

Bulletin

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Aquaculture Association of Canada

Association Aquacole du Canada

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Bulletin de l'Association aquacole du Canada

decembre 1999 (99-4)

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Front cover: Racks used by Cultivos Marinos Achao for the intertidal culture of Crassostrea gigas in Curaco de Velez, Chile (DE Aiken photo). Inside covers: Images from Aquaculture Canada '99 (photocollage by Chris Hendry, photos by John Castell and Chris Hendry).

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Mia Parker
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Melinda Jacobs
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and many other volunteers

Aquaculture Canada '99

Meeting Report

Aquaculture Canada '99 began at the Victoria Conference Centre on the evening of 26 October with a reception sponsored by Future SEA Technologies. Chief Robert Sam and elders from the Songhees First Nations Band welcomed delegates to Victoria — their ancestral homeland — and offered a blessing for a successful conference.

The Opening Ceremony the following morning had presentations from Dr. Jay Parsons, President, Aquaculture Association of Canada; Mr. Yves Bastien, Commissioner for Aquaculture Development; Ms. Liseanne Forand, Assistant Deputy Minister, Policy, Department of Fisheries and Oceans; Mr. Bill Valentine, Deputy Minister, who substituted for the ailing Honourable Dennis Streifel, Minister of Fisheries for the Province of British Columbia; and Mr. Marc Kielley, President, Canadian Aquaculture Industry Alliance. The Honourable Dan Miller, Premier of the Province of British Columbia, made an unexpected — but welcomed — appearance and offered his support to the BC salmon and shellfish aquaculture industry.

Over 550 delegates attended the AAC conference from across Canada, the United States and Europe. The meeting was co-hosted by Master Promotions and was held in conjunction with the successful Pacific Exposition and Trade Show. An additional 230 people were directly associated with the trade show which had 103 exhibitors displaying aquaculture technologies and products.

Sessions were devoted to the conference theme *Aquaculture — A Future in Fisheries* and the program featured over 150 oral and 10 poster presentations in 25 sessions. A debate was "won" by Yves Bastien, Aquaculture Commissioner, and Mike Hunter who defended the topic *Be it resolved that aquaculture and fisheries be united into a single industry by 2020*. Ted White and Jan Negrijn spoke against the issue.

The AAC Student Endowment Fund provided travel support to a number of graduate students presenting papers at the conference. Recipients were Miranda Pryor, Eddy Kennedy, Nancy Mouland, Melissa Mooney, Julie Bertrand, Louise Copeman and Shelby Temple-Banner from Memorial University, Chris Hendry from the University of New Brunswick — Fredericton, Jennifer Ramsay from the Uni-

versity of Prince Edward Island, Kristopher Chandroo from the University of Guelph, Virginia Eccleston from Malaspina University College, Rachