduction

American eel is a species of increasing economic importance in Atlantic Canada's aquaculture industry. Market value for this fish is high due to growing demand from Asia and Europe for both juveniles and adult fish. Presently, American eels farmed in Atlantic Canada are fed commercial diets formulated for other fish species, namely Japanese eel *(Anguilla japonica)* and the salmonids. This is predominantly due to the fact that American eel culture is a relatively new industry and, thus, there is a lack of nutritional information.

Protein is the most abundant and expensive ingredient in eel diets and the dietary protein requirement for this species is not known. This information is required to develop cost-effective feed formulations that optimize fish growth and protein retention, and reduce the soluble and solid load of nitrogenous compounds in the water used for eel culture systems, particularly in recirculation systems. The present study was designed to determine the quantitative dietary crude protein requirement for optimum growth of juvenile American eel in a practical fishmeal-based diet.

Materials and Methods

Five isocaloric diets were formulated[3] to supply crude protein levels of 35, 39, 43, 47 and 51%. American eel elvers (n=675), initial mean weight 8.11 ± 0.07 g, were obtained from Springhill Fish Farms Ltd. and housed in fifteen 40-L cylindrical tanks at an initial density of 18 kg/m^3. Freshwater was supplied at 1 L/min and maintained at 22.0 ± 0.2°C. The fish were hand-fed to apparent satiation twice daily during the week and once daily on weekends. The growth trial lasted for 84 days.

Data were collected daily to determine feed intake

per tank and the fish in each tank were individually weighed at 28-day intervals. Intake data and body weight data were then used to determine apparent feed intake (AFI), mean weight (MW), mean weight gain (MWG), specific growth rate (SGR) and feed conversion ratio (FCR).

Digestibilities of organic matter (OM), crude protein (CP) and energy (E) of the diets were estimated after the feeding trial, employing an indirect method using chromic (III) oxide (Cr_2O_3, 5 g/kg) as the digestion indicator. Fish were housed in 60-L glass aquariums and temperature was maintained at 22°C. Apparent digestibility coefficients (ADC) were then calculated.

Fish were sampled at the beginning and end of the trial for body composition analysis. Carcass protein gain (PG), lipid gain (LG) and energy gain (EG) were calculated.

Statistical analysis was done using SAS software[5] and involved a randomized block design, the General Linear Models (GLM) procedure, repeated measures analysis, and pdiff tests. The confidence level used was 95%.

Results and Discussion

After 56 days of feeding, the mean weight of fish on the 47% and 51% protein diets were significantly higher (P=0.0333) than fish fed the other diets (Fig. 1). This trend persisted with final mean weights of fish on the 47% and 51% crude protein diets being similar to each other and approximately 18% higher (P=0.0070) than those on the other diets.

Dietary protein level showed significant effects on both growth performance variables measured (Fig. 2). Specific growth rate was found to be 19% higher (P=0.0087) when dietary crude protein was 47% and 51% as opposed to the lower levels. Optimum feed conversion ratio was achieved when feeding 47% crude protein and was significantly lower (P=0.0078) than that of the 51% crude protein diet at a margin of approximately 18%.

A common trend was observed with the growth performance data where the final mean weights, specific growth rates, and feed conversion ratios of fish fed 47% crude protein reached a plateau and either leveled off or declined thereafter. Based on these results, increasing dietary crude protein beyond the 47% level provides no significant benefit in terms of growth.

The fact that growth performance reached a plateau and leveled off is consistent with results obtained by other researchers which indicate that extremes of protein are not used for normal protein metabolic functions, such as tissue growth, but are instead used as a comparatively inefficient energy source[6] and that excess protein is not likely to be beneficial.[7] In the case of the feed conversion ratio, where performance not only leveled off but actually declined after 47%, other researchers have attributed similar effects in channel catfish[8] and Japanese eel[9] to high levels of dietary protein.

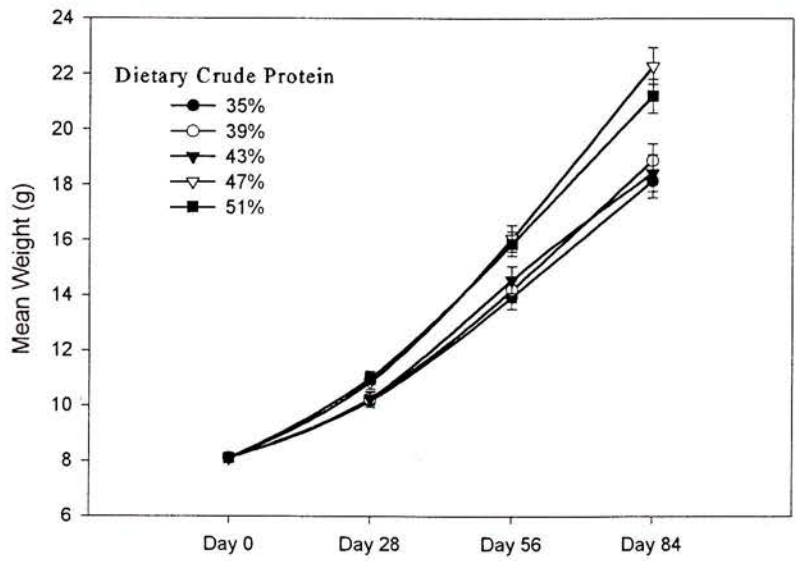

Figure 1. Effect of dietary protein level on growth of American eels at various sampling times during the experiment (Values are lsmeans ± SEM of three replicates)

Apparent digestibility coefficients (ADC) of OM, CP and E for the 39 to 51% crude protein diets averaged 88%, 91% and 90%, respectively. This indicates that, for the most part, the diets were of high quality in terms of nutrient availability. The ADC obtained for OM and CP verify earlier results[10] where ADC for OM and CP of 87% and 94% were obtained for European eel using a similar fishmeal-based diet. The significantly lower digestibility of the 35% crude protein diet may be due to the way the diets were formulated. Carbohydrate in the form of corn starch was used to make the diets isoenergetic; whereas the dietary protein level increased from 35% to

51%, carbohydrate declined from 32% to 22%. Because eels are carnivorous, it is likely that the fish could not digest the high level of carbohydrate as well as the lower levels. From these results, it appears as if nutrient digestibility was not significantly affected by the level of protein in the diet when carbohydrate was below the 30% level. These findings are in general agreement with those of other researchers[11] who concluded that nutrient digestibility was independent of dietary protein level using similar experimental diets.

Nutrient retention data showed a similar pattern to that of the growth performance data where the highest carcass protein gain (P=0.0013) and carcass lipid gain (P=0.0013) was achieved at the 47% crude protein level, again indicating that by increasing dietary crude protein beyond the 47% level, there is no significant benefit in terms of growth. Little difference in carcass energy gain was observed.

Summary and Conclusion

The 47% dietary protein treatment resulted in the best performance as no significant benefit in terms of growth, digestibility, and nutrient utilization was observed at the 51% protein level. Therefore, based on these observations, it was concluded that the optimum dietary protein requirement for juvenile American eel is 47%, also expressed as 22 g digestible protein/megajoule digestible energy.

The results support the findings of other authors[9] who reported that beyond 45% crude protein no further growth increase was observed in Japanese eel fed casein at 25°C. The 2% difference is related to protein source and water temperature. We would expect the requirement to be slightly higher in our study because we used fishmeal as the protein source and raised the fish at a lower water temperature.

When the protein requirement established here is compared with that of the protein requirement found earlier by Wattendorf[6] for the same species of eel, the requirement is practically identical at 47% and 48%, respectively. Strangely, this result cannot be found in the primary scientific literature.

There is a clear indication that commercial eel grower diets are providing crude protein in excess of that required by juvenile American eel. This information, if utilized, may increase protein efficiency, decrease ammonia excretion, ultimately resulting in reduced nutrient load on recirculation systems and more cost-effective diets for American eel.

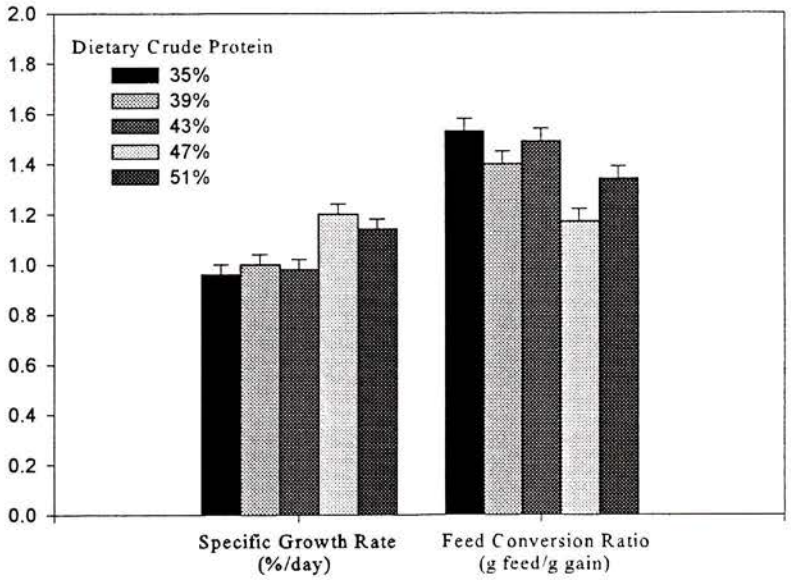

Figure 2. Effect of dietary protein level on growth performance of American eels fed the five experimental diets after 84 days (Values are lsmeans ± SEM of three replicates)

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Status of commercial tilapia culture in Canada

T.T. George,[1] C. Weaver,[2] and T. Shaw[2]

In 1995, the Province of Ontario amended its regulations to permit the culture of tilapia. In 1997, approximately 30,000 kg was marketed in Toronto by Northern Tilapia Inc. using an intensive recirculation system. At present, there are several other tilapia farms under construction or in the process of producing tilapia. This paper reports on these farms, their culture systems and status of production, explains why tilapia culture is now expanding in Canada, and lists industry concerns and recommendations.

Introduction

Tilapia, native to Africa and the Middle East, is the second most common farm-raised food fish in the world.[3] It has recently become important in Canada as a culture species and also as a tool for diabetes research at Dalhousie University.[4] Ontario, the first province in Canada to permit tilapia culture,[5] now has 6 farms: Northern Tilapia Inc. (NTI) at Lindsay, Integrated Aquatics Inc. (IAI) at Port Hope, Canadian Tilapia International (CTI) at Simcoe, Phoenix Fish Farm (PFF) at Tyendanaga, Chatham Aquaculture (CA) at Chatham and Mississauga First Nation (MFN) at Blind River. These farms are either producing tilapia for the Toronto market or are under construction. NTI is the only farm operating a hatchery; all the others purchase fingerlings from NTI or the United States.

System Type, Set-up and Operation

All farms raise tilapia in greenhouses or steel buildings using intensive recirculation to optimize production under controlled conditions. All have systems with the same waste treatment processes: removal of waste, fine/dissolved solids and carbon dioxide; pH adjustment; oxidation of ammonia and nitrite nitrogen; addition of pure oxygen; and foam fractionation (except IAI). Only IAI combines the culture of tilapia and hydroponic vegetables/herbs in a closed synergistic system. Therefore, NTI and IAI are described hereinafter.

The NTI system is based on a prototype developed at Cornell University.[6] The growout system consists of 37,500-L circular tanks fitted with simple drains that collect the solids. The solids are removed to a single drum filter in a flow that equals approximately 25% of the total flow leaving the tank. The clarified water is pumped to downflow bubble contact reactors for oxygenation with pure oxygen before returning to the tanks. Water from the upper standpipe entrance is pumped to packed column aerators to remove carbon dioxide. The water falls onto the top of two 1.2-m diameter downflow polystyrene floating bead biological filters located adjacent to each culture tank and overflowing back into the tanks. Effluent passes through a foam fractionator to remove fine particles. The growout tanks are stocked with advanced fingerlings (~100 g) and, by reducing fish density as the fish grow, are harvested live at 590-680 g.

The IAI system closely mirrors that of NTI, except for the hydroponic component, and consists of 1600-L circular tanks for early rearing, 55,000-L concrete raceways for grow-out, a rotating drum screen filter, a biofilter, 4 low-head oxygenators, a 400-L pumping reservoir, a 3/4 hp high centrifugal pump for the circular tanks, and a 2 hp axil flow pumps for the raceways. The water is heated via two 4.06×10^8 joules (3.85×10^5 btu) natural gas pool heaters.

Effluent from the raceways is passed through the drum filter to reduce suspended solids and BOD, and through the biofilter to oxidize ammonia and nitrite. A portion of the water then flows through the vegetation production units where dissolved nutrients are removed. Finally, water collects in a reservoir and is returned to the raceway. The plants are cultured by NFT technique which uses narrow plastic troughs in which the roots are exposed to a thin, flowing film of nutrient solution applied intermittently. IAI applies a multiple rearing units stock management method whereby each raceway is divided into 2 to 4 compartments with movable plastic screens. As the fish grow, the compartment size is increased and the fish are moved closer to one end of the raceway from where they are eventually harvested.

In both systems, ammonia and nitrite are maintained below 1 ppm and 0.1 ppm, respectively, effluent oxygen levels rarely fall below 5 ppm, feed conversion

ratios range from 0.8 to 1.0-1.2 kg feed/kg of fish for small and large fish respectively. Feeding of fish is spread over time to maintain consistent water quality. Unlike NTI, IAI uses concrete raceways for growout and one large recirculation system for both early rearing and growout. Separate biofilters are not required for aquaponics as the surface area of the hydroponic tanks and ammonia uptake by the plants provide biofilteration; however, IAI still uses them. Foam fractionation is not required either. The double drain design is not appropriate for raceways, so IAI filters water through the drum filter. Although the circular tanks used by NTI are more efficient in removing solids due to the cyclonic effect of water flow, IAI chose raceways to increase production area by using both vertical and horizontal space. It is also much easier to apply the concept of multiple stock rearing management and accessing and harvesting plants in raceways is easier and less labour intensive. Suspended solids leaving the facility as concentrated sludge are treated in a small aerobic sludge lagoon; NTI discharges directly to the municipal treatment system.

Status of Tilapia Production and Economics

At present, the actual production in kilograms for NTI, IAI, CA, CTI, and PFF is 30 000, 2800, 4500, 1400, and 19 000 respectively, while the production target in this order is 91 000, 91 000, 82 000, 45 000 and 45 000 kg; the MFN target is 80 000 kg, but the farm is still under construction. IAI also produced 114 kg of chives out of a 1900-kg target. NTI established a joint venture with International Aqua Foods Ltd., Vancouver, to expand its operation in Canada and the USA.[7] However, production costs have not been determined for any of these farms.

Tilapia Market and Industry Concerns

Toronto, with its mix of immigrant communities, is the largest tilapia niche market in North America and Big Land Farm is one of the largest wholesale buyers. In 1997, it imported 680,000 kg of tilapia from the United States. By 1999, its operation will expand to 1.4 million kg.[8,9] However, there is concern in Canada of importing tilapia infected with *Streptococcus iniae* because tilapias are more susceptible to this bacterium than any other fish species.[10] Also, Canadian farmers may face stiff competition in the live market from massive tilapia operations in the USA. The lack of veterinary and nutritional expertise on tilapia in Canada is a major constraint as is the reluctance of federal and provincial governments to acknowledge aquaculture as a primary producing industry, comparable to livestock production. This means the legal, administrative, and investment framework is often inappropriate to industry's needs.

Discussion

It is assumed that aquaponic systems will be more profitable because they produce higher yields of fish and vegetables per unit volume of water, have vegetables that remove nutrients which would otherwise inhibit fish growth and have negative effects on the environment, eliminate the need for separate biofilters and foam fractionators, reduce water consumption, and share management and infrastructure expenses.[11] However, economic analyses are necessary to secure investment capital. Accurate records of capital costs, operating expenses, product output, and sale price will allow budget development and risk assessment. Market research will assist in the development of production strategies and pricing polices which respond to the nature of the consumer demand.

The cost of tilapia production in closed systems is high, but market prices for live fish can justify the investment. Tilapia offers a variety of product options and should not be limited to niche markets. However, if the live market does not expand as production increases, many farmers may be put out of business. Tilapia farms in the US using recirculating systems require about US$4.40/kg to make a profit.[12] Thus, Canadian producers have a competitive advantage in terms of both freight and fish condition because they are located close to key markets. They can also achieve cost-effective production through proper management and avoidance of superfluous expenses.

Economic analyses suggest that factors related to fish biology and management of production capacity will affect profitability more than improvements in the engineering components of the culture system.[13] Maintaining optimum temperature and using genetically improved tilapia can be used to increase growth rate. Feed accounts for 20-35% of production costs and changes in feed costs or conversion efficiency will have profound effects on profitability.[14] To date, no feeds have been developed specifically for tilapia and there are no vaccines. Also important are costs for transportation and fingerling production. As mentioned, producers close to the market have the competitive advantage. Using organic fertilizers (broiler litter) to rear fingerlings to stocker size provides significant savings in time and expenses. Fabricating equipment (tanks, biofilters, etc.) lowers capital costs as does utilizing pre-fabricated buildings or existing structures as culture facilities (e.g. greenhouses, hog barns, chicken houses). Inexpensive and robust monitoring and control systems may reduce requirements for labourers.[15]

Foam fractionation of the effluent from the biofilter

removes fine particles and reduces turbidity and volatile compounds that adversely affect fish taste.[16] Addition of ozone to the air stream of the foam fractionator enhances foaming in the contact chamber and removes colloidal organic matter. While not necessary in intensive recirculating systems, ozone has been used to remove colour in the water, reduce dissolved organic loading, oxidize nitrite nitrogen, and control disease.[17] However, according to Holder[18] ozone or UV will not work for disinfection in tilapia recirculating systems and a quarantine facility must be available.

Unless cultured tilapia are subjected to environmentally induced or other stressors, they are generally disease resistant. Red tilapia is resistant to *S. iniae,* but inaccurate media comments about *S. inae* causing "mad fish disease" in farmed tilapia have temporarily affected the tilapia market. It is important for the aquaculture industry to work collectively to counteract unfounded negative attitudes about farmed tilapia.[10]

According to Moccia and Hynes,[19,20] aquaculture is livestock agriculture and should be treated as such by the federal and provincial governments in Ontario, especially regarding environmental concerns. It also should be recognized as a business quite distinct from the management of the wild fishery. Strong arguments can be made for the administration of aquaculture being in the hands of either the agriculture or fisheries departments. However, the advantages of being allied to agriculture increase with intensive aquaculture. This is because research and development are most needed in the areas of genetics, nutrition and health, which rely more on animal husbandry expertise than fishery science; the same applies for training and extension services. Also, the same concessions which apply to intensive livestock production seem appropriate to extend to aquaculture.

Recommendations

1) To ensure profitability and competition in the marketplace tilapia farmers:
   a) should cooperate through the OAA Promotion Committee, manage their systems at the lowest cost, and determine the economic feasibility of their recirculating systems on a commercial basis;
   b) reduce production costs by improving survival, weight gain, feed conversion efficiency, and altering the genetic make-up line with market requirements;
   c) adopt health maintenance procedures to prevent *S. iniae* infection or by culturing the resistant red tilapia (which also has a higher market price than other tilapias);
   d) use ozone with the air stream of the foam fractionator to reduce turbidity and volatile compounds that affect fish taste.
2) Feed companies should produce special tilapia feed, as it will be less expensive than trout feed, so that farmers can compete in the marketplace.
3) Markets for live tilapia should be expanded from ethnic niches into wider ethnic and socioeconomic groups including the traditional outlets of restaurants and seafood counters.
4) Federal and provincial government departments should recognise aquaculture as a primary producing industry akin to livestock agriculture and rationalize their legislation and regulatory policies, especially regarding environmental concerns, so that aquaculture can thrive as a business.

This paper is dedicated to the owners of the tilapia farms in Canada. We thank them for providing production data, especially Gary Chapman of NTI. We also thank Michael Lam, Big Land Farm, for providing import records of live tilapia from the USA..

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Essential fatty acids in plankton from cold ocean coastal environments

S. M. Budge,[1,2] C. C. Parrish,[2,1] R. J. Thompson[2] and C. H. McKenzie[2]

The fatty acid nutritional requirements of marine finfish and shellfish are of utmost importance. The long-chain polyunsaturated fatty acids (PUFA), arachadonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) are essential for animal growth and survival. Insufficient amounts of these acids in the diet have a variety of deleterious effects, including reduced feeding efficiencies, liver damage and behavioural changes. Studies have suggested that for broodstock conditioning optimum levels of ω3/ω6 PUFA in finfish diets are in the range of 5:1 to 10:1, while the recommended ratio of the specific fatty acids DHA/EPA is 2:1. In adult bivalves, more appropriate ratios seem to be 5:1 to 15:1 and 1:1, respectively, while in larvae the correct ratio of DHA/EPA may be as low as 1:2. Such requirements are often overlooked when establishing bivalve aquaculture sites in the natural environment. A former aquaculture site in Notre Dame Bay, Newfoundland, was investigated to determine typical fatty acid compositions of plankton. The quality of plankton-produced fatty acids in the natural environment fluctuates both spatially and temporally and we have found that the ratios in plankton (>10 μm) in this area have a variety of values. PUFA levels in plankton were high, ranging from 33 to 57% of total fatty acids with an average of 44%. Ratios of ω3/ω6 PUFA varied from 4 to 22 with an average of 12, while the ratio of DHA/EPA ranged from 0.1 to 2.4 with an average of 1.0. The average values of these ratios compared well with the corresponding values found in mussels (*Mytilus edulis*) in Notre Dame Bay.

Introduction

Numerous studies [3-6] have shown that certain long-chain polyunsaturated fatty acids (PUFA) are essential for growth and reproduction of bivalves. Bivalves have an absolute requirement for arachadonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) that may be partly met through dietary uptake of phytoplankton. Most shellfish farmers are aware of these requirements but the fatty acid composition of natural plankton populations is rarely, if ever, considered when aquaculture sites are being selected.

Fatty acid nutrition in finfish has been extensively studied. The ratios, DHA/EPA and ω3/ω6 PUFA, are useful parameters in evaluating fish diets and Sargent[7] has recommended levels of DHA/EPA and ω3/ω6 PUFA of 2:1 and 5:1 to 10:1, respectively, in broodstock diets. Similar recommendations for optimal values in bivalves have yet to be made, but it is possible to compile a range of commonly encountered values in the literature. Several studies[3, 8-11] report a ratio of DHA/EPA of 0.5 in eggs and female gonads of various bivalves while others[5, 12-15] seem to suggest a slightly larger ratio of 1 to 1.5 for adult bivalve growth. A ratio of ω3/ω6 PUFA of 5:1 to 15:1 is commonly found in the same references for both broodstock conditioning and growth in adults. The fatty acid composition of naturally occurring plankton populations in Notre Dame Bay, Newfoundland, are evaluated here in terms of these parameters.

Methods

Plankton samples were collected at a former blue mussel (*Mytilus edulis*) farm in Notre Dame Bay using a 20-μm mesh net. Samples were taken on four separate dates from August 1995 to November 1995 and were immediately filtered and stored in chloroform. The filters were then extracted using a modified Folch[16] method to yield lipid extracts. Fatty acid methyl esters (FAME) were produced from these ex-

tracts by transesterification using boron trifluoride in methanol. FAME were analyzed using a gas chromatograph equipped with flame ionization detection, a temperature programmable injector and a gas-line oxygen scrubber.

Subsamples of phytoplankton were preserved in Lugol's iodine and 10% buffered formaldehyde for microscopic analyses. Samples were dominated by small (< 30 μm diameter) centric diatoms and larger (up to 300 μm diameter) dinoflagellates. Four blue mussel samples were also collected in the fall of 1995 at the same location in Notre Dame Bay.

Results and Discussion

The fatty acid parameters, DHA/EPA and ω3/ω6 PUFA, of plankton samples are presented in Figure 1. The plankton collected in the periods of August to October had an ω3/ω6 PUFA level from 7 to 10, which is within the commonly encountered range of 5 to 15 in bivalves. The November sample was beyond this range with a value of approximately 21. This increased proportion of ω3 PUFA in the bivalve diet may be beneficial. Water temperatures are colder in late fall and it is commonly thought that larger amounts of ω3 PUFA are required to maintain membrane fluidity as temperature drops.[17]

Also shown in Figure 1 are the corresponding ratios of DHA/EPA. In Newfoundland, blue mussels generally spawn in July so the presence of very young larvae would be expected in August. These young larvae would have a DHA/EPA requirement similar to that of developing eggs, i.e. a value of 0.5 in the plankton would be beneficial. As the larvae grow, their requirements would more resemble those of the adult bivalve and a higher DHA/EPA ratio of 1 to 1.5 would be appropriate. The plankton seem to have provided a DHA/EPA level corresponding to these needs, as a value of 0.5 is obtained in August with increasing levels in early and late October. The very low value in November may also be related to the decreased water temperature. The ω3/ω6 PUFA ratio suggests that more ω3 fatty acids are produced in November and it would seem that increased amounts of EPA, rather than DHA, are being generated.

It is also possible to evaluate the plankton data in terms of adult bivalve growth. The mean of the fatty acid plankton data is shown in Figure 2. AA and total PUFA, expressed as a proportion of total fatty acids, as well as DHA/EPA and ω3/ω6 PUFA levels, are included. These parameters are also shown for adult mussels collected in the same area in Notre Dame Bay. The fatty acid profile of the blue mussels is typical of those collected elsewhere[14] and is not unique to Notre Dame Bay. It is obvious from Figure 2 that the plankton are providing fatty acids in proportions very similar to those accumulated by the mussels. PUFA comprise 44% of total fatty acids in the plankton and 52% in the mussels, while the DHA/EPA levels in both mussels and plankton have a value of 1. Less similar levels, however, are observed for AA and ω3/ω6 PUFA. The higher level of AA in the mussel than in the plankton raises some interesting possibilities and suggests that either the mussel is very efficient at selectively retaining AA or it is capable of synthesizing AA from a fatty acid precursor. The second possibility is quite important because it implies that AA is not essential in bivalve diets. There is some evidence in the literature[18] that bivalves do contain the necessary enzyme, a 5 desaturase, to synthesize this acid, but

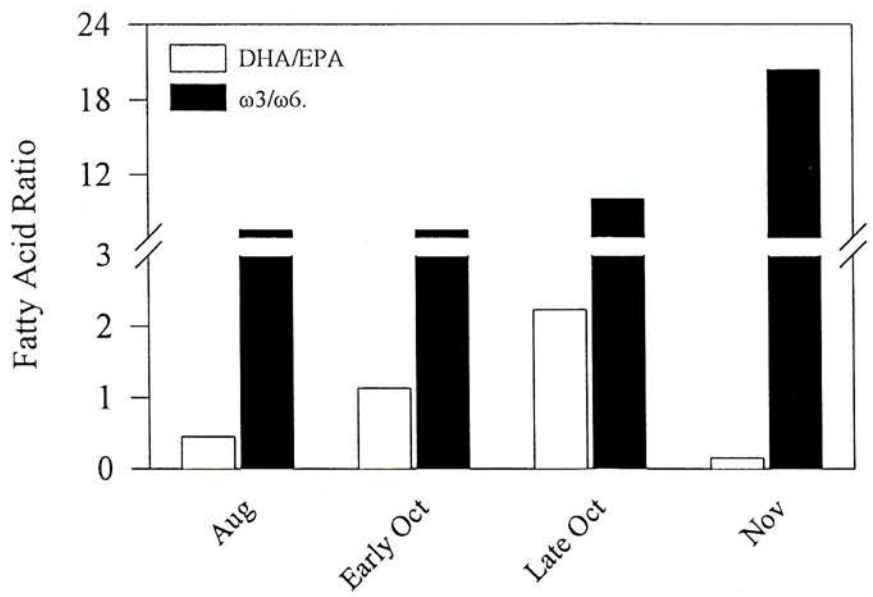

Figure 1. Temporal variations of fatty acid parameters in plankton (n = 1-3).

further study is required before AA can be eliminated as a dietary essential fatty acid. This higher level of AA in the bivalve is the cause of the reduced ω3/ω6 PUFA level because AA is an ω6 fatty acid.

While the fatty acid composition of the blue mussels collected in Notre Dame Bay was typical of healthy mussels found elsewhere, the fatty acid composition of the plankton was exceptional. For comparison, the fatty acid composition of plankton collected at a mussel farm in Ship Harbour, Nova Scotia[19] is also shown in Figure 2. Ship Harbour plankton contained approximately 6-fold less PUFA, a larger DHA/EPA ratio and no AA. The colder average water temperature in Notre Dame Bay may play a role in the increased PUFA values and likely influences the levels of the other parameters. Whatever the cause, the fatty acid profiles of plankton in Notre Dame Bay suggest that the plankton in that area are providing fatty acids in proportions that closely resemble those of the mussel. This in turn suggests that a mussel in Notre Dame Bay could more easily acquire the necessary fatty acid composition and, conceivably, this could correspond to less stress on the mussel and enhanced growth rates. It is known that the blue mussels produced in Ship Harbour are healthy with a normal growth rate,[20] so it is obvious that factors other than fatty acid proportions in plankton are important. In terms of fatty acids, for example, absolute PUFA concentrations should be determined in addition to proportions. Certainly, if two potential aquaculture sites were equal in all respects except the fatty acid proportions of naturally occurring plankton populations, the site whose planktonic fatty acids more closely resemble those of the bivalve should be chosen.

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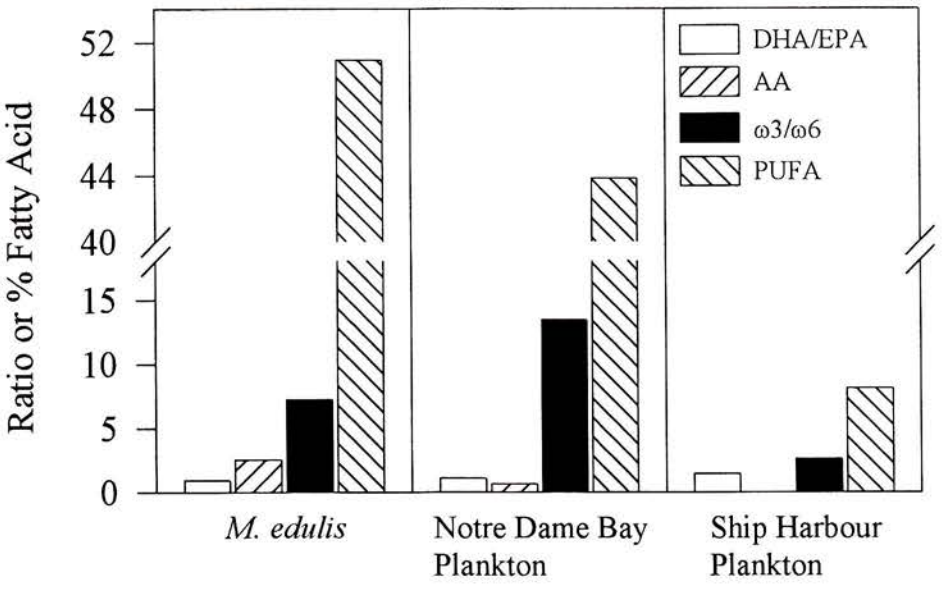

Figure 2. Fatty acid composition of mussels and plankton (n = 4-10).

Factors influencing cultured mussel meat yields and recommendations for a standard method

Diego Ibarra and Cyr Couturier[1]

Newfoundland cultured mussels (*Mytilus edulis*) were used to assess the factors influencing meat yields. The effect of cooking time on meat yield was assessed first on individual mussels, then on 1-kg samples. Visual categories of cooking status were developed and for meat yield studies it is recommended that cooking be continued for 3 minutes after the mussels are completely cooked (category 4). The effect of "post-cooking, air-drying time" was assessed; yield losses were minimal after 2.5 minutes. The debyssing process decreased yields by approximately 0.5% but did not affect shelf-life for up to 8 days. Time out of the water prior to meat yield determination had no influence on the yields in either debyssed mussels or in the control (unprocessed) mussels. Conversion formulas between the North American, European and dry methods were developed and a standardized method for determining cooked meat yields is presented.

Introduction

Meat yield is a measurement that relates the amount of shell to the quantity of living tissue of a bivalve.[2] It is used in the bivalve farming industry as a measurement of quality and condition.[3-6] The problem is that meat yields can be calculated in several different ways[2,7,8] and each formula can be applied using different procedures. The result is that comparison between meat yields is difficult.

Mussel growers, processors, customers, and researchers should use a standard procedure to calculate meat yields so that results can be compared. As well, conversion factors are needed to enable comparison with results obtained from other meat yield formulas, such as those being used in Europe.

Materials and Methods

From January to March 1998, 8 lots of mussels were analyzed. The mussels (*Mytilus edulis*) were harvested from 4 commercial sites in Notre Dame Bay, Newfoundland.

The effect of cooking time on meat yield was assessed by recording yield losses during the cooking process. Individual mussels were studied first, then 1-kg samples of mussels. Individual mussels were used because mussels in a pot cook unevenly. This was determined in a small experiment in which thermocouples recorded the temperature in 5 regions within the pot and by visual inspection of the mussels during the cooking process.

A total of 24 mussels were individually cooked using a 300-mL glass beaker adapted as a steamer. Each mussel was placed in the beaker when the water inside (50 mL) was boiling vigorously. At 1-minute intervals, the mussel was taken out, drained, weighed, visually inspected and returned to the beaker to continue the cooking process.

Cooking time was then determined using 1-kg samples of mussels. The mussels were placed into a 5-L steamer pot in which 500 mL of water was boiling heavily. A total of 9 samples were cooked for either 9,12 or 15 minutes (0, 3 and 6 minutes after all mussels were fully cooked (category 4)). Once the desired cooking time was reached, the mussels were shucked by hand.

The effect of the length of time between the end of the cooking process and the post-cooking, air-drying time was assessed. A total of 9 samples were analyzed. Once each sample was cooked, weight losses were recorded every minute during and after shucking.

Shell and meat dry weights were determined after the mussels were dried in a preheated oven (85°C) until they reached a constant weight. The North American (N), European (E) and dry (D) meat yields were calculated for all the 1-kg samples processed:

N = (cooked meats x 100) / (cooked meats + cooked shells),
E = (cooked meats x 100) / (fresh weight), and
D = (dry meats x 100) / (dry meats + dry shells).

The effect of debyssing and the length of time the mussels were out of the water prior to meat yield determination were also assessed. A lot of unprocessed mussels was divided into two parts. One group was debyssed by hand and the other group was left intact. Meat yields were calculated (triplicates) for each half-lot at days 1, 4 and 9. A shelf-life study was performed using two 100-mussels samples, one of debyssed and the other of non-debyssed mussels. The samples were maintained at 4°C.

Results and Discussion

When the cooking process started, there was a period of time when the meat yields did not change. For our purposes, this period was called the "warming phase". It varied from 0 to 3 minutes and was mainly dependent on the initial temperature of the mussel. During this phase, the temperature of the mussel increased until it reached the temperature at which the tissue started to denature (beginning of the cooking phase), water began to evacuate from the tissues, and water soluble proteins and salts leached out.[9] This dehydration process was rapid at the beginning of the cooking procedure, when the mussel tissues were fully hydrated, but as the tissue dehydrated, the rate of weight loss decreased. The regression of the average of the 24 individual samples was $\log y = -0.172 \log x + 1.642$ ($R^2 = 0.963$; $P<0.01$), were x is time and y is meat yield. The warming phase was not included in this regression.

The next step was to determine the cooking time. However, the time required to cook mussels is variable and depends on the power of the stove, the size and shape of the pot, the initial temperature of the mussels, etc. To solve this problem, it was decided to link the visual appearance of the mussel meats to the cooking time. In other words, the mussels would not be considered to be cooked until they achieved a certain appearance, regardless of the time required to reach that state. Five categories of cooking status were used:

0) *Raw* — The valves are closed and the mussel is considered to be alive.
1) *Mantle separated from the shell* — The mussel mantle starts to cook, shrink, and separate from the shell.
2) *One set of adductor muscles is separated from the shell* — The anterior or posterior adductor muscle is detached from the shell; the external appearance of the meat is wet and mushy.
3) *Cooked outside but raw inside* — The external surface of the meat has a firm consistency and can be dry. However, if the mantle is opened with a pair of tweezers, the gills, foot and inside walls of the mantle appear raw (wet and mushy).
4) *Totally cooked* — The outside of the mussel appears the same as in category 3, but the inside is cooked. The gills are shriveled or shrunk together and all internal structures have a firm, dry consistency (Fig. 1).

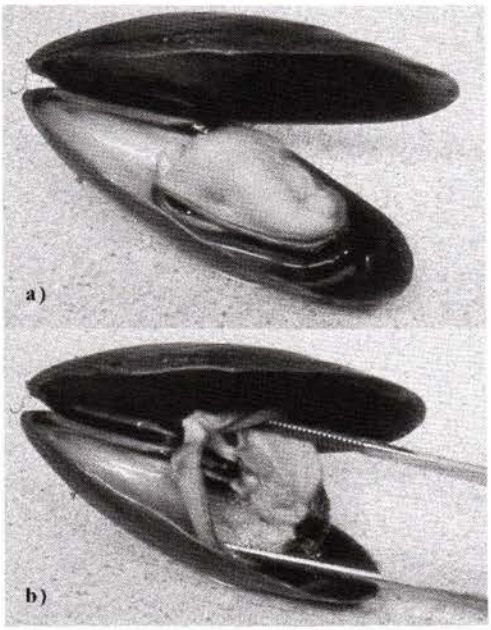

Figure 1. External (a) and internal (b) appearance of a category 4 mussel.

The proposed cooking time in terms of the visual aspect of the mussel meats can be defined as 3 minutes after all mussels have reached category 4 (Fig. 2). This suggested cooking time is an approximation. A variation in the cooking time of 6 minutes (3 minutes over and 3 minutes under the proposed cooking time) will generate a variation in meat yield of only 3.15%. These results were tested in 1-kg samples, which is the proposed sample size for determining meat yield.

In the experiment using 1-kg samples, the analysis of variance (ANOVA and Tukey test)[10] showed significant differences only between samples cooked 9 minutes and those cooked 12 and 15 minutes ($P = 0.02039$). Therefore, the proposed cooking time of 3 minutes after all mussels are fully cooked (category 4) also works well with 1-kg samples. A similar cooking time (10 minutes) was used by Bernard.[11]

Yields decreased by approximately 1% with the air-drying process. However, most of the decrement occurred in the first 2.5 minutes. Since the time required to shuck a kilogram of mussels is about 4 minutes, it is recommended that the shells and meats be weighed immediately after being shucked. The regression of the average of the 9 samples was $\log y = -0.0117 \log x + 1.523$ ($R^2 = 0.956$; $P<0.01$), were x is drying time and y is meat yield.

The analysis of variance showed a significant de-

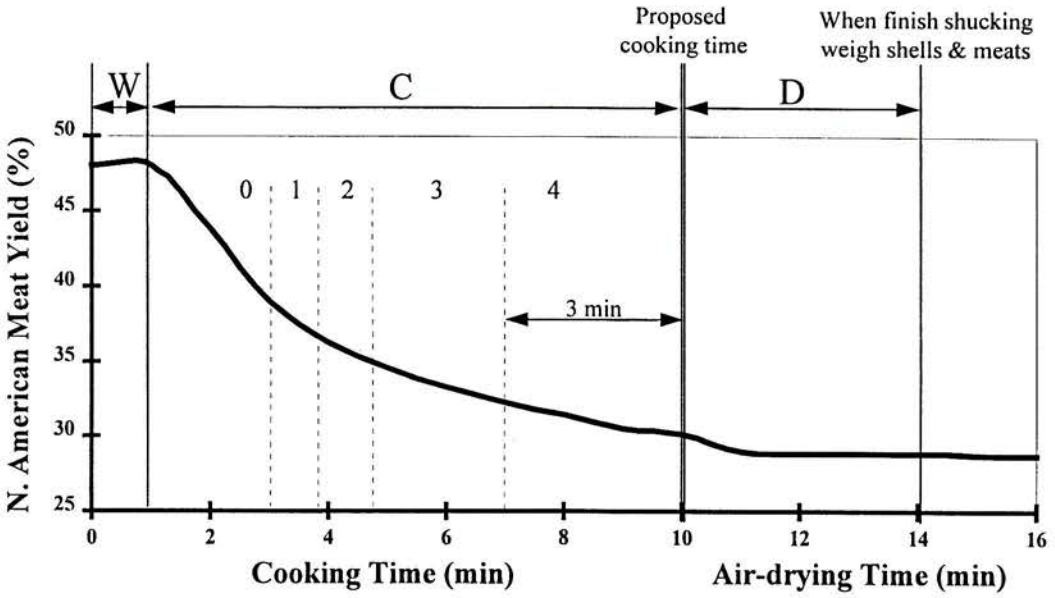

Figure 2. Meat yield profile during cooking and air-drying processes. The warming (W), cooking (C) and drying (D) phases are shown above the graph. The dotted lines are divisions between the categories of cooking status. The proposed cooking time is 3 minutes after the mussels reached category 4 (fully cooked).

crease of 0.5% in meat yield due to debyssing (P = 0.0136). However, yields are not affected by time out of the water (P=0.3442), at least during the first 8 days.

There was no significant difference in shelf-life between debyssed and non-debyssed mussels (shelf-life of 10% of mortality).[12] The sample size of the experiment was too small to make any conclusions. However, we recommend further assessment of this, since it is contrary to the shelf-life of European mussels.

The European and dry methods of determining meat yield are well correlated with the North American method ($R^2 = 0.903$, $P<0.01$, and $R^2 = 0.894$, $P<0.01$, respectively). The conversion formula is presented below and it allows comparison between results obtained with the three methods:

$N = 0.953\ E - 10.27 = 0.549\ D - 6.130$.

Conclusion

The traditional cooking time of 5 to 6 minutes (until mussels opened) should be increased to 10 to 12 minutes (3 minutes after all mussels reach category 4) when determining meat yields.

If the processing factors influencing meat yields are better understood and the same procedure to calculate yields is employed by everyone, it will be easier to study the biological factors that affect meat yields (e.g., reproductive status,[6] health and pollutants,[8] etc.) and therefore improve farming practices.

We want to thank the Marine Institute of MUN for providing the funding for this research.

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Flow rate reduction in scallop grow-out trays

John Brake [1,2] *and G. Jay Parsons* [1]

Scallops are primarily cultured in Newfoundland using pearl nets. Recently, however, there has been interest in using trays to rear scallops. Hence it is important to examine the parameters that would affect the profitability of this type of culture. Feeding rates of scallops, and consequently growth, tend to increase with increased current velocity until an optimum level is reached, after which any increase in velocity results in decreased feeding. Therefore, the effect of the gear on the internal current velocity is an important factor in selecting gear type. In this study, the reduction in flow rate through 6 types of grow-out trays was determined using 10 experimental current velocities. The position within the tray where the measurement was taken and the tray type were both found to be statistically significant in terms of the reduction in flow rate. In all but 2 tray types, the reduction in flow rate was lowest near the front of the tray and highest in the back of the tray. In the Norwegian black tray, the greatest reduction in flow rate was on the sides of the tray, and in the bread tray the greatest reduction was in the front of the tray. There was a significant correlation between the surface area of the tray (% opening on bottom half; $R^2 = 0.71$, $P < 0.05$) and the reduction in flow rate, suggesting that mesh size is an important criteria in choosing tray type. These results can be used as a guide to help growers choose the best possible grow-out tray for their site.

Introduction

Scallops are subtidal, benthic, suspension-feeders that depend upon water currents to bring them food.[3] In scallop aquaculture, food availability is primarily controlled by the current velocity.[4] Although the velocity of the current cannot be altered directly, gear types, which impede the flow of water over the scallop, may be modified in some way. Such alterations, or the choosing of more suitable gear, can lead to higher food flux and better growth.

Feeding rates tend to increase with increased current velocity until an optimum velocity is reached.[5,6] This fact can be used to help ensure an optimal growth level for each site. For example, if a site has a current velocity and food concentration close to the optimum, then a gear type that allows good flow should be used. Where current velocity is too high and impedes feeding, a gear type that reduces flow to a more optimum level should be used. Therefore, the availability of different gear types, with varying capabilities of reducing flow, would allow aquaculturists to optimize growth under different conditions.

Pearl nets are currently used in Newfoundland to culture scallops but interest has recently developed in tray culture. The advantages of using trays are that a number of designs are available and trays are less expensive, require less labour, and are more durable than pearl nets. The effect of the trays on current flow is unknown. The experiments reported here evaluated the effects of and the interaction among current velocity, tray type, and position within the tray on reduction in flow rate.

Methods

Experiments were conducted in the model flume tank at the Marine Institute, a 1:8 scale model of the flume tank in the Fishing Technology Unit of the institute that is used for testing fishing gear and boat hulls. A stainless steel tray holder and plexiglass top were constructed to facilitate the various measurements in the various tray types. Six trays were studied: Norwegian design — black, Norwegian — grey, Mexican, Irish, British Columbian Dark Sea, and Newfoundland commercially-used bread tray (Table 1).

Flow rate reductions in these trays were tested at 3

positions (front, side and back), and each of these positions were tested at 10 current velocities (10 to 100 cm/s, in 10 cm/s intervals). All trays were tested in triplicate, and each trial was replicated. An impeller current meter was used for all measurements of current velocity. Percent surface area was measured only for the bottom half of the sides of each tray as this represented the area in which flow would have an impact on scallops held in the trays.

The data were analysed using the SPSS statistical package. Multi-factor ANOVA, regression and descriptive statistics were all used in the analyses. The level of significance was set at $\alpha = 0.05$.

Results

Both tray type and position within the tray were found to be significant ($P < 0.001$). When the descriptive statistics for these factors were examined, a general pattern became evident. For most trays, the mean velocity was highest at the front of the tray, lower in the middle of the tray, and lowest near the back of the tray. There were two exceptions: the Norwegian black tray had the lowest velocity in the middle of the tray and the highest in the front of the tray, and the bread tray had the highest velocity in the back of the tray and the lowest in the front. For all trays, the effect of position within the tray on current velocity was found to be statistically significant.

A significant correlation ($R^2=0.55$; $P<0.001$) between external velocity and internal velocity was found. The percent reduction was then regressed against the external velocity for each tray in order to determine the relationship between these two factors. There was no correlation between flow reduction and external velocity in any of the trays examined, indicating that the reduction in the tray as a whole (i.e., excluding position within the tray) was independent of the current velocities. The relationship between the surface area of the lower half of the tray and percent current reduction was significant ($R^2 = 0.71$; $P<0.05$) (Fig. 1).

Table 1 is a summary of some of the notable factors, such as the presence or absence of dividers or pole holders, which impede flow through the trays.

Discussion

In this study, flow rate reduction differed significantly with both tray type and position in the tray. Each tray had a different mesh shape and size, and some trays had dividers and some did not. All of these factors contributed to the differences in the reduction in flow rate among the various types of trays. The significant differences between the flow rate reduction and position in the tray was due to five of the six trays having a central sheath and/or dividers which blocked the current. As well, after the flow hit the front mesh of the tray, it likely became turbulent and caused the formation of eddies and current fluctuations in different parts of the trays.

Food uptake by scallops is one of the most important

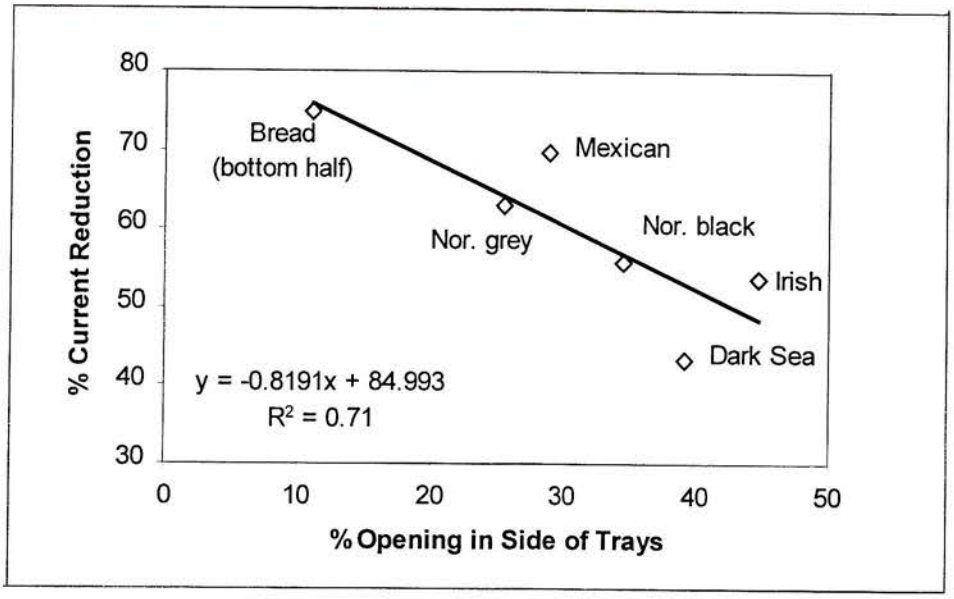

Figure 1. Relationship between surface area (% opening) and percent current reduction.

Table 1. Characteristics affecting the flow through the six trays examined for current reduction.

Tray Type	% Current Reduction	% Open Side	Dividers (yes/no)	Pole Holder (yes/no)	Additional Features
Dark Sea	43.24	39.12	yes	yes	Deep, large holes; large dividers and pole holder; corners angled at 45°
Norwegian Black	55.55	34.51	yes	yes	Small mesh, small pole holder
Norwegian Grey	62.94	25.51	yes	yes	Round holes/mesh, relatively high dividers
Mexican	69.59	28.73	yes	yes	Round holes/mesh; relatively high dividers
Bread	74.75	11.00	no	no	No mesh; upper portion of the side panel open, lower portion has no holes; rectangular
Irish	53.47	44.75	no	no	Smallest tray; mesh relatively large compared to tray size

factors determining the feasibility of grow-out strategies. To assess the use of trays in the grow-out of scallops it is necessary to know the effect of the trays on the water currents which carry the food to the scallops. Wildish and Saulnier[6] found optimal currents to be 3 to 6 cm/s, and scallops ceased feeding at currents of 30 cm/s. In our study the velocities used were higher than 3 to 6 cm/s. The implications of this study are that if a particular farmer would like to culture scallops using one of the six trays examined, there is now a basis on which to form a decision on which tray to use. Bread trays showed the highest percent current reduction and therefore may be useful in areas of higher than optimal flow. Dark Sea trays showed the lowest percent current reduction, and therefore may be more useful in areas of low flow, where flow should not be impeded through the tray.

Another consideration is the variability of flow rate reduction in the trays. Trays such as the bread tray and the Norwegian black tray showed more variation in the amount of flow reduction among the different positions than did the Norwegian grey and Irish trays. The ramification of this is that trays with greater variability may show more variability in scallop growth, as growth is partially dependent upon flow rate.[7] More research in the understanding of flow variation within the trays is required.

Conclusions

All six tray types have characteristics that may affect flow rate as water currents pass through them. In this study it was found that, with the exception of the Norwegian black trays and the bread trays, the current was reduced the most near the back of the tray and the least near the front of the tray. In the bread trays, the reduction was greatest near the front, while it was greatest along the sides of the Norwegian black trays. Both tray type and position within the tray (front, side and back) were found to significantly affect flow through the tray. The correlation between tray surface area (% opening) and flow reduction should serve as a good criterion for the selection of the appropriate tray type at the various grow-out sites. This work provides a baseline for future research on scallop tray culture and should be useful to scallop farmers considering grow-out in trays.

Future work should be conducted on phenomena such as turbulence and variability between trials. Dye tests may help to show where turbulence occurs within the different tray types. As well, more work on *in situ* flow patterns and feeding trials with scallops will help make the knowledge gained in these studies more applicable to growers.

We would like to thank George Legge, Ray Fitzgerald, Keith Rideout, Gus Yetman, Alistair Struthers and the Marine Institute aquaculture and library staff for their assistance throughout this project.

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Effect of algal harvest phase on larval and post-larval growth of giant scallops (*Placopecten magellanicus*) in a commercial hatchery

Catherine M. Ryan,[1] *Jay Parsons*[1] *and Pat Dabinett*[2]

The effect of diets of algae harvested from log and near stationary phase cultures on growth of scallop larvae and spat, and the relationship of spat growth to lipid class composition of the diet was investigated. In 3 out of 4 experiments, larvae fed log phase algae were significantly larger than those fed stationary phase algae. Similar results were obtained with spat from four feeding trials. Lipid analysis of the diet indicated that significant positive correlations were obtained for spat growth and quantity of methyl ketones and sterols, and significant negative correlations were obtained for spat growth and alcohols, ethyl ketones, free fatty acids, hydrocarbons, total acyl lipids, phospholipids, and sterol and wax esters. Acetone mobile polar lipids, diacylglycerols, and triacylglycerols were not significantly correlated with growth.

Introduction

The major problem facing the giant scallop industry in Newfoundland, Canada, is the unpredictability of the availability of spat.[3] If spat could be reliably grown in a hatchery situation, a reliable source of seed would be ensured to the farmers and the seed supply problem would be eliminated.

One important procedure with regards to the rearing of marine bivalves is the production of unicellular algae. Algae used as food must supply both energy and essential nutrients. Conditions under which algae grow affect the biochemical composition, energy, and nutrient value.[4] The biochemical composition of microalgae is influenced by such factors as the culture medium, temperature, light intensity, photoperiod, and phase of harvest.[5]

This study investigated the effect of algal harvest phase (logarithmic and near stationary growth phases) on growth of *Placopecten* larvae and spat.

Materials and Methods

Larvae were batch fed 20 cells/µL of a standard diet from the Seasalter algal system, under both logarithmic (0.40 divisions/day) and stationary (<0.20 divisions/day) growth phases, consisting of a mix of equal numbers of six algal species (*Isochrysis galbana*, *Isochrysis* (clone T.ISO), *Pavlova lutheri*, *Thalassiosira pseudonana* (clone 3H), *Chaetoceros muelleri*, and *Chaetoceros ceratosporum*) in four growth trials.

Juvenile scallops (spat) were stained for a duration of 72 hours using calcein (0.15 g/L of seawater), a non-toxic stain which is bound during calcification of the shell and which fluoresces bright yellow-green under epifluorescence microscopy.[6] The resulting fluorescent band was used as a benchmark from which to measure new growth during feeding trials. Calcein stained spat were batch fed 40 cells/µL of a standard diet in four growth trials.

Samples of algae were taken during two of the spat trials and analysed for lipid class composition. Total lipids were extracted with a mixture of chloroform and methanol. The Chromarod-Iatroscan TLC/FID system was used to identify and quantify lipid classes.[7] The relationship of spat growth to lipid class composition of the diet was explored using the Spearman rank correlation coefficient.

Results and Discussion

In 3 out of 4 experiments, larvae fed log-phase algae were significantly larger than those fed stationary-phase algae (*t*-test, $P<0.05$) (Fig.1). Similar results

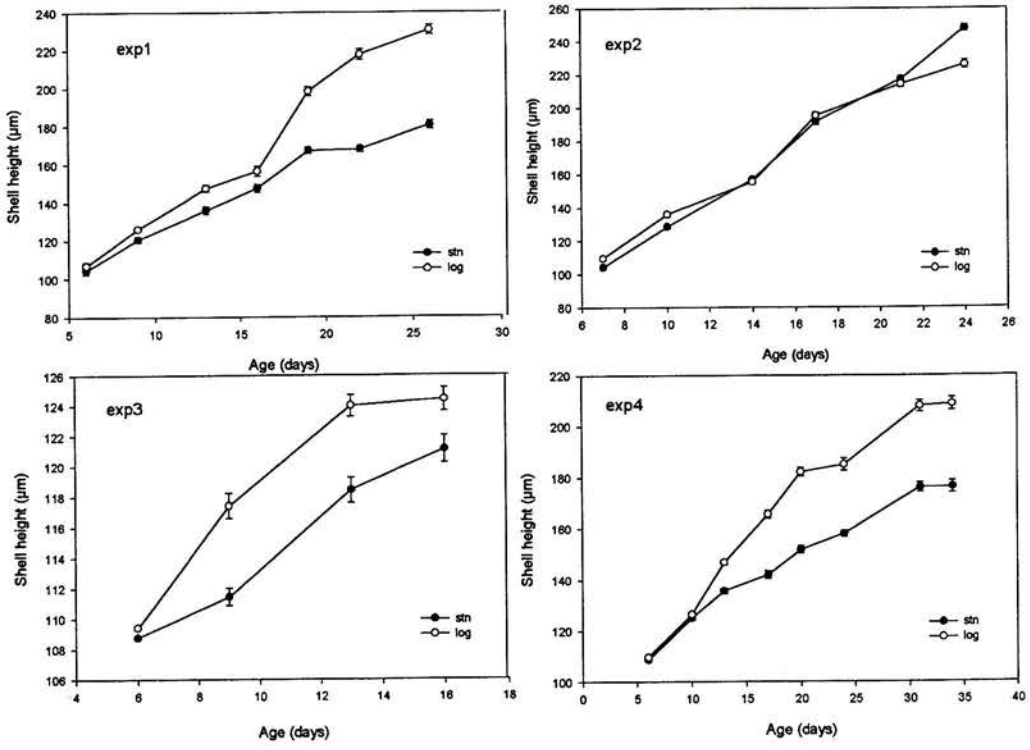

Figure 1. Larval growth: algal harvest from stationary/log phases

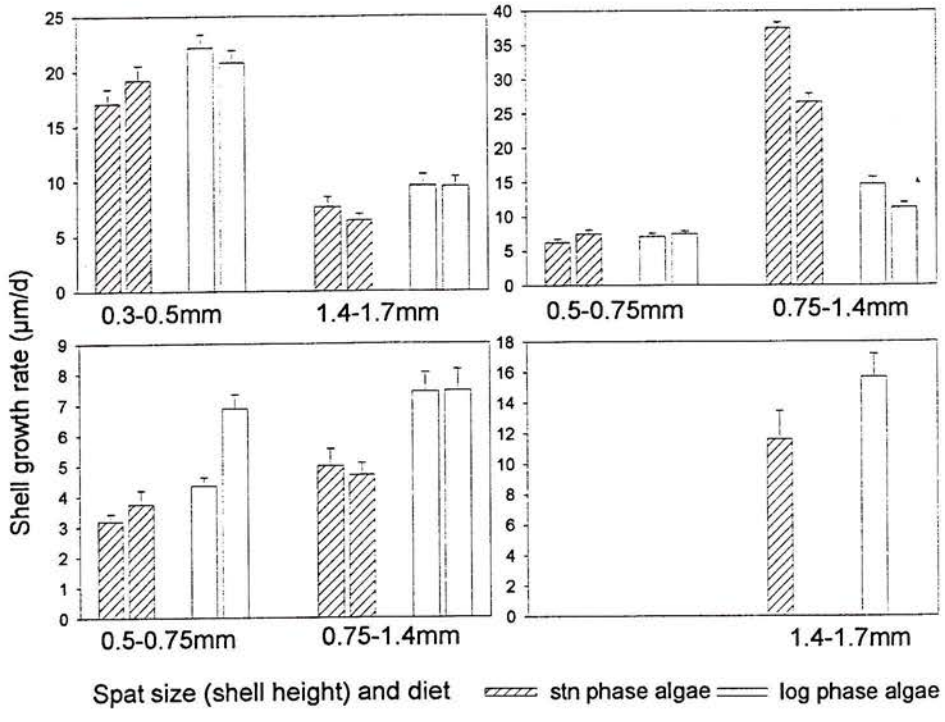

Figure 2. Scallop spat: shell growth and algal harvest phase

were obtained with spat from four feeding trials (*t*-test, $P<0.05$) (Fig. 2). These results are important with regards to operating continuous algal culture systems because such systems can be operated in either log or stationary phase but are difficult to operate in both phases simultaneously. These results suggest the systems be run in log phase to obtain optimal growth rates of scallop larvae and spat.

The lipid class composition of microalgal cells at the different growth phases can differ significantly,[8] and harvesting at specific growth phases may enable the lipid class composition of microalgal cultures to be tailored for specific purposes, such as obtaining optimal larval and spat growth rates. The preliminary lipid results illustrated that significant ($P<0.01$, 2-tailed) positive correlations were obtained for spat growth and quantity of methyl ketones and sterols, and significant negative correlations for spat growth and alcohols, ethyl ketones, free fatty acids, hydrocarbons, total acyl lipids, phospholipids, and sterol and wax esters. Acetone mobile polar lipids, diacylglycerols, and triacylglycerols were found not to be significantly correlated to growth.

The implication of these findings with regards to *Placopecten* larval and spat growth rates is that growth rates can be optimized by feeding algae harvested during the logarithmic growth phase. This in turn will assist in optimizing commercial production by reliably growing larvae and spat in a hatchery.

We would like to thank ACERA and CCFI for funding this research, and the staff of the Belleoram Sea Scallop Hatchery for their assistance.

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Toxicity of selected cryoprotectants on trochophore larvae of *Mytilus edulis*

Brent Tompkins and Cyr Couturier[1]

The toxicity of three known cryoprotectants was examined as a preliminary step to establishing a protocol for the cryopreservation of *Mytilus edulis* trochophore larvae. Trochophore larvae were subjected to ethylene glycol, dimethyl sulfoxide, or sucrose, at three concentrations, and the effect of equilibration temperature (4°C and 15°C) on the survival of cryoprotectant–challenged trochophores was assessed. Larvae were considered to have survived if they underwent metamorphosis to the D-stage. There were no significant differences in toxicity among the three cryoprotectants. Survival varied inversely with cryoprotectant concentration in all treatments. Equilibration temperature had little effect on the toxicity of ethylene glycol and dimethyl sulfoxide. In contrast, the survival of larvae exposed to sucrose was higher at 4°C than at 15°C. The inverse relationship between cryoprotectant concentration and survival suggests the cryoprotectants cause both osmotic and biochemical stress to the trochophores. The cryoprotectants probably act indirectly as well by altering membrane permeability to selected ions. Lower equilibration temperatures are believed to reduce metabolic demands and hence biochemical toxicity, which would account for the reduced toxicity of sucrose at the lower temperature.

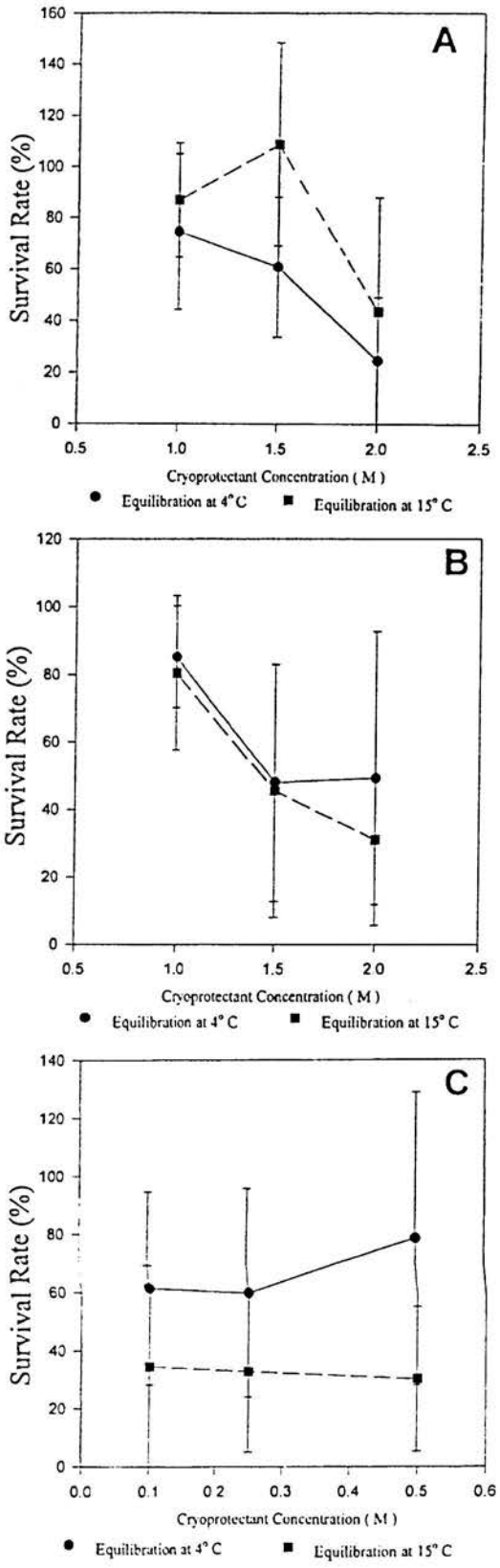
Introduction

Cryopreservation of marine bivalve embryos and larvae may offer tremendous potential for aquaculturists and non-aquaculturists alike. Species or stocks with desirable traits such as rapid growth or disease resistance could be kept almost indefinitely in cryostorage for later use.

Bivalve hatcheries could improve their production by preserving batches of embryos/larvae during peak periods when larval quality is high and rearing space is limited. Trochophore larvae of marine bivalves contain high levels of HUFAs essential for marine larval fish development: current suppliers of cryopreserved trochophores produce warm water species, such as *Crassostrea gigas*, but there has been little success in introducing these species to cold-water marine fish larvae reared at temperatures below 8°C.[10] Finally, bivalve embryos are often employed in marine toxicity assays due to their sensitivity, but these assays suffer from seasonal variation in the supply of the larvae of interest. Cryopreservation may offer a means to provide a continuous supply of similar quality animals for year-round toxicity assays, enabling more reliable comparisons among assays.

Cryopreservation of marine invertebrate gametes and embryos has been achieved with variable success in a few species of sea urchins,[2] bivalves,[3,4] and crustaceans.[5] Cryoprotectant chemicals are employed to prevent cell damage during the freezing and thawing processes. Though cryoprotectants offer some degree of cellular protection during cryopreservation, they may be toxic, particularly at the high concentrations required for cryoprotection. Few studies have attempted to assess the relative toxicity of cryoprotectants prior to establishing freezing protocols, particularly in marine bivalve embryos or larvae.[4,6,7] Moreover, equilibration temperatures employed during cryoprotectant exposure are generally above 20°C, leading to enhanced toxicity.[8]

The goals of the present study were to evaluate the toxicity of selected cryoprotectants on trochophore larvae of *Mytilus edulis*, a cold-water bivalve species, to ascertain the optimal concentrations of the cryopro-



tectants for possible use in cryopreservation trials, and to determine the influence of low equilibration temperatures on cryoprotectant toxicity.

Methods

Thirty adult mussels (50 to 60 mm shell length) were held in a glass aquarium and conditioned for one month in unfiltered seawater at 12°C and 32 ppt salinity. Aeration was provided and animals were fed a daily ration of 50 L of concentrated algae (*Isochrysis galbana* at 2 million cells/mL).

Spawning was achieved by placing mussels directly in heated seawater (26°C), filtered to 1 µm. Eggs were fertilized with a mixture of sperm and excess sperm, faeces, and debris were removed prior to incubation at 14°C in basket-type chambers.[6] The fertilized eggs were incubated for 24 h to the trochophore stage.

Trochophores were challenged for 30 minutes with three cryoprotectants, ethylene glycol (EG), dimethyl sulfoxide (DMSO), and sucrose (SU), at three concentrations (EG and DMSO at 1.0 M, 1.5 M, and 2.0 M; SU at 0.1 M, 0.25 M, and 0.5 M). Two equilibration temperatures were employed (4°C and 15°C) and each treatment was done in triplicate. Controls were manipulated in an identical fashion to other treatments, but without exposure to cryoprotectants.

Trochophores were allowed to develop for an additional 48 hours, to the D-stage, and were then preserved in formalin for later determination of survival, expressed as a normalized percentage of control mussels. Cryoprotectant toxicity was analyzed with a Model I 3x3x2 factorial ANOVA.

Results

- The type of cryoprotectant had no significant effect on survival to the D-stage ($P = 0.3112$).
- Survival varied inversely with concentration of ethylene glycol and dimethyl sulfoxide (Fig. 1, $P < 0.05$).
- Survival was independent of sucrose concentration (Fig. 1, $P > 0.05$).
- Survival in ethylene glycol and dimethyl sulfoxide was not influenced by equilibration temperature (Fig. 1, $P > 0.05$).

Figure 1. Survival of *Mytilus edulis* trochophores to D-stage following 30–minute exposures to: (A) ethylene glycol, (B) dimethyl sulfoxide, and (C) sucrose at two equilibration temperatures. Data are normalized to the controls. Values represent the mean ± s.d. (n=3). Note different x-axes.

- Survival was significantly lower for sucrose at 15°C compared to 4°C (Fig. 1, P = 0.03).

Discussion

The toxicity of ethylene glycol has been shown to increase in oyster, clam, and scallop embryos above concentrations of 1.0 M.[6] In the present study, ethylene glycol was not toxic to mussel embryos at concentrentrations below 2.0 M.

The toxicity of dimethyl sulfoxide to mussel embryos increased with higher concentrations but was not significantly different from the toxicity of the other cryoprotectants. In contrast, previous studies have found DMSO to be toxic to bivalve embryos at any concentration.[6]

Sucrose has rarely been used in cryopreservation studies with marine bivalves. The toxicity depends to a large extent on the species, the quality of the embryo, and the concentration of cryoprotectant.[3,4,6] Mussel embryos were unaffected by the concentrations of sucrose employed in the present study.

In mammalian models, lower equilibration temperatures are thought to reduce cryoprotectant toxicity by reducing poisoning of metabolic pathways, since the penetration rate of cryoprotectants is unaffected by temperature.[9] The results obtained here with sucrose suggest a similar mechanism of toxicity reduction in mussel embryos.

Conclusions and Recommendations

- Several cryoprotectants were found to have relatively low toxicity to mussel embryos. Advantages may be gained by employing mixtures of cryoprotectants, which have different protective mechanisms (e.g., EG and SU).
- Cryoprotectant toxicity was reduced at the lower equilibration temperature, supporting mammalian studies.
- Further studies related to equilibration temperatures and exposure times are warranted before cryopreservation trials are undertaken.

We would like to thank Donna Somerton for helping with algal culture and maintenance.

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Studies of the turbellarian *Urastoma cyprinae* in relation to the presence of mucus on the gills of oysters *Crassostrea virginica*

Nicole T. Brun,[1] Andrew D. Boghen[1] and Jacques Allard[2]

Urastoma cyprinae occurs on the gills of oysters *Crassostrea virginica* and is believed to feed on the mucus secreted by the host. Studies were undertaken to establish if related activities by *U. cyprinae* support this contention. Results indicate that there is a positive chemotaxis by *U. cyprinae* to oyster gills, and more specifically to isolated oyster mucus. Moreover, zymography studies using electrophoretic techniques of mucus previously exposed and not exposed to *U. cyprinae* demonstrate that there is a greater level of protease activity in mucus that had previously been in contact with starved worms compared to mucus that had been exposed to fed worms.

Introduction

Urastoma cyprinae has been reported on the gills of bivalves throughout the world.[3-5] In Atlantic Canada, it is recurrent in the eastern oyster, *Crassostrea virginica*.[6, 7] Contrary to the early belief that *U. cyprinae* is a commensal,[8, 9] recent investigations have demonstrated the the "gill-worm" can induce damage to mussels and is therefore parasitic.[10] Our previous work has shown that there is a strong attraction by *U. cyprinae* to oysters,[11] thus dispelling the likelihood that its presence in the host is coincidental. Some authors have suggested that the worm feeds on the mucus secreted by the gills;[12] this, however, has never been the subject of scientific scrutiny.

The objectives of the study were: 1) to determine if *U. cyprinae* is attracted to oyster mucus, and 2) to identify if there are biochemical changes in oyster mucus that may be attributable to the presence of the worms.

Methods

U. cyprinae were isolated from the gills of oysters collected at Shippagan Bay (New Brunswick, Canada) during the summer of 1997. The worms were maintained in filtered sea water (25 ppt) at 22°C for 12 hours. Chemotactic responses were determined using specifically designed two- and four-well glass chambers.[11,13] The stimulants tested were: a) isolated oyster mucus vs body of the oyster (gills removed), b) isolated oyster mucus vs gills, and c) isolated oyster mucus vs gills vs mucus-free gills vs sea water. The above stimulants were homogenized (except the mucus), and centrifuged in the following proportions: 1:10 (v/v) substrate:filtered sea water (25 ppt). The concentrates were placed in the wells of the chambers, and *U. cyprinae* were added through the central opening. After 60 minutes, the wells were drained and the worms were counted. A total of 10 replicates (60 worms/replicate) were tested using the two-well glass chambers, and 6 replicates (100 worms/replicate) were tested using the four-well glass chambers. The Student's *t*- test was employed.

Electrophoretic techniques[14,15] were used to compare protease activity in the following samples: isolated oyster mucus (control), oyster mucus previously exposed to starved *U. cyprinae*, and oyster mucus previously exposed to fed *U. cyprinae*. Electrophoresis was performed for 45 minutes at 100 volts. The gel was stained for 30 minutes, destained for several hours, and subsequently analyzed.

Results and Discussion

Findings reveal that *U. cyprinae* display a definite attraction to isolated oyster mucus compared to the oyster body with the gills removed (P= 0.004). More specifically, the worms are attracted to the following stimulants in a descending order of preference: iso-

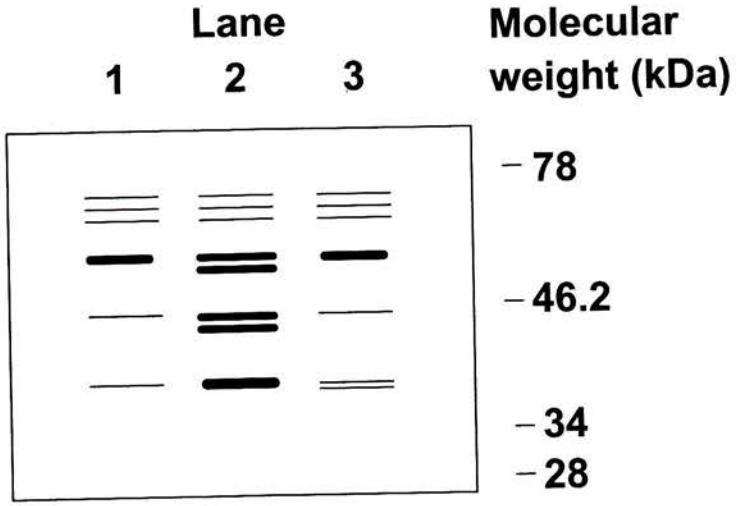

Figure 1. Zymogram comparing protease activity of isolated oyster mucus (control) [lane 1], oyster mucus previously exposed to starved worms [lane 2], and oyster mucus previously in contact with fed *U. cyprinae* [lane 3].

lated mucus, gills, mucus-free gills and sea water. The difference in the degree of attraction is less evident, however, between the isolated oyster mucus and the gills (P= 0.174). Since the gills of oysters are coated with mucus, it is likely that the mucus may be contributing in attracting *U. cyprinae* to the host.

No obvious differences were found between the banding patterns of isolated oyster mucus (control) and mucus previously exposed to fed worms (Fig. 1). However, when the banding patterns of mucus that had previously been exposed to starved *U. cyprinae* are compared to mucus that had been exposed to fed *U. cyprinae* and the control, greater protease activity was detected in the mucus sample that had been in contact with starved worms. This is reflected by the appearance of two double bands between the 44- and 60-kDa range, and an additional strongly-staining band near the 38 kDa level (Fig. 1). While the precise role of the proteases has not been defined, the latter may turn out to be a consequence of digestion-related activities induced by *U. cyprinae*.

The electrophoretic component of this project was conducted in collaboration with Dr. Neil Ross (IMB-NRC). Financial support provided to the senior author by the Faculty of Research and Graduate Studies of the Université de Moncton is greatly appreciated.

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Effect of deployment date and environmental conditions at a farm–based nursery on growth and recovery of hatchery-reared sea scallops *(Placopecten magellanicus)*

L. Levy,[1] *G. J. Parsons* [1] *and P. Dabinett* [2]

The effect of deployment date on growth and recovery was studied to provide information for management of nursery-size scallops, which range from 1.4 to 7.0 mm in shell height. The objective of this study was to find a time period for deployment on a farm-based nursery that would yield commercially acceptable growth and recovery rates. Spat of the same size class were deployed at the same stocking density over consecutive 18–day intervals beginning in August. Environmental factors were monitored weekly. The shell height and recovery of the scallops were determined at the end of each interval. After sampling, the collectors were re-deployed. The final sample was taken on November 8, 1997. Results indicated significant differences among scallop growth rate and recovery for the five consecutive intervals. Significant correlations were found between both growth rates and recovery rates and some of the environmental conditions. By November 8, only the scallops deployed in August were above 7 mm in size and thus could be sorted and transferred to larger mesh equipment. The findings of this study indicate that early farm-based nursery deployment allows for high growth and recovery rates. But deployment later than early September would require that scallops be overwintered in the nursery.

Introduction

Hatchery rearing of *Placopecten magellanicus* has been researched since 1980. Little is known about nursery culture, which is the transition between a controlled hatchery setting to a less controlled, intermediate culture environment. The Belleoram Sea Scallop Hatchery (BSSH) has defined the nursery stage as the period of growth from 1.4 to 7 mm in shell height. To minimize this transition period, nursery strategies must optimize growth and recovery rates. The BSSH operators deploy nursery-sized, hatchery-reared scallops in mesh equipment at a farm-based nursery. In using the ocean as a scallop nursery, growth and recovery rates are functions of measurable natural factors such as water quality, food availability, and presence of potential predators.

Several studies have shown that growth rates of scallops vary seasonally due to natural fluctuations in food and temperature levels.[3,4] Growth rates of *P. magellanicus* are highest in the summer and lowest in the winter[5-7] and show no increase during the autumn bloom.[8] For hatchery-reared *Patinopecten yessoensis*, growth rates are dependent on the timing of deployment to the farm-based nursery.[9]

Salinity and the presence of predators impact the recovery of scallops. Long term exposure to salinity concentrations below 18 parts per thousand (ppt) has been found to cause mortality in scallops.[10] Sea stars are an important predator of sea scallops when they settle on rearing equipment and prey on scallops.[6,11]

Timing of deployment of nursery-sized spat is critical for optimizing growth and recovery rates. The goal of this study was to find a window of opportunity for deployment of hatchery-reared sea scallops at a farm-based nursery that enhances growth and recovery rates.

Methods

Scallops between 1.4 and 2.0 mm in shell height were obtained from BSSH. Scallops (n=500) were stocked in 1.2 mm collector bags containing Netron and left over night in ambient water. Initial shell height

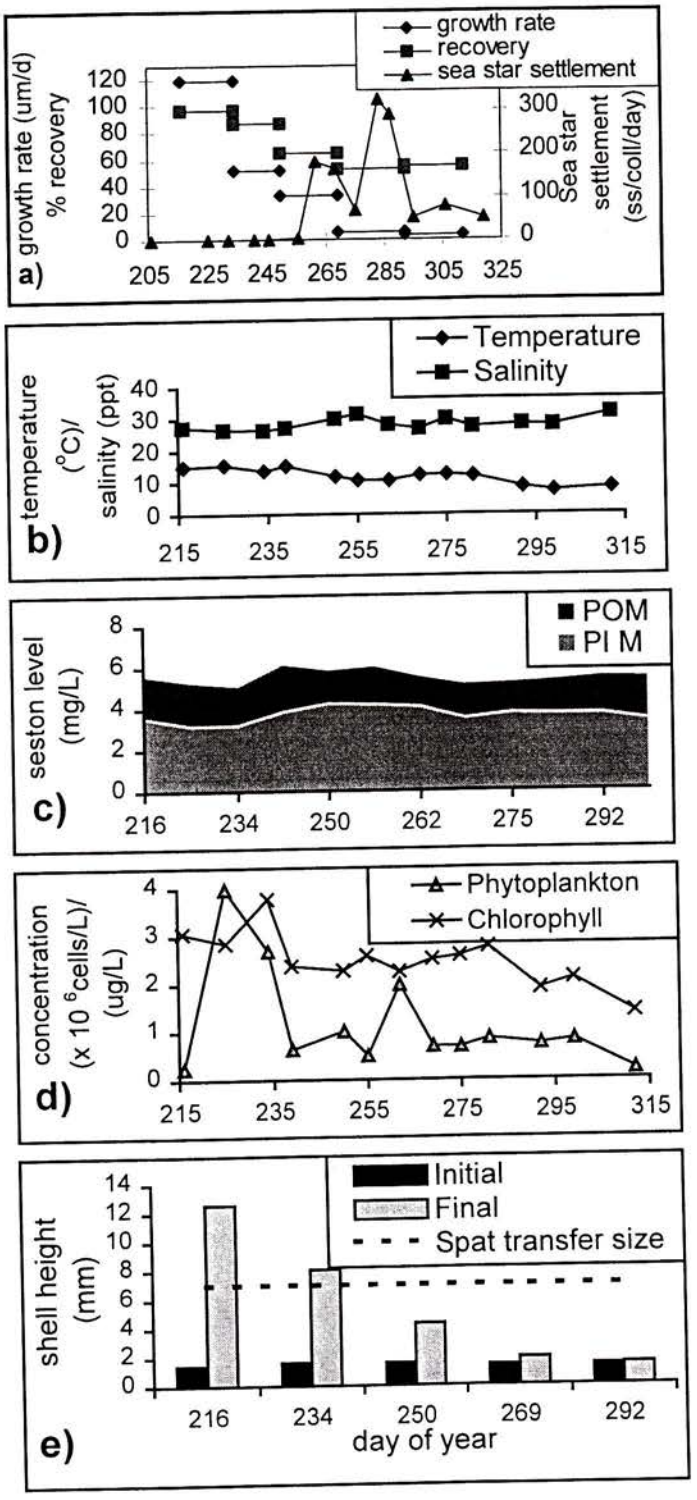

Figure 1. a) Scallop growth, recovery and sea star settlement rates, b) temperature and salinity, c) seston levels (PIM+POM=TPM), d) phytoplankton and chlorophyll *a* concentration, and e) shell heights of scallops at Pool's Cove, Newfoundland, on November 8.

was determined for each replicate (n=30). The number of replicates varied due to scallop availability, but there were at least two replicates for each of the five 18-day intervals. The intervals began on August 4, 1997, and ended on November 8, 1997. Scallops were deployed at a depth of 5 m at Shell Fresh Farms, Pool's Cove, Newfoundland. At the end of each interval, the scallops were counted for recovery and measured for shell height (n=30). Scallops were re-deployed and measured again on November 8. Over the study, phytoplankton, total particulate matter (TPM), particulate inorganic matter (PIM), particulate organic matter (POM), chlorophyll *a*, temperature and salinity were measured weekly. Eight pearl nets, filled with gill netting, were deployed weekly and collected bi-weekly to measure sea star settlement. Variation in growth and recovery was analyzed using an ANOVA while equality of means was analyzed using independent sample *t*-test. Correlation analyses were also performed with growth and recovery and environmental conditions.

Results

Growth and recovery rates declined over all intervals (Fig. 1). Variation for both growth and recovery rates was significantly different among dates ($P<0.01$), but not replicates ($P=0.859$; $P>0.05$). Final interval shell heights were significantly different from initial shell heights ($P<0.01$). All final interval shell heights were significantly different from each other except for the September 26 and October 19 deployments ($P=0.084$). A one-way ANOVA indicated significant differences in recovery between interval dates ($P<0.01$). A comparison of initial stocking density with final recovery for each interval indicated significant differences in means ($P<0.05$).

Over the study, a declining trend was found in temperature (°C) and food levels (phytoplankton count (cells/L), chlorophyll *a* (µg/L) and POM (mg/L)), while an increase occurred for salinity (ppt) and sea star settlement

Table 1. Pearson's coefficient of correlation for growth and recovery rates, with environmental factors (* indicates significant correlation).

Parameter	Growth Rate (μm/day)	Recovery (transformed)
Temperature	0.840*	0.828*
Salinity	– 0.826*	– 0.698*
TPM	– 0.043	0.233
PIM	– 0.573	– 0.358
POM	0.700*	0.714*
Phytoplankton	0.996*	0.922*
Chlorophyll a	0.901*	0.849*
Sea star	– 0.796*	– 0.890*

(sea stars/collector/day), which peaked between September 19 and October 23 (Fig. 1). An ANOVA indicated significant differences in settlement among sample periods. Several of the environmental factors correlated to growth and recovery rates (Table 1).

Discussion

The highest growth and recovery rates occurred when scallops were deployed early in the season. The high rates may be explained by the environmental factors examined. It is not unexpected to have higher growth rates earlier in the season when temperatures are high and more food is present. Sea scallops clear more food during higher temperatures[12] and increased food availability promotes higher growth rates.[13] It is unlikely that sea star settlement, which had a significant negative correlation with growth rate, had any effect on growth as there was no difference in the size of empty shells compared to the size of live scallops.[14] Studies show that small scallops are more vulnerable to sea star predation.[15] This needs further study for settling sea stars and nursery-size sea scallops.

Recovery was correlated with temperature, food levels, and sea star settlement; however, all may not have reduced recovery. The decline in recovery rates with the increase in sea star settlement may be explained by predation. Scallops are a primary prey of sea stars,[6,11] hence sea star settlement on suspended scallop equipment may result in scallop predation. Temperature and food levels were not low enough to cause mortality, but poor acclimation to the lower food and temperature levels at later intervals may have resulted in mortality. This requires further investigation.

The negative correlation of salinity with growth and recovery may not be causative as the natural range of tolerance for juvenile sea scallops to salinity is 18 ppt and greater,[10] which was lower than salinity levels during the study.

Implications for Hatchery Management

Based on this study, management at the BSSH may want to start broodstock conditioning and spawning earlier in the year to produce nursery-size scallops for an early summer deployment. Also, they should monitor the environmental conditions to determine when the window of opportunity for deployment exists (August to mid-September in 1997). If transferring scallops to the farm-based nursery during sub-optimal conditions, the scallops should be acclimated (after mid-September in 1997). Most importantly, the early deployment of nursery-sized scallops should be recognized as a strategy for growing, sorting, and relaying scallops by autumn, such that they can be available in greater numbers for the growers due to higher growth rates and minimal loss to sea star predation.

We would like to thank ACERA for research funding and Belleoram Sea Scallop Hatchery and Shell Fresh Farms for scallops and research sites. L.A.L. thanks J. Parsons and P. Dabinett for advice in the field and in analysis of data.

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Giant scallop, *Placopecten magellanicus*, gonad maturation from juveniles to adults

Leslie-Anne Davidson[1] *and Yves Poussart*[2]

The progressive development of the gonadal tissue of juvenile giant scallops, shell height 5 mm to 80 mm, was observed and described in relation to their size and the time of year. The sexual development of the young scallops with shell height of 21 mm or greater was synchronized with that of the adults. The first time female scallops released sex cells (oocytes I) their shell height measured 31 to 40 mm (age of 21 to 25 months). The first time male scallops released sex cells (spermatozoa) their shell height measured 26 to 30 mm (age of 9 to 13 months). Scallops in size classes 61 to 70 mm and 71 to 80 mm filled their follicles with mature sex cells and they released the majority of these cells during the spawning period. Scallops in the size classes between 21 mm and 60 mm accumulated sexual cells but they did not release the majority of these cells during the spawning period. Larger scallops developed a greater number of sex cells than smaller scallops.

Introduction

The giant scallop, *Placopecten magellanicus*, is commercially fished off the east coast of Canada and the United States and has recently become popular as a cultured species. The reproductive cycle of commercial-sized scallops has been extensively studied but less is known about the gonadal development of pre-recruit sizes of scallops (< 80 mm). There are, however, some recorded sporadic observations. In the past, it was difficult to obtain samples of pre-recruit scallops as scallops can only be collected from wild beds by dragging or scuba diving and pre-recruit scallops are often difficult to locate in the wild. Scallop aquaculture has made it possible to obtain scallops of all sizes at the desired time of the year.

The objective of this study was to perform an in-depth investigation of the gonadal development of young giant scallops (< 80 mm) in relation to their size and to the time of year.

Methods

The young scallops (shell height 5 to 80 mm) investigated in this study were obtained at an aquaculture site in Piccadilly Bay located within Port au Port Bay, Newfoundland. They were maintained at low densities of 5 to 10 scallops per pearl net. During the reproductive season, scallop samples were obtained from the following size intervals: 5 to 20 mm, 21 to 25 mm, 26 to 30 mm, 31 to 40 mm, 41 to 50 mm, 51 to 60 mm, 61 to 70 mm, and 71 to 80 mm shell height. Biweekly samples were collected in July and October 1991 and weekly samples were obtained in August and September 1991. Ten scallops from each size class were collected at each sampling date. Gonadal sections of each scallop were processed using histological techniques and were stained with hematoxylin-eosin. Each slide was examined to determine the sex and the development stage based on criteria described by Davidson and Worms.[3] Gonad development stages were only determined for scallops with a shell height greater than 41 mm. Image analysis was employed to measure the area occupied by the follicles, the sex cells, and the interfollicular and innerfollicular space in representative gonad tissue from each class collected from July through September, 1991. For females in each size class sampled on August 28, September 17, and October 17, 1991, the diameter of 30 free oocytes I with visible nuclei was measured using an ocular micrometer.

The reproductive cycle of adult scallops can be monitored by following the changes in the gonosomatic index (GSI).[4] The GSI is calculated by dividing the weight of the gonads by the weight of the soft tissue and expressing the result as a percentage.[5]

During the commercial fishing season, the GSI of adults was monitored to determine the peak development date and the spawning period of the scallop population in Port au Port Bay. The GSI was also evaluated for scallops in the following size classes: 41 to 50 mm, 51 to 60 mm, 61 to 70 mm, and 71 to 80 mm.

Results and Discussion

Using histological techniques, sexual differentiation was observed in scallops as small as 6 mm. A sex ratio of 1:1 was observed in all size classes of scallops greater than 21 mm. In the smallest size class (5 to 20 mm), the sex of 49.1% of the individuals could not be determined, 47.4% were males, and only 3.5% were females. Individuals containing both male and female sex cells were not observed. The results confirm that the giant scallop is a dioecious species.

The adults reached their peak GSI on August 29, were in the process of spawning on September 19, and were spent on October 11, 1991. Young scallops (40 to 80 mm) synchronized their reproductive cycle with the adults and the GSI value of the young scallops increased as the shell height increased. In adults, the GSI is independent of the shell size.[4]

Results of the GSI and the summary of the development stage classification technique showed similar results. In general, for each size class, the peak GSI values occurred when the highest number of individuals reached stage 4 (ripe). Using the GSI technique, a spawning period was identified for scallops in each size class (41 to 50 mm, 51 to 60 mm, 61 to 70 mm and 71 to 80 mm). Using the development stage classification, the spawning period could not be determined for scallops in size class 41 to 50 mm and 51 to 60 mm because the majority of individuals did not reach stage 6 (spent); therefore the end of the spawning period was not identified. The majority of scallops in size class 61 to 70 mm and 71 to 80 mm reached stage 6 (spent).

Histological examination of gonad tissue revealed that gonad development in young scallops, with a shell height of 21 mm or greater, was synchronized with that of the adult. The shell height of the smallest male with sex cells was 6 mm while the shell height of the smallest female with sex cells was 8 mm; however these sex cells were in early development. Male scallops were observed to release mature sex cells (spermatozoa) at a shell height of 26 to 30 mm (an age of 9 to 13 months). Female scallops were observed to release mature sex cells (free oocytes I) at a shell

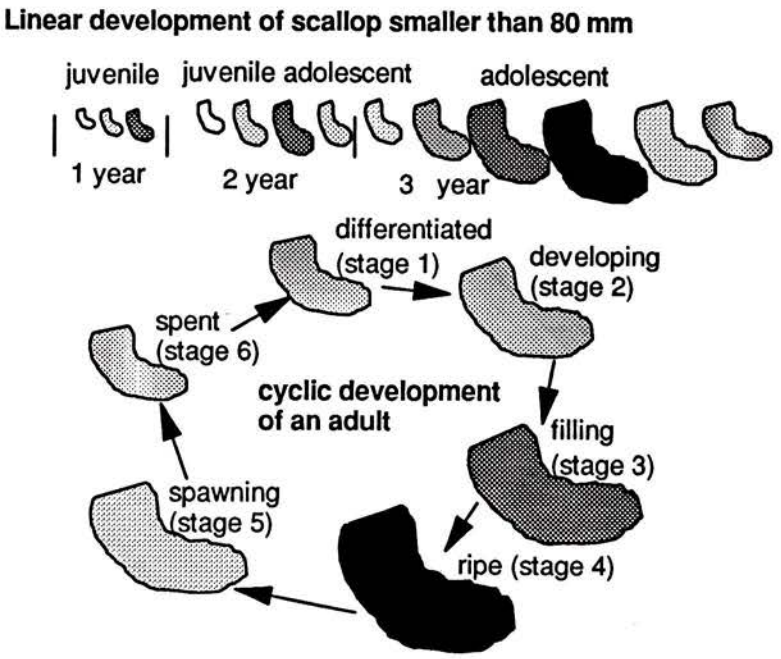

Figure 1. Schematic of gonad development in the giant scallop.

height of 31 to 40 mm (an age of 21 to 25 months). Only female scallops with a shell height greater than 31 mm possessed free oocytes I. In female scallops of size classes 31 to 40 mm, 41 to 50 mm, 61 to 70 mm, and 71 to 80 mm, the average diameter of the free oocytes I decreased from August 28 to September 17, 1991. The average oocyte diameter increased in scallops of the size class 31 to 40 mm, but the change was not significant. The decrease in size of the average free oocyte I diameter observed on August 28 to September 17, 1991, in female scallops in classes 41 to 50 mm, 51 to 60 mm, 61 to 70 mm, and 71 to 80 mm was most likely because the larger oocytes I had already been released. Scallops in size class 31 to 40 mm probably did not release large numbers of free oocyte I.

The area occupied by the follicles and sex cells increased as the shell height increased in the size classes 5 to 20 mm, 21 to 25 mm, 26 to 30 mm, 31 to 40 mm, and 41 to 50 mm. During peak development, the follicles appeared to be filled to capacity with sex cells in gonad tissue of scallop in the 51 to 60 mm, the 61 to 70 mm, and the 71 to 80 mm size classes. Scallops in the size classes between 21 mm and 60 mm accumulated sexual cells but did not release the majority of these cells during the spawning period. Scallops in the size classes 61 to 70 mm and 71 to 80 mm filled their follicles with mature sex cells and released the majority of these cells during the spawning period.

Sexual development is a progressive process in the giant scallop. However, the following stages have been identified:

- ***Juvenile*** (5 to 20 mm)
  Gonads contains only a few follicles and the presence of the sex cells does not seem to be synchronized with that of the adults.
- ***Juvenile adolescent*** (21 to 60 mm)
  Gonads can be filled with follicles. Production of sex cells is synchronized to the reproductive cycle of the adults. The majority of individuals do not empty their follicles during the spawning season.
- ***Adolescent*** (61 to 80 mm)
  Gonads can be filled with follicles that are packed with mature sex cells. The follicles can be completely emptied during the spawning season. The GSI is dependent on the shell size.
- ***Adults*** (81 mm and larger)
  Gonads are completely functional like those of the pre-adults. The GSI is independent of the shell size.[4]

In previous studies,[6,7] the sexual development of young scallops had been included in the first three stages of the reproductive development described for adults. The development of the young scallop is linear rather that cyclic. Scallops greater than 21 mm had sexual development that was synchronized with that of the adults; however, they had distinctive histological images specific to their size. Figure 1 illustrates the linear development of young scallops and the cyclic development of the adults.

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Evaluation of the recapture rate of seeded scallops *(Placopecten magellanicus)* during commercial fishing activity in Îles-de-la-Madeleine, Québec

M. Nadeau and G. Cliche [1]

Commercial seeding of the giant scallop, *Placopecten magellanicus* (Gmelin), has been conducted annually in the Îles-de-la-Madeleine, Québec, since 1993. In July 1997, the areas seeded in 1993, 1994, and 1995 were re-opened to commercial fishing. A concerted effort was made to evaluate the success of the seeding strategy. Data provided by the recapture of scallops tagged before seeding showed that growth was in the range required for economic profitability. Scallops collected in 1992 reached 96 mm after 4.6 years. There appears to be little dispersal of the seeded scallops as most were recaptured on the seeded area. About 6% of the scallops seeded in 1993 and less than 1% of the 1994 and 1995 seedings have been recaptured. Recapture rates of 20 to 30% are required to be profitable. Many factors, including seeding strategy, time of seeding, scallop vitality, and predation may be associated with these recapture rates. Some technical improvements were made during the 1996 seeding and an experimental survey conducted 8 months later showed encouraging results.

Introduction

Commercial bottom seeding of juvenile giant scallops, *Placopecten magellanicus,* has occurred annually in the Îles-de-la-Madeleine, Québec, in an area closed to fishing activity since 1993. In July 1997, the area seeded in 1993, 1994, and 1995 was re-opened to fishing. Special efforts were made to collect data to evaluate recapture rate. This information is particularly important for estimating the profitability of the bottom seeding strategy.

The project described in this paper was a component of a large research program (REPERE) for seeding and enhancement in Îles-de-la-Madeleine. The main partners were the Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec based in Îles-de-la-Madeleine, the local scallop fishermen's association, and the federal Department of Fisheries and Oceans.

Method

Commercial seedings were conducted in an area 10 km southeast of Îles-de-la-Madeleine on a natural scallop ground known as "Chaîne-de-la-Passe" (Fig. 1). The eastern part of the area received the 1993, 1994, and 1995 seedings and was re-opened to fishing in July 1997. A total of about 1.7 million scallops measuring 25 to 40 mm were seeded on this area at densities between 0.1 to 4.5 individuals/m^2. To estimate the recapture rate, 20 000 to 30 000 scallops were marked each year with a plastic tag glued on the upper valve. Tags of a different color were used each year so that the year-classes could be identified.

To evaluate the fishing rate in the area, an inventory was conducted before and after the fishing season using double-netted drags. Also, a few days before fishing began, 3 057 adult scallops were marked with a plastic tag on the upper valve and an "X" was engraved on the lower valve. These scallops were spread over the fishing area to evaluate the tag loss caused by the drag. Finally, special efforts were made to sensitize each of the 23 fishermen to the presence of the tagged scallops and their importance in determining the success of the seeding project.

During the fishing period, tagged scallops, shell samples, and dragging location were collected by 12 scientific observers onboard fishing boats. The ob-

Table 1. Evaluation of the recapture rates from tags recovered.

Scallops	Year of seeding		
	1993	1994	1995
Tagged (N)	29 600	27 387	19 871
Recaptured (N)	1 267	57	53
Recaptured (N) (corrected number)*	1 729	79	71
Recapture rate (%)	5.8	0.3	0.4

* corrected for tag loss of 7.8% and boats without observers.

servers were particulary useful because most fishermen remove the scallop "meat" by holding the scallops with the lower valve on top and, by doing so, may not notice that the scallop is tagged. The dragging location and the tagged scallops from those boats without observers (10 boats) were collected at the wharf. A correction factor was calculated from the returns of tagged scallops from boats with observers compared to the number of returns from boats without observers. The correction factor was then applied to the data from the boats without observers.

Results and Discussion

The majority of the scallop landings occurred during the first two weeks of the fishing season and the fishing rate was determined to be more than 80%. During this period, there was dragging activity over

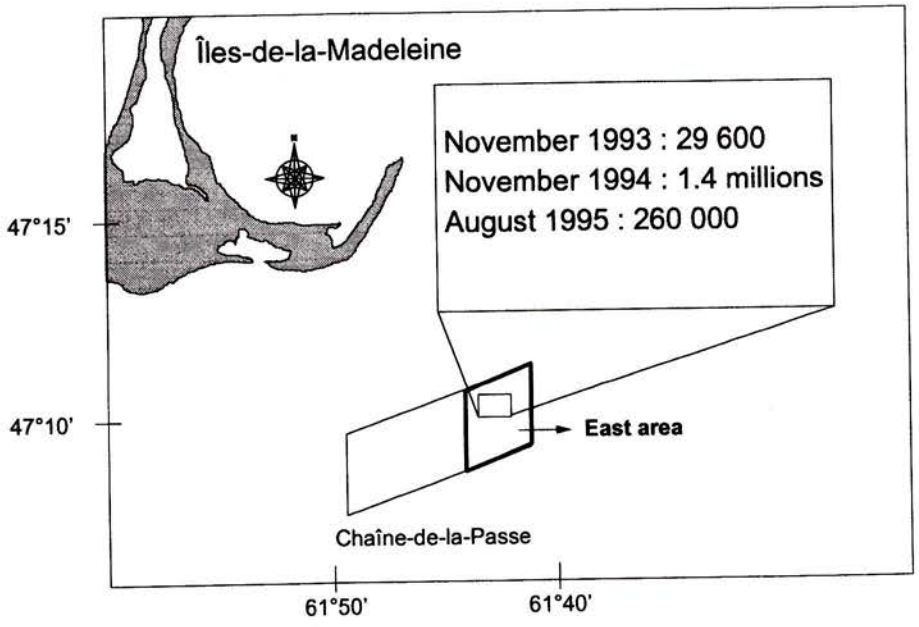

Figure 1. Approximate location of the area (darker square) re-opened to fishing in July 1997 and which supported the 1993, 1994 and 1995 seedings.

During this period, there was dragging activity over the entire area. The growth of the tagged scallops was in the range of what was initially used in the economic model: scallops seeded in 1993 reached 96 mm 4.6 years after spat collection, whereas scallops from the 1994 and 1995 seedings reached 81 mm after 3.6 years. In comparison, wild scallops only reach 100 mm after 7 years on this ground.[2] The dispersal of the seeded scallops appeared to be relatively limited. Most of the tagged scallops were brought in by boats fishing over the seeded area. In contrast, boats that brought back only a few tags tended to drag outside the seeded area.

Recapture rates of 20 to 30% are required for bottom seeding to be profitable. Unfortunately, the recapture rates estimated from the return of tagged scallops were low. About 6% of the scallops seeded in 1993 and less than 1% of those seeded in 1994 and 1995 were recaptured, including the corrections made for tag loss and for boats without observers (Table 1). Tag loss during dragging was estimated to be 8%. Shell samples confirmed these results as they did not show any particular concentrations of scallops from the size classes corresponding to those that had been seeded.

Despite these relatively low recapture rates, the results are encouraging. In comparison, the first seeding trials performed in Japan[3] had recapture rates of about 5%. The Japanese have since controlled predation, a major factor in the 30% recapture rate now being obtained.

In our study, many factors such as seeding strategy, seeding period, scallop vitality, and predation may have affected the recapture rates. During the 1994 seeding, the fishermen had problems precisely locating the areas to seed and no rigorous protocol for spreading the seed over the ground was followed. Since then, the seeding strategy has been more precisely defined and a technician is always onboard to co-ordinate the seeding activty.

The 1995 seeding was done in August which is the is the warmest period of the year. As it is well known that the activity of predators is related to sea temperature,[4,5] future seedings will be done earlier in the spring or later in the fall when temperatures are lower.

In 1994, the vitality of the scallops during the grow-out period was negatively affected by the presence of massive quantities of mussel spat that had settled on the pearl nets and the scallops. This problem has been resolved by stocking the pearl nets after the period of mussel spat settlement. In addition, trials of simple methods to evaluate the vitality of the scallops before seeding are underway. The results will be helpful in improving seeding practices and increasing the survival of the scallops on the sea bed.

Finally, predation by sea stars seems to be a major factor in the loss of seeded scallops. In some areas the density of sea stars can reach as much as 0.5 sea stars/m^2. Therefore, the effect of predator removal by dragging or other means just before seeding will be tested. Afterwards, the impact of this operation on scallop survival will be evaluated.

We would like to thank the technical staff of the Station Technologique Maricole des Îles-de-la-Madeleine for their valuable participation in this project. We also thank all the observers and the scallop fishermen for their good collaboration.

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Recommendations for the management of bivalve aquaculture to minimize exposure to paralytic shellfish poisoning: Site selection, harvest and transport

C.H. McKenzie,[1] *R.J. Thompson*[1] *and C.C. Parrish*[1,2]

A three-year multidisciplinary study (biology, chemistry, physics) was conducted in Notre Dame Bay at several bivalve aquaculture sites to determine factors which influence the distribution of *Alexandrium fundyense* vegetative cells and cysts. This study included water, sediment, sediment trap, and mussel tissue analyses, as well as an investigation of the circulation patterns in the region. The goals of this study were: a) to determine desirable aquaculture site characteristics which minimize exposure of cultured bivalves to paralytic shellfish poisoning (PSP); b) to determine a time-frame for the safe harvest of bivalves after exposure to PSP; and c) to develop methods to determine the risk of transferring toxic cysts during the transportation of bivalves. The study has been completed and the results and recommendations for the management of bivalve aquaculture are discussed.

Introduction

During the winter of 1992, several aquaculture sites in Newfoundland were closed when high levels of PSP toxicity were detected in harvested mussels. Examination of the stomachs of these toxic mussels revealed the presence of *Alexandrium* hypnozygotes which had presumably been resuspended from the sediment.[3] A three-year (1995 to 1997) multidisciplinary study was conducted to determine factors which influence the distribution of these cysts and to characterize the bloom dynamics of *Alexandrium* in this area of Notre Dame Bay. The objective was to develop aquaculture management practices which would minimize the risk of PSP contamination in bivalves. The goals were to determine desirable aquaculture site characteristics which minimize exposure of cultured bivalves to PSP; to determine a time-frame for the safe harvest of bivalves after exposure to PSP; and to determine factors which influence the risk of transferring toxic cysts during the transportation of spat or product. Harvesting and transport of bivalves could be conducted more cost effectively if "high risk" periods for *Alexandrium* blooms and the role of bivalves as hypnozygote vectors could be determined. Site selection, harvest and transportation: each stage of product development can minimize bivalve exposure to PSP. The following recommendations address each stage of bivalve product development.

Materials and Methods

The project study area included Barred Island Cove, a former aquaculture site now permanently closed due to high concentrations of *Alexandrium* cysts in the sediment. It is located near several commercial aquaculture sites in Notre Dame Bay, Newfoundland. In 1996, to determine dispersal and transportation of the cysts and vegetative cells through the transfer of mussel seed (spat) between sites and the transportation of harvested mussels from sites and to processing plants, two sites (Charles Arm and Fortune Harbour) where major processing plants were located were included in the study. SCUBA divers collected bottom sediment cores by hand, with modified 50 cc centrifuge tubes. The *Alexandrium* cysts were concentrated by the Schwinghamer density gradient method.[4] Material settling from the water column was collected in sediment traps (2 unpoisoned, 2 poisoned with mercuric chloride) moored at 3 m and 15 m. During the pre-bloom, bloom, and post-bloom periods, July through November, extensive sampling of the con-

taminated site and the surrounding areas, including three other commercial aquaculture sites, was conducted. CTD profiles were obtained using a SEABIRD at 28 stations, and inorganic nutrients were determined from whole water samples (Niskin bottles). Seston collected from Niskin bottles, vertical net tows (20 µm mesh) and sediment trap contents were subjected to chlorophyll, lipid class, and floristic analyses. Counts of *Alexandrium* cells/cysts and other floristic analyses were performed with a Zeiss inverted microscope. Samples of the cells and cysts were critical point dried and examined with a Hitachi S570 SEM.[5] Nutrient samples were analysed with a Perstorp nutrient autoanalyzer.

Recommendations on Site Selection

Information useful for site selection can be obtained by considering the following factors:

1. *Sediment type as an indication of system flushing and potential for resuspension*
If the sediment is soft silty clay there is little system flushing and cyst resuspension will be an ongoing problem. The silty sediment at Barred Island Cove is over 1 m deep, and every time the sediment is resuspended the bivalves are exposed to the cysts. Thus a site should be selected which does not have a silty bottom; alternatively, the site should be deep enough to minimize resuspension.

2. *Depth as an indication of resuspension as well as potential thermal and salinity stratification*
The deeper the site, the less chance of resuspension of any cysts that are in the sediment. How deep is "good enough" depends on the amount of wind, wind direction, currents and tidal action.

3. *Sediment trap data to determine the sedimentation rates of phytoplankton and resuspended cysts*
To determine resuspension due to tidal action, wind, or currents, a sediment trap can be suspended at the depth that the lowest sock would be located. At least one short-term (tidal cycle) check should be conducted.

4. *Microscopic and chemical analysis of sediment to evaluate the potential for exposure of bivalves to toxic cysts*
Before a site is chosen as a bivalve aquaculture site the sediment should be examined microscopically to check for the presence of potentially toxic cysts.

5. *A video survey of the bottom to establish the "environmental health" of a site*
During our benthic video survey we observed several different bottom types within the area. A "healthy" site was indicated by an active benthic population (i.e. anemones, starfish, crabs, fish), evidence of water movement, and an active and diverse food chain. The Barred Island site was characterized by little or no benthic plants or animals, and had accumulated man-made and natural "trash". The protected nature of this site and its lack of flushing was the cause of the toxic cyst and vegetative cell accumulation that led to its closure as an aquaculture site.

Recommendations on a Time-frame for the Safe Harvest of Bivalves

1. *Determining the vegetative bloom risk season*
Vegetative cell flux calculated from sediment trap contents (48 hour deployment August 29 to August 31, 1995) was 4.72 x 10^5/m^2/d. Hypnozygote flux was 3.14 x 10^5/m^2/d. The water temperature at the site was 11°C to a depth of 8 m and the incident light peaked (100 µE/m^2/s) during this bloom period in 1995. In the weeks before the initiation of the bloom, the oxygen saturation was 110% near the bottom. This may have triggered the resting cysts in the silty sediment to begin excysting, since an oxygen-rich environment has been shown to be an absolute requirement for cyst germination.[6] In 1996 and 1997 the bloom occurred when the temperature, light, and oxygen saturation (100%) at the bottom were similar to values for the initiation of the bloom in 1995.

2. *Identification of the life stage of the cells*
Alexandrium cells were detected in October 1996 poisoned sediment trap samples in what appeared to be various stages of encystment. These samples contained 7.4 x10^5 cells/L *Alexandrium* vegetative cells and 13.6 x 10^5 cells/L forming cysts or emerging from the thecal plates. The trap samples also contained 4.3 x 10^5 hypnozygotes/ L that were either oval or capsulate. Scanning electron microscopy (SEM) of the hypnozygotes revealed the roughened cyst wall characteristic of newly developed cysts.[7]

3. *Depuration period for bivalves and egestion of viable cysts*
Measurements have been made of gut residence time, clearance rate and absorption rate for mussels *(Mytilus edulis)* and scallops *(Placopecten magellanicus)* feeding on suspensions containing *Alexandrium* cysts.[8] The thick-walled dinoflagellate cysts egested in faeces germinated in about 10 to 14 days. The estimated gut retention time of these cysts is 9 hours. The egestion of viable cysts from mussels could have serious implications for current aquaculture practice.[8] Similar results were reported on the effects of gut passage on dinoflagellate vegetative cells.[9]

4. *Determining a safe time frame for bivalve harvest*
Under laboratory conditions, the mussels have been shown to egest the cysts after 9 hours, but in the wild the time for depuration is much more variable. Determining the end of the vegetative cell bloom must be done microscopically to be accurate. The 9-hour period does not include the depuration time required for the toxin that may have accumulated in the tissue itself from the vegetative cell. If a site is contaminated with toxic cysts and resuspension is a factor, the time-frame for safe harvest would be very difficult to determine, which could lead to the loss of all stock at the site, as occurred at Barred Island Cove before this study. Therefore, site selection is more critical in dealing with the year-round problem of toxic cysts than in eliminating the seasonal vegetative cell bloom. The time-frame for safe harvest ultimately depends on the type of contamination, i.e. whether it is a seasonal harmful algal bloom or resuspended toxic cysts.

Recommendations on the Transportation of Bivalves

The dispersal and transportation of cysts and vegetative cells through the transfer of mussel seed (spat) between sites and the transportation of harvested mussels from sites to processing plants is one of the most serious threats to the spread of toxic species throughout an area. This was also discussed by Scarratt et al.[10] Growers are therefore cautioned to evaluate the product to be processed and the spat or seed transferred to a site using the following criteria:

- *The location and history of the original site*
  (see recommendations on site selection)
- *The time of year in which bivalves are transported should be selected to minimize contamination of new sites*
  (this is not particularly effective with cyst transport)
- *Strength and direction of recent wind events to assess resuspension and contamination of bivalves*

An understanding of the essential site characteristics and the toxicity status allows more informed management of each site. The site selection guidelines are vital to growers with existing sites and growers who will be selecting sites. Discussions on toxic algae increase the awareness of present and future growers to the problems, particularly the transportation of "spat" and product between sites. An informed manager is a prepared manager.

The authors would like to thank the mussel growers who allowed their sites to be used for this study. We also thank the OSC dive team, as well as Elizabeth Hatfield, Mike Riehl and Jeanette Wells for their excellent technical assistance. Funding for this study was provided by an NSERC Strategic Grant to R.J. Thompson et al.

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Preliminary ultrastructural studies of the surface mucus of Atlantic salmon

Margaret M. Horne and David E. Sims[1]

The objectives of this study were to examine the ultrastructure of healthy skin mucus of Atlantic salmon (*Salmo salar* L) and to investigate possible changes induced by infestation with sea lice (*Lepeophtheirus salmonis*). Until recently, there have been no methods for reliable preservation of fish skin mucus. A fixation method that uses a non-aqueous solvent and osmium tetroxide has been developed for this purpose. Results indicate that the surface mucus of Atlantic salmon is multilayered. Mucus cells probably contribute most of an outer, dense layer, while other epithelial cells likely secrete materials into a lower lucent zone between the epidermal ridges. When compared with non-infested fish, Atlantic salmon with moderate to severe infestation of sea lice have significantly thinned or "washed out" mucus.

Introduction

Mucoid substances such as fish skin mucus have historically been problematic to histologists and ultrastructuralists, due to their propensity for dissolving in aqueous fixatives more readily than they can be stabilized. With recent development of methods for preserving glycoproteins and proteoglycans for ultrastructural assessment[2,3] we have examined the skin of Atlantic salmon, where mucus is a primary locus of defense and osmoregulation. The long range goals of this research program are to elucidate structure/function correlates in fish skin mucus, and to determine changes that occur in stressful conditions such as sea lice infestation. This paper presents preliminary observations on the ultrastructure of skin mucus on normal and sea lice-infested Atlantic Salmon.

Methods

Skin samples from Atlantic salmon that had been anesthetized or pithed were quickly and carefully removed, then either immersed in a non-aqueous fixative or placed in its fumes. The fixative consists of FC-72, a perfluorocarbon solvent (3-M Co., London ON) with 0.5% w/v dissolved osmium tetroxide. After 20 min of osmication, the tissues were cut to suitable size for embedding, then processed with conventional methods. Atlantic salmon for this study were obtained from a commercial fish aquaculture site and averaged 4 kg in weight. After selecting skin samples from fish with moderate to heavy sea lice infestation (usually around the anus or other ventral positions), equivalent sites were sampled from fish lacking sea lice or evidence of recent sea lice infestation. Actual lesions caused by sea lice attachment and burrowing were not taken. Instead, we sampled skin that was unbroken, though often reddened, 1 to 2 cm away from acute lesions.

Results

The mucus coat on the surface of Atlantic salmon is heterogenous in terms of thickness and composition. The simplest version is a denser, outer glycoprotein layer (Fig. 1, arrowhead) that is affixed to the tips of microridges, with a more lucent or clear zone beneath (arrow). Mucus is thicker and more variable below scale folds and on about half the surface, due to the addition of dead and dying cells. Sloughed cells and cell remnants may compose as much as 50% of the mucus coat.

Mucus from Atlantic salmon that are infested with sea lice is "washed out" or less dense (Fig. 2). It may or may not be as thick in terms of dimensions, but is more sparse, with little or no distinction between a dense outer layer and a lucid inner layer.

Discussion

The lucent layer beneath a denser glycoprotein layer may have an important role in defense and osmoregu-

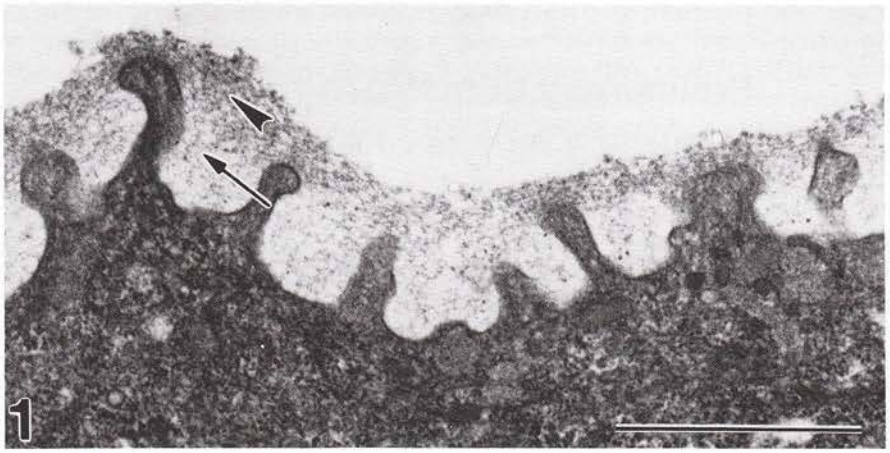

Figure 1. Mucus on the surface of a healthy Atlantic salmon has two layers, an outer dense layer that is affixed to the tips of microridges (arrowhead), and a less dense layer (arrow) between microridges. Both micrographs are enlarged to X 27,000. The magnification bar represents 1μm.

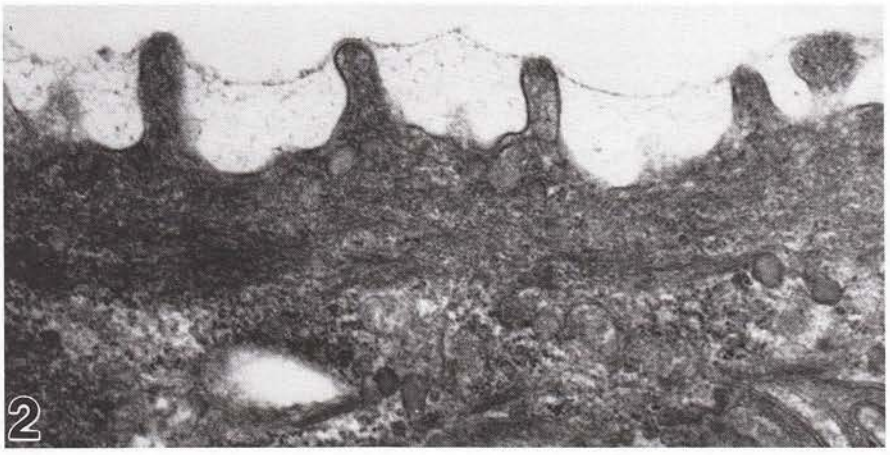

Figure 2. Mucus on the surface of Atlantic salmon infested with sea lice is significantly thinner, presenting a "washed out" appearance.

lation. Epithelial cells other than mucous cells likely contribute to the lucent layer, where there may be little turnover or diffusion into the water beyond the outer dense layer. Atlantic salmon stressed by sea lice infestation appear to have lost the distinct lucent layer, as well as much of the outer dense layer.

With the ability to preserve mucus, its role in the health and disease of fish may now be analyzed. Ongoing studies seek to quantify mucus architecture, and to determine the molecular components in healthy and sea lice-infested fish.

We thank Drs. Ahmed Mustafa and John Burka for field support. This research is financed by an NSERC/NRC Research Partnership Grant.

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Canadian doctors confirm the infection and effects of *Streptococcus iniae* in fish and humans

Thomas T. George[1]

Streptococcus iniae, a β-hemolytic bacterium, was first reported in 1976 to cause "golf ball disease" in freshwater dolphins *(Inia geoffrensis)* kept in aquaria in the United States. Later, it was found to be a major pathogen responsible for mortalities in freshwater and marine fish species. The first recognized case of *S. iniae* infection in humans occurred in Texas, USA, in 1991 and a second case occurred in Ottawa, Canada, in 1994; however, potential sources of the infections were not determined. In the winter of 1995-1996, Canadian doctors found that *S. iniae* infection can be spread to humans through a cut in the skin when handling infected fish. Unfortunately, this pathogen and its associated disease can pose serious constraints on the profitability of culturing fish and also be the cause of direct health risks to humans. This paper provides an account of the history and taxonomy of *S. iniae*, its infection and effects in fish and humans, its impact on the aquaculture industry, methods of control in the aquatic media, and strategies for protecting humans from becoming infected.

Introduction

Streptococcus iniae is a bacterium that has caused global infections and high economic losses in freshwater/marine and wild/cultured fish. It is not an obligate fish pathogen; it infects stressed fish, especially in intensive water recirculation systems, and causes invasive septicaemia disease.[2] In such systems, it affects the "miracle fish" tilapia[3,4] more severely than other fish species and tilapias in saltwater are more susceptible than those in freshwater. Currently, *S. iniae* is the most serious bacterial pathogen in intensively cultured tilapias in the United States. More attention has been focused on this bacterium in North America since Canadian doctors discovered and confirmed in 1996 that *S. iniae* also infects humans.[5-7]

Characteristics, History and Taxonomy

S. iniae is gram-positive, β-hemolytic, non-sporulating, facultatively anaerobic, chain-forming cocci that is catalase negative and positive to starch hydrolysis.[6] It was first isolated as a new species in 1976 from skin lesions of Amazon freshwater dolphins, *Inia geoffrensis,* suffering from "golf ball disease" at aquaria in San Francisco, California and New York.[8] The first streptococcal infection in fish was reported in 1958 in cultured rainbow trout in Japan, while the first report of infection in tilapia was in 1970.[9] Thereafter, disease outbreaks in tilapia occurred in Japan (1981), Taiwan (1985), Israel (1986), the United States (1992), and Saudi Arabia (1992).[5,10] The species was renamed *S. shiloi* in Israel in 1986, but following taxonomic validation in 1995 the name *S. iniae* was retained because it was published before *S. shiloi.* The species are phenotypically identical.[11]

Affected Fish and Distribution

Worldwide, streptococcal infections are reported in about 22 fish species. The most seriously affected fish are yellowtail, eel, tilapia, striped bass, rainbow trout and turbot. The most affected countries are Japan, Israel, United States, South Africa, Australia and Spain.[6]

Infection Discovery in Humans

The first cases of *S. iniae* infection in humans occurred in Texas, USA, in 1991 and in Ottawa, Canada,

in 1994. However, the causative agent was not determined in either of these cases.[12] In 1995-1996, Canadian doctors in Toronto discovered and confirmed through DNA analysis that a clone of *S. iniae* causes invasive disease in humans and fish.[7,13] Fourteen patients were infected when they cut their hands with knives or fish fins while preparing fresh fish for cooking (eating cooked fish or handling fish without incurring a wound poses no threat).[6,12] Consequently, it was published in local newspapers and magazines that *S. iniae* caused "mad fish disease", that tilapia transmitted the bacterium to humans, and that this bacterium will have a major impact on the aquaculture industry.[14,15]

These news reports affected Canadian and American tilapia markets for several weeks.[16] To clarify the scientific facts for the public, I published a report in the Toronto Star stating that infections in fish were not restricted to tilapia"[17] and D. McGrogan reported that "the so-called mad fish disease was unlike mad cow disease."[18] Also in response to the news reports, the American Tilapia Association issued a press release stating that the report of "mad fish disease is inaccurate, highly inflammatory and must be withdrawn; streptococcus makes neither humans nor fish mad."[16]

Transmission, Infection and Signs

Streptococcal infections are horizontally transmitted from fish to fish. Streptococci released from dead or carrier fish and mud/water around culture facilities are infection sources. *S. iniae* may colonize the surface of the fish or cause invasive disease associated with mortalities of 30 to 50%. In humans, transmission occurs only as mentioned above and may cause cellulitis at the injury site or meningitis while circulating in the blood.[6,12]

Moribund fish swim erratically and display a whirling motion at the water surface. External signs include darkening, dorsal rigidity, swollen abdomen, exophthalmic eyes, corneal opacity, rupture of the eyes, and haemorrhage in the lower jaw, abdomen, opercles, anus, and the base of fins. Internally, there are bloody ascites in the abdominal cavity, a pale liver, and enlarged spleen; fish die within several days of infection.[5,6,10] Infected humans show signs of cellulitis within 16 to 48 hours after injury, elevation of leucocyte counts, and usually have a fever.[7,12]

Impact on the Aquaculture Industry

Annual worldwide loss estimates caused by *S. iniae* in fish species being intensively cultured exceed US$150 million. In the United States, tilapia farmers lost more than US$10 million when *S. iniae* caused mortalities of 30 to 50% in fish cultured in ponds and 75% in fish reared in closed systems. [6]

Preventive Measures and Treatment

Minimizing fish stress from factors such as crowding, mishandling, fluctuating temperature, low water quality, chemicals and drugs, etc., will reduce or prevent streptococcus infections in fish.[4,5] In Israel, treatment with feed medicated with tetracycline eradicated the disease effectively in tilapia.[19] In humans, streptococcal infection can be prevented by wearing heavy latex gloves when preparing live fish for cooking; infected patients can be treated with antibiotics and penicillin.[7,12,13]

Discussion

Canadian doctors confirmed that *S. iniae* infects both fish and humans and that the patients in Toronto were infected with it after they handled infected tilapia imported from farms in North Dakota, Tennessee, Arkansas, Delaware and Illinois. In Canada, the federal and provincial fish health protection and inspection regulations apply only to salmonids. Therefore, this paper calls for cooperation between Canada and the United States to reduce the risk of introducing infectious diseases, to make federal regulatory changes, and to enhance provincial inspection programs.

The serious problem of *S. iniae* in American tilapia farms may be due to a local bacterium strain or an imported strain since the United States imports tilapia from Israel.[11] *S. iniae,* first isolated in the United States in 1996, may have come from Israel in fish eggs imported in the early 1980s. Similarly, *S. iniae* may have come to Saudi Arabia from Taiwan. Also, African and other countries where tilapias are native, may face serious problems from inadvertent introduction of the disease with "genetically improved" tilapias from countries where their culture is more advanced.[19] Therefore, both developing and developed countries should carefully address this issue and develop a technique for early detection of the disease. In Japan, several antibiotics are used to treat streptococcosis in fish, but currently no antibiotics are licensed for use on tilapia in Canada or the United States.[5,19] However, antibiotic treatments have not been entirely effective in eliminating streptococcal problems, especially in closed culture systems, because *S. iniae* survives in macrophages, which are not affected by antibiotics, and diseased fish do not eat the medicated feed. Hence, antibiotic treatments appear to suppress a disease outbreak, and favour bacterium resistance to antibiotics and the development of carrier fish which serve as infection reservoirs for future outbreaks.[6]

Researchers and fish farmers should be aware of these facts.

S. iniae can cause meningoencephalitis in many fish species but is not analogous to "cow mad disease" or BSE which is caused by a prion.[18] *S. iniae* is not restricted to tilapia and has been reported in other fish species. Its spread to humans will certainly motivate aquaculturists to produce healthy tilapia through good health management procedures rather than application of chemicals/drugs which have potential deleterious effects on fish and consumers.[5,17] This approach should be supported by the development of live bacterial vaccines or DNA vaccination for effective protection against streptococcal disease in cultured tilapia. However, no vaccines other than that for rainbow trout have been developed for cultured tilapias or humans against streptococcus.[6,19,20] Hence, a combination of immunization, maintenance of environmental conditions, and prudent use of drugs would certainly be the way fish farmers should follow to produce healthy fish in Ontario, Canada, or elsewhere.

Recommendations

1. Canada and the United States should adopt a joint policy on introductions and transfers of aquatic organisms.

2. Fish health protection regulations in Canada should include farmed fish species other than salmonoids and ban importation of any live fish species that may pose serious health risks to humans and local fish.

3. Ontario should enact legislation that requires imported farmed fish to be certified free from infectious organisms, particularly *S. inae*; enhance provincial inspection programs; and quarantine all suspected imported live fish.

4. Ontario tilapia farmers are advised to reduce stress and apply good health management procedures to control *S. iniae*; also imported tilapia seed should be quarantined before it is stocked into the production systems.

5. African and other countries where tilapias are native should ban the importation of tilapia to avoid the introduction of *Streptococcus iniae.*

6. Methods aimed at diagnosing subclinical infections (e.g. fluorescent antibody and ELISA techniques) should be developed to detect streptococcal disease at an early stage.

7. Research is needed to: a) find effective ways for using antibiotics in closed culture systems to control *S. iniae*; b) develop vaccines for humans and tilapias against *S. iniae*; c) find a method for detecting fish carrying *S. iniae;* and d) study the epidemiology of streptococcal infections and determine why tilapia is more susceptible to *S. iniae* than any other fish species.

8. People should be enlightened on how to handle live fish to avoid *S. iniae* infections.

I am deeply indebted to Prof. Donald Low, University of Toronto and Microbiologist-in-chief, Mount Sinai and Princes Margaret Hospitals for providing me with his publications and slides on S. iniae.

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Effects of sea lice infestation on macrophage functions in Atlantic salmon

A. Mustafa, J. Bowers, C. MacWilliams, N. Fernandez, G. Conboy, and J. F. Burka[1]

An experiment was conducted to determine the effects of sea lice, *Lepeophtheirus salmonis*, on non-specific defense mechanisms in Atlantic salmon, *Salmo salar*, by experimentally infesting 180 hatchery-reared post-smolts with laboratory-grown infective copepodids at moderate to high infection intensities ranging from 15 to 285 lice per fish. The effects of sea lice-induced stress were investigated by measuring the blood levels of cortisol and glucose as indicators of primary and secondary stress responses, and by changes in macrophage respiratory burst activity and phagocytosis as indicators of tertiary stress responses as well as non-specific defense mechanisms. Fish were sampled prior to sea lice infestation and at days 3, 7, 14, and 21 post-infestation. Blood levels of cortisol and glucose were found to be significantly increased in sea lice-infested fish throughout the experiment while respiratory burst and phagocytic activities were found to be significantly decreased at day 21. The reductions in both respiratory burst and phagocytic activities are presumably the results of chronic stress induced by sea lice. The results from this study also indicate that sea lice have effects on the development of chronic stress and on the suppression of host defense mechanisms when infested with moderate to high parasite intensities.

Introduction

The absence of completely effective and safe methods for treating sea lice infections emphasizes the need to develop alternate methods. The development of such methods is limited by deficiencies in our understanding of many aspects of the basic biology of *Lepeophtheirus salmonis*, especially its effects on host defense mechanisms. In all vertebrates, environmental stressors cause neuroendocrine and autonomic changes that modulate both non-specific and specific defense mechanisms, which is often considered the cause of higher susceptibility of stressed individuals to disease.[2] In aquaculture, fish are exposed to stressors such as transport, handling, marking, grading, etc., on a regular basis which elicit the release of corticosteroids from the hypothalamus-pituitary-interrenal axis and catecholamines from the autonomic nervous system. [3,4] These stress hormones can modulate macrophage functions. Increased production of cortisol and glucose and decreased macrophage functions in salmonids have been well documented.[3,5] However, there have apparently been no reports on assessment of stress on immune responses due to sea lice infestations in salmonids. In this study, the effects of sea lice infestations on plasma cortisol concentrations and changes in the host non-specific defense mechanisms, i.e., respiratory burst and phagocytic activities of macrophages have been examined along a sea lice development gradient under laboratory conditions.

Materials and Methods

Approximately 300 Atlantic salmon smolts with a mean weight of 680 g were obtained from a Prince Edward Island hatchery and randomly assigned among five 1500-L tanks. Fish were acclimated to a gradual increase in artificial seawater (Instant Ocean®) over a one-week period and then acclimated

Table 1. Plasma concentrations of cortisol and glucose, and macrophage repiratory burst activity and phagocytic competence in Atlantic salmon.

Day of sampling	Plasma cortisol concentration (nmol/L)		Plasma glucose concentration (nmol/L)		Respiratory burst activity (% positive cells)		Phagocytic capacity (> 5 bacteria/cell)	
	Controls	Infested	Controls	Infested	Controls	Infested	Controls	Infested
Day 0	28.52	57.0	3.77	3.29	75.00	75.00	60.00	58.00
Day 3	43.10	121.73*	3.78	3.97	79.30	76.40	75.66	57.33
Day 7	46.12	101.32*	3.37	3.88*	85.00	89.10	28.00	29.00
Day 14	39.05	46.53	3.45	3.74*	84.30	89.11	84.66	71.28
Day 31	63.06	181.10*	3.55	4.53*	85.60	49.80*	83.50	59.25*

in 30 ± 2 ppt at 10 ± 1°C for a further 10 days prior to sea lice infestation. Fish were fed daily to satiation and cared for according to the guidelines of the Canadian Council on Animal Care.

Ovigerous sea lice were collected from Atlantic salmon from aquaculture sites in the Bay of Fundy and infective sea lice larvae (copepodids) were grown under laboratory conditions. Fish from three of five designated tanks were infested by adding approximately 20,000 infective copepodids to each tank.

At day 0, i.e. prior to sea lice infestation, and days 3, 7, 14, and 21 post-infestation, 10 fish from each of five tanks were randomly sampled. Each fish was collected individually with a white 70-µm Nitex mesh net and placed immediately into a white plastic bucket containing a lethal dose of anaesthetic (MS-222; ~200 mg/L). Each fish was measured for length and weight and bled to measure plasma levels of cortisol and glucose. Head kidneys from each fish were removed aseptically for macrophage assays. Sea lice were counted from each fish as well as from each net and bucket corresponding to that particular fish.

Plasma levels of cortisol and glucose were analyzed using validated and characterized radioimmunoassays. Macrophage cells were isolated from head kidneys and characterized for respiratory burst and phagocytic activities using methods described by Secombes[6] and Brown et al.[7]

Analyses were carried out using Student's *t*-test, ANOVA, and post-ANOVA multiple comparison test. Difference was considered significant when $P<0.05$.

Results

Only copepodids were found on fish at day 3. At day 7 sea lice were at both copepodid and chalimus stages while at day 14 all lice found were at chalimus stages. The lice were mostly pre-adults by day 21. Most of the copepodid and chalimus larvae were found attached to the gills and fins whereas most pre-adults were found on the body surface. The overall range of infestation was between 15 and 285 lice per fish with an average intensity of 106.

Plasma cortisol concentrations increased following sea lice infestation and remained significantly elevated ($P<0.05$) in the infested groups throughout the experiment, except at day 14 (Table 1). Plasma glucose concentrations also increased with time post-infestation (Table 1). However, glucose concentrations in both infested and control groups appeared to fall within the "normal" reference range values for Atlantic salmon in seawater (standards developed by Diagnostic Services, Atlantic Veterinary College).

Respiratory burst activity remained unchanged until day 14 between infested and control groups, but a significant suppression ($P<0.05$) was observed in the infested groups at day 21 (Table 1). Phagocytic capacity (>5 intracellular bacteria) showed a similar pattern to respiratory burst activity with a significant suppression ($P<0.05$) at day 21 (Table 1) in the infested groups compared to that of controls.

Discussion

The prevalence of sea lice infestation in this study was 100%. During copepodid and chalimus stages, most lice were recorded from gills and fins, and during mobile pre-adult stages, most lice were recorded from body surfaces, especially the head, external operculum, between dorsal and adipose fins, and peri-anal regions. These findings are similar to those of Johnson and Albright,[8] and Grimnes and Jakobsen.[9] Mobile stages appear to prefer these regions because the thin epidermis with no or fewer scales makes feeding easier.[10]

Plasma cortisol concentrations, indicator of primary stress, increased significantly post infestation with the highest level at day 21 when most lice were at pre-adult stages. Plasma glucose concentrations, indicator of secondary stress, also increased similarly with the highest level at day 21. On the same day (i.e., day 21) macrophage respiratory and phagocytic activities were also found to be significantly lower for the lice-infested fish compared to those of controls. These findings are consistent with the findings of Grimnes and Jakobsen, who suggested that the pre-adult and adult stages of sea lice have greater impact on the physiology of fish than the earlier stages.[9] The cortisol elevation at days 3 and 7 may have been due to an unforeseen handling stressor: the dip-nets had an extremely small mesh size to avoid any loss of sea lice, which slowed down the removal of fish from the tanks. Regular nets were used for all control fish, and thus cortisol concentrations remained consistent. After day 14, the increased cortisol in infested fish was probably due to the stress caused by mobile sea lice. Plasma glucose concentrations, even though increased over controls post infestation, were within the "normal range".[11] On the other hand, the higher elevation of glucose concentration at day 21 correlated with the elevation of cortisol. Cortisol is gluconeogenic; therefore prolonged increase in cortisol results in elevation of glucose.[12] Thus, the increase in plasma cortisol and glucose concentrations during the earlier days post-infestation is probably the effect of acute stress caused by sampling, and during the later days the effect of chronic stress caused by mobile sea lice stages.

Persistence of stressors generally cause biological tolerance limits to be exceeded, resulting in a maladaptation with adverse physiological and behavioral consequences. Prolonged increased cortisol concentrations can induce a generalized immune suppression.[2,4,13] Thus, the decrease in macrophage respiratory burst and phagocytic activities could be a consequence of increased cortisol concentrations. This is consistent with Barton et al.[5] It is more likely that fish were acutely stressed, perhaps due to handling in the beginning, but during the later days stress became chronic with the increased size or development of sea lice into mobile stages and their grazing, which eventually suppressed the immune system by reducing the respiratory burst and phagocytic activities of macrophage cells.

This research was funded by a grant from the NSERC/NRC Partnership Program.

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Changes in protease activity in the skin surface mucus of Atlantic salmon (*Salmo salar*) during sea lice (*Lepeophtheirus salmonis*) infestation

Kara J. Firth,[1,2] Neil W. Ross,[1] John F. Burka,[2] and Stewart C. Johnson[1]

Skin surface mucus contains a number of biologically active substances, including proteases, shown or suspected to have a role in the innate (non-antibody) defense system of fish. The objective of this study was to determine whether protease activity in the skin surface mucus of fish is altered as a result of sea lice infestation. In two experiments in which Atlantic salmon were infested with *Lepeophtheirus salmonis*, samples taken from infested fish showed increased protease activity compared to controls using two different assay methods. In particular, zymography of samples from infested fish showed a series of low molecular weight (17-20 kDa) proteases that were not present in control samples.

Introduction

Infestation of sea-farmed salmonids with the salmon louse (*Lepeophtheirus salmonis)* remains an important problem for the salmon industry throughout the Northern Hemisphere. In Canada, disease outbreaks caused by *L. salmonis* have occurred on both coasts and cost salmon growers millions of dollars in losses.[3] All stages of *L. salmonis* feed on host mucus, skin and blood, and this highly destructive feeding behavior can lead to the development of open lesions.[4]

The innate defense system is important in fish and other cold-blooded animals because the low environmental temperatures result in slow development and activation of acquired immunity. Therefore, it is innate factors that initially assume the significant role in defense against invading organisms.[5] Skin surface mucus contains a number of biologically active substances shown or suspected to have a role in the innate defense system of the fish,[6] including proteolytic enzymes (proteases).[7,8] Since lice feed on host mucus and skin, it seems reasonable to expect that some of the biological interactions between sea lice and their salmonid hosts are taking place in the skin surface mucus of the fish. The objective of this study was to determine whether protease activity in the skin surface mucus of the fish is altered as a result of sea lice infestation.

Materials and Methods

Sea Lice Challenges. Two sea lice challenges were executed, one at the Institute for Marine Biosciences (IMB) and one at the Atlantic Veterinary College (AVC). For the IMB challenge, sea lice were obtained from aquaculture sites in the Bay of Fundy and cultured in a flow-through seawater system at 12°C. Sea lice for the AVC challenge were obtained and cultured as per Bowers et al.[9] Pre-smolt Atlantic salmon (32 to 75 g), obtained from Mersey Hatchery, NS, were maintained at IMB in a flow-through system using ambient seawater at 12°C. Following smoltification fish were randomly divided into two groups of 40 fish each. One group of fish served as controls and the other was infested with newly molted copepodids. A high infestation level was used, with an average of 100 lice per fish (lice were counted at time of sampling). Seven fish were sampled from each group at 3, 5 and 10 days post-infestation (dpi). The sea lice challenge at AVC was carried out as per Bowers et al.[9]

Mucus Collection. Fish were anesthetized in 0.005% (w/v) MS-222 in individual buckets, then placed in a plastic bag containing 5 mL of 100 mM NH_4HCO_3, pH 7.8 for approximately 1 min. Mucus accumulated on the sides of the bags. The bags were placed on ice and upon return to the laboratory an additional 5 mL of buffer was added. Mucus and buffer were removed from the bags, centrifuged, aliquotted and stored at –80°C until use.

Zymography. Zymography was used to visualize

protease activity in our samples as per Kleiner and Stetler-Stevenson.[10] Samples were thawed on ice and protein concentration of samples was determined using the Bradford Assay.[11] After mixing 1:1 with SDS-PAGE sample buffer, an equal amount of protein for each sample was loaded onto a 12% SDS-PAGE gel containing 0.1% gelatin. Gels were run at 4°C and 150 V for 1 h. Following electrophoresis, gels were washed 3 times at 4°C with 2.5% Triton X-100, 50 mM Tris-HCl, pH 7.5, then incubated in the same buffer containing 50 mM $MgCl_2$, 6.25 mM $CaCl_2$, on a shaker at 30°C for approximately 19 h. Gels were stained in 0.1% amido black in MeOH/H_2O/AcOH (45:45:10) for 1 h and destained with MeOH/H_2O/ AcOH (50:48:2).

Azocasein Hydrolysis. An assay involving azocasein hydrolysis was used to quantify the protease activity in the samples.[12] An equal amount of each sample was used, and final concentration of azocasein in the assay was 0.35%. Tubes were placed on a shaker at 30°C for approximately 19 h. The reaction was stopped by adding trichloroacetic acid (20% final concentration) and tubes were placed on ice. Samples were centrifuged and 100 µL of each supernatant was added to 100 µL of 0.5 M NaOH in microplate wells. Optical density was measured at 450 nm on a Molecular Devices microplate reader.

Results and Discussion

In the challenge performed at IMB, zymography at 3 and 5 dpi showed a slight increase in the amount of protease activity in samples taken from infested fish compared to controls. At 10 dpi, a substantial difference in protease activity was observed between samples taken from control fish and those obtained from the fish infested with *L. salmonis* (Fig. 1a). This difference in protease activity between control and infested samples at 10 dpi was determined to be significant (*t*-test, $P<0.05$) (Fig. 1b). There were more protease bands present in infested samples; most notable is a series of low molecular weight (<18.5 kDa) bands exhibiting high activity.

A representative zymogram of some of the 300 samples generated in the challenge performed at AVC is

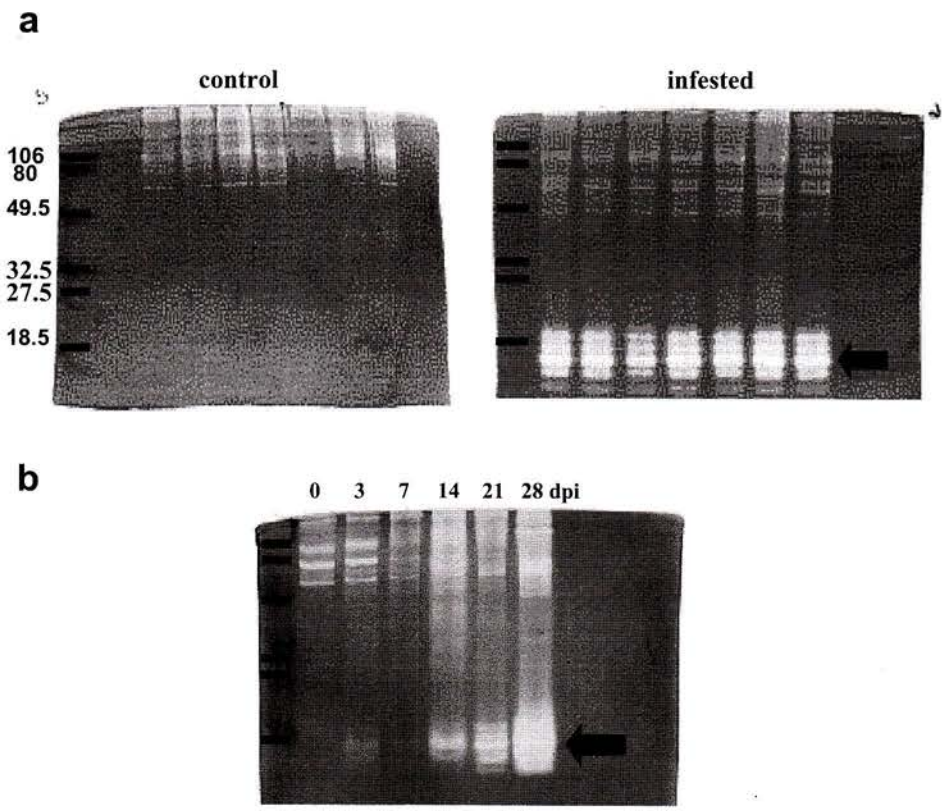

Figure 1 Zymograms of mucus samples collected from individual fish during (a) IMB sea lice challenge at 10 dpi and (b) AVC sea lice challenge. Molecular weights are given in kilodaltons (kDa). Arrows indicate series of low molecular weight (17-20 kDa) bands observed only in samples taken from fish infested with *L. salmonis*.

shown in Fig. 2(a). There were progressive increases in the amount of protease activity found in samples taken from infested fish over the course of the infestation. There was little difference in mucus protease activity between uninfested fish (0 dpi) and infested fish at 3 and 7 dpi, but by 14 dpi there was a large increase in the amount of protease activity. There were more protease bands, including the multiple low molecular weight bands observed in the IMB challenge samples. The increase in protease activity continues through 21 and 29 dpi. Mucus taken from control fish showed no increase in protease activity (data not shown). The trend of increasing protease activity in the mucus of fish infested with *L. salmonis* observed in the AVC challenge was found to be significant (linear regression, $P<0.001$), while no trend was found among control samples (Fig. 2b).

There are two possible sources of the proteases that appear in the mucus of fish infested with *L. salmonis*. Fish may secrete proteases in response to stress or disease; these proteases may be responsible for the thinning and shedding of mucus that has been reported in highly stressed and diseased fish.[13] Shedding of mucus may remove parasites such as *L. salmonis* from the host surface. Since sea lice feed on host skin and mucus, they may also be attacked internally by proteases in the mucus they ingest. Proteases in the mucus of infested fish may also come from the lice. There is protease activity in homogenates of whole *L. salmonis*.[14] Lice may secrete proteases to break up the mucus and skin on the fish surface as an aid to their feeding activities. Numerous parasites are known to secrete proteases as an aid in infection and for avoidance of host immunological responses.[15,16] Our investigations of the source and possible role(s) of these proteases in the relationship between *L. salmonis* and Atlantic salmon are ongoing.

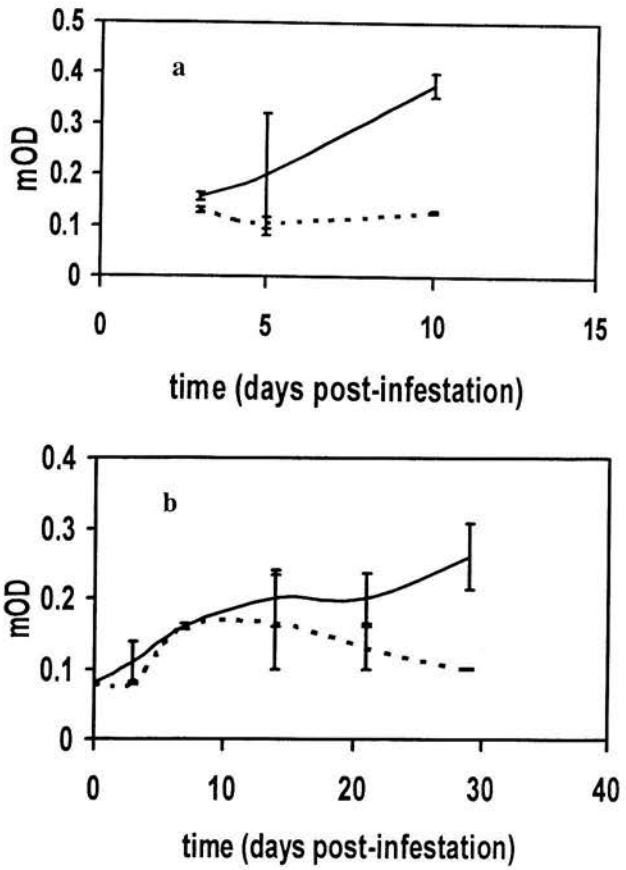

Figure 2 Azocasein hydrolysis assays of mucus samples collected from individual fish during (a) IMB sea lice challenge and (b) AVC sea lice challenge.

This project was supported by a grant from the NSERC/NRC Partnership Program.

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Conditioning green sea urchins in tanks: Water quality tolerance limits

S. Motnikar,[1] R. Marsan,[2] and F. Tétreault[1]

Little is known about sea urchin (*Strongylocentrotus droebachiensis*) responses to suboptimal water quality conditions and the effect these may have on their survival, gonadal quality, and development during conditioning trials in tanks. The four experimental parameters of this study consisted of two salinities (29‰ and 19‰), two densities of urchins (45 kg/m^3 = 1 and 90 kg/m^3 = 2), two water exchange rates (one tank volume/hour = C and one tank volume/12 hours = S) and either the presence or absence of food (fed = O and non-fed = N). Water quality (oxygen levels and ammonia) was monitored regularly and the mortality of urchins was followed throughout the study. At the end of 29 days, mortality, the gonad index (G.I.) as well as the acceptability of gonad color of surviving urchins were analysed. The results show that mortality was significanly higher in all the groups having a low water exchange rate. Low salinity and high density can be limiting factors when combined. The presence or absence of food does not appear to be a critical factor for urchin survival. However, the non-fed groups appear to have a lower gonadal quality. As for the quality of gonad color, the gonads from the treatment 292CN (29‰, 90 kg/m^3, water exchange rate of one tank volume/hour, non-fed) are more often rejected due to unacceptable colour, as compared to the gonads from the initial group. It appears that a combination of critical factors are a cause of mortality, rather than any factor by itself, except perhaps the low water exchange rate.

Introduction

The development of long term storage and enhancement techniques for the green sea urchin is needed in order to improve gonad quality and to obtain a higher value product. In order for these operations to be successful, the animals have to be kept under optimum conditions of density, feeding schedule, and water quality, and the tolerance limits and optimum levels need to be established. The immediate objectives are to determine which environmental conditions limit survival and gonad quality. The long term objective is to determine the optimal environmental requirements of green sea urchins during conditioning in land-based facilities. The study tested the hypothesis that suboptimal water quality, such as low water exchange and low salinity, high urchin density and the absence of food will all have a negative effect on the survival rate and gonadal quality of sea urchins kept in tanks.

Materials and Methods

The 29-day study took place in 1997, during the optimum conditioning period of September and October. Divers collected 640 sea urchins from the Gaspé Bay (Qc), which were acclimated for a period of 14 days prior to the start of the study. They were then distributed at random into 64 compartments and exposed to two variants of four parameters. Each treatment was repeated four times. The parameters consisted of two salinities (29 ‰ and 19 ‰), two densities of urchins (45 kg/m^3 = 1 and 90 kg/m^3 = 2), two water exchange rates (one tank volume/hour = C and one tank volume/12 hours = S) and either the presence or absence of food (fed = O and non-fed = N). The sea urchins in the appropriate groups were fed three times a week with the semi-moist diet D3-92.[3] Water quality was monitored by measuring the pH, temperature, salinity, dissolved oxygen and ammonia levels twice a week. The mortality of the sea urchins was followed

Table 1. Final mortality observed in each of the treatments having an initial sample of 40 sea urchins and the day of the study at which it was reached.

Treatment	Mortality		Day
	Number	%	
291CN	12	30	20
191CO	14	35	29
191CN	16	40	28
291CO	19	48	18
292CN	19	48	29
292CO	30	75	28
192CN	30	75	29
291SO	37	93	12
192CO	39	98	29
291SN	40	100	18
292SO	40	100	10
192SN	40	100	29
292SN	40	100	11
192SO	40	100	15
191SO	40	100	11
191SN	40	100	11

throughout. At the end of the study, the sea urchins from the five treatments having a sufficient number of survivors were sacrificed and the gonads extracted in order to determine their gonad index (G.I.) as well as their color. The gonads were then graded into A, B, C and rejected classes, as they would have been by a buyer.

Results and Discussion

In our study, pH and temperature were those of sea water in the natural sea urchin habitat and did not seem to have an effect on the urchins. Oxygen content, however, varied with each treatment and seemed to become limiting when the saturation level in the water fell to values generally accepted as suboptimal in the aquaculture industry (about 65 %).[4] To that effect, the treatments which maintained high oxygen levels (291CN, 191CN, 191CO) had the lowest mortality rates. It is therefore possible that the low oxygen levels in certain treatments, especially those of low water exchange, where a greater than 90% mortality rate was observed ($P < 0.05$) compared with high water exchange groups, contributed to urchin mortality (Table 1). An accumulation of ammonia was observed in the same tanks as well. Kaplan-Meier analyses also showed that one water exchange rate per 12 hours is not sufficient for urchin survival and that a higher water flow ensures an adequate oxygen supply and regular removal of ammonia. This agrees with the observation made by Leahy[5] who noted that the optimal exchange rate is one tank volume per hour for an urchin density equivalent to 60 kg/m^3.

Survival table (Kaplan-Meier) comparisons show that there is a significant difference between some of the 16 treatments as compared two by two, according to the four parameters. Apart from the water exchange rate already discussed above, the other two parameters of this study which seem to influence urchin survival are urchin density and water salinity. In comparing groups with different densities, it was noted that mortality was higher in the treatment 192CO as compared to 191CO ($P = 1.7E-07$) and that 192CN was higher as compared to 191CN ($P = 0.001$). The higher the density, the greater the amount of oxygen that is required and the greater the amount of ammonia that is produced, as was noted in the preceding groups. When comparing salinity, it was noted that the mortality in treatment 192CO was higher than in 292CO ($P = 0.005$), as was that of treatment 192CN compared to 292CN, ($P = 0.014$). Sabourin and Stickle[6] observed that under low salinity conditions urchins tend to reduce or maintain constant their oxygen consumption as well as nitrogen excretion. It was furthermore noted that the effect of high density was observed only under low salinity conditions, while the effect of low salinity was observed only in the high density groups. Higher mortality rates were obtained in the low salinity and high density conditions although the lower salinity tolerance limit for the green sea urchin is 12 to 13‰.[6] It appears that, as Vernberg[7] noted, each of these conditions by itself has little effect, while in combination they have a lethal effect. Also, it is not surprising that starvation alone was not a cause of mortality, since urchins tolerate low food availability fairly well and adapt by turning towards other sources of food.[8] As expected, more ammonia was produced by the groups that were fed, which in turn diminished water quality.

None of the treatments permitted somatic or gametogenic growth of urchins great enough to show a measurable difference as compared with the initial

Table 2. Mean gonad index, classification of urchin gonads (A,B, C or rejected) and the results of the comparison with the chi-square test of each treatment with the initial group.

Treatment	n	Gonad Index	A	B	C	Accepted	Rejected	% Accepted	χ^2	P
291CO	21	8.4 ± 3.1	1	14	3	18	3	86	0.32	0.570
291CN	27	7.4 ± 2.2	0	12	5	17	10	63	2.65	0.104
292CN	21	6.4 ± 2.2	0	9	0	9	12	43	9.54	0.002
191CO	25	11.6 ± 4.0	3	13	0	16	9	64	2.26	0.133
191CN	24	7.2 ± 3.0	5	9	0	14	10	58	3.86	0.049
Initial	50	9.0 ± 3.1	7	23	10	40	10	80	–	–

values. For example, the initial sample has a higher G.I. than the final results of treatment 292CN ($P < 0.05$). Also, the results of a non-parametric multiple comparison test, preceded by a Kruskall-Wallis test, indicate that treatment 191CO has a significantly higher ($P < 0.05$) mean G.I. than the treatments 292CN and 291CN, but is not significantly higher than the initial value (Table 2). The results therefore indicate that the treatments undergoing starvation generally produced gonads having a low mean G.I. and color quality. As for analysis of gonad color, the results of the Chi-square comparison (Fisher Method) show that treatments 292CN and 191CN consisted of gonads which were more often rejected due to inacceptable colour than the initial group; ($P = 0.002$) and ($P = 0.049$) respectively. This may be due to the fact that the urchins, in the absence of food, resorb their gonadal and gut tissues in order to gain the energy necessary for maintenance, as was suggested by Kato and Schroeter.[9]

Conclusions

It appears that the most critical of the parameters examined is the water exchange rate. In order to avoid mortality, a sufficient water exchange rate should be maintained at all times. If this is not possible, additional oxygenation might be advisable. Also, ammonia levels should be kept at a minimum and buildup may be avoided by decreasing urchin density in tanks, as it may also become limiting. Furthermore, in a commercial situation of urchin gonadal enhancement or long term stocking, the temperature and salinity of sea water should be maintained at optimum levels, or at least approximating natural conditions. Our results indicate that the parameters which can be critical and have to be closely followed are the oxygen and ammonia levels. Although the study does not permit us to determine exact minimum oxygen saturation limits, it is certain that maintaining oxygen saturation levels above 65% saturation is desirable.

These interventions may reduce mortality, but might not be sufficient to augment and maintain optimum gonad quality in terms of G.I. and colour acceptability. None of the four treatments applied in this study indicate clearly that they had a definitive effect on this requirement.

In order to better define the precise environmental conditions required for urchin survival and good gonadal quality, exact physiological limits of tolerance should be determined in regard to oxygen and ammonia levels in tanks.

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Pilot-scale commercial sea urchin roe enhancement — ocean corral trials

C. J. Bridger,[1] R. G. Hooper,[2] and T. J. McKeever[1]

New Ocean Enterprises Ltd. (NOEL) of Placentia, Newfoundland, a commercial partner of the Marine Institute of Memorial University of Newfoundland sea urchin research team, tested the results obtained from small-scale roe enhancement studies on a pilot semi-commercial sea urchin ranch. Sea urchins were maintained in corrals and fed *Laminaria digitata*. Gonad yield was greater in all fed treatments than in the deeper, wild sea urchins sampled. Gonad quality was also better; however, the quality within treatments was variable. Sea urchins held and fed in deep, barren environments gave yields comparable to those held in more favorable environments. In addition, there were indications that the sea urchins held in the deeper, more constant environment may have given better results if the feeding trials had begun earlier in the season. Sea urchins harvested from the ranch received favorable market prices, providing incentive for the venture to expand for the next season.

Newfoundland waters hold a large stock of sea urchins, but only a low proportion has the quality and quantity of roe necessary to attain the top prices in the market.[3] It was previously determined on a small scale that it is possible to increase the roe production of wild, market-sized sea urchins in a relatively short period of time.[4]

The current project was undertaken to test the effectiveness of ranching sea urchins on a commercial scale and to determine the economics of such a venture. The selected site for the ranch was near Cooper Island on the west side of Placentia Bay, Newfoundland. This site is a large natural sea urchin bed that allowed for the successful containment of the sea urchins. The water depth and bottom type also allowed testing of these two factors in close proximity and within diving safety limits. In addition, the site was near sufficient supplies of both *Laminaria digitata* and *L. longicruris* beds to feed sea urchins on a commercial scale.

Sea urchins were contained within corrals constructed of a braided 3.8 cm (1.5") nylon mesh approximately 1 m high. The foot rope consisted of two lead ropes of appropriate weight to keep the net on the bottom. Gill net buoys were attached to the head rope to allow underwater flotation of the net. Four square corrals were built with 15.2-m (50-foot) sides. Some stocking of the corrals was necessary to ensure the project was conducted at a semi-commercial scale. Approximately 4,536 kg (10,000 lbs.) of sea urchins were held for roe enhancement from September to December 1997.

Diving was the primary method used for harvesting kelp. Several methods were employed involving various numbers of divers and tenders and differing methods of collection (bagging or pumping). The best strategy was when the tasks of cutting and pumping the kelp were separated; this approach resulted in up to 1,270 kg (2,800 lb) of kelp being harvested in 2 hours of bottom time. This involved either one or two divers cutting at the same time and, on a second dive, one diver pumping up the kelp, while the other diver assisted the boat tender. Bagging the kelp on the bottom is also feasible when pumps are not available, although it is much more labor intensive. If excess kelp was cut or it was too rough to feed the kelp immediately to the sea urchins, the kelp could be stored for up to 13 days before it began to discolor and break down.

Feeding the sea urchins was also most effectively performed by divers. However, with the proper water conditions, the shallow corrals could be fed from the surface. Weather conditions on the site limited the number of times the sea urchins were fed during the project, reinforcing the importance of starting commercial farming earlier in the year when better weather can be expected.

Table 1. Chores involved in maintaining a semi-commercial sea urchin ranch and associated man-hours.

Chore	Man-hours	
	Underwater	Tender
Corral set-up	21	9
Cage set-up	3	5
Corral maintenance	5	4.75
Cage maintenance	0	0
Environmental monitoring	–	1.75
Sea urchin roe monitoring	2.25	33.25
Feeding corrals	7.25	8.25
Feeding cages	0.25	0.5
Kelp harvesting	28.75	34.75
Kelp storage	–	2.5
Sea urchin harvesting	37.5	81

Attempts were made to take a sample of sea urchins from each of the corrals at regular intervals throughout the project. At these times, measurements of sea urchins and gonad weight were taken, and gonad quality was assessed. Samples of 20 sea urchins were taken each time and compared to two control sample areas. The first control consisted of sea urchins close to the kelp bed. These were considered to be the best sea urchins on the site and those that would be harvested in the wild fishery. The other control was sea urchins from deeper water, outside the deep corrals, considered to be barren and having the least appealing roe for the Japanese market. All factors of date, area, and interactions affected gonad quantity throughout the experimental period ($P<0.05$). Due to the high variation among individuals in the small samples, a larger sample of 100 sea urchins from each area was taken in December. From this sample, it was apparent there were significant differences among the treatments and the controls ($P<0.05$). Those sea urchins from the kelp bed had the highest roe content of all the samples and no significant difference was observed between these sea urchins and those in the upper corral. For the deeper corrals, sea urchin gonad yield was not much lower than that in the shallow corrals and was significantly greater than yields from the deep control sample. This is a critical result and demonstrates that aquaculture could be employed to enhance the roe content of sea urchins in Newfoundland waters. These sea urchins were held in less favorable deep water conditions on a barren substrate away from any appropriate food supplies. Through feeding, we created an artificial kelp bed, within these unfavorable conditions, to produce higher gonad yields than those in a comparable environment, the deep control. Results on gonad quality were not as good; however, sea urchins in the deep corrals were fed less than those in the upper corrals, which may have contributed to the results. In addition, the analysis of quality is somewhat subjective and tends to be more conservative than the actual results achieved in the market.

Sea urchins were harvested from the ranch in the usual manner employed in the wild fishery, with divers collecting sea urchins and placing them in grab bags which are then lifted to the surface by the dive tender. A total of 4,427 kg (9,760 lb) of sea urchins were harvested and 2,023 kg (4,460 lb) were sent to be packaged for market, a discard rate of 45%. From this, the importance of removing undersized sea urchins from the farm prior to feeding is obvious. The harvested sea urchins were transported to the School of Fisheries processing plant at the Marine Institute to be packaged for shipment to the Japanese market (F.O.B. Tokyo).

An essential part of this project was to determine the commercial viability of roe enhancement utilizing open-ocean corrals. All man-hours were recorded throughout the project to determine the work effort

Table 2. Revenues received for sea urchin roe from the pilot commercial-scale farm.

Harvest Date	Quality (% AB)	Price Received (US$/kilogram)	Total Boxes	Total Revenue
January 10-11	15	7.55	103	$14 850
February 5-6	12	7.55	57	$8 217

required for such an operation. Associated man-hours are listed in Table 1. Cages were also present on-site, however they were neglected due to time constraints during the project. It was apparent that corrals required more time to set-up and maintain than cages. However, the corrals could hold a larger biomass which helped to offset the extra effort and less time is required to feed sea urchins held in corrals. Again, the necessity of removing undersized sea urchins should be emphasized, as extra time was spent on the surface during harvesting, culling out small sea urchins. Diving is an important tool in this form of aquaculture as it is required for nearly every task of the operation. However, diving is expensive in terms of both labor and cost and maintenance of gear, and is limited by the cumulative bottom time. It is therefore recommended that other methods be found to decrease the amount of diving necessary. To complete the economic analysis, data were recorded on the harvest. Table 2 illustrates the associated returns of the harvest. This roe was readily accepted in the Japanese market and fetched some of the highest prices of the year for Newfoundland roe.

From the results of this pilot-scale sea urchin ranch, it was apparent that such a venture could potentially be feasible in Newfoundland. With proper quantities of feed provided to the corralled sea urchins, areas outside of the expected wild harvest could be utilized for the farm. It is expected that sea urchins maintained on flat, sandy bottoms in low surf areas would give better roe enhancement results because these sea urchins would be more capable of actively moving towards the feed than those in areas with high water movement. Operations which require stocking of the site with sea urchins from other areas should take special care with handling urchins to prevent any unnecessary problems associated with mortality. Future research should concentrate on the development and use of artificial feeds to decrease the diving effort and prevent variable quality.

Sincere appreciation is expressed to Brian Burke of the Canadian Centre for Fisheries Innovation and Paul Strickland of the Atlantic Canada Opportunities Agency for their support throughout this project. This project would not have been possible without Mr. Ken Bruce and the crew of New Ocean Enterprises Ltd.

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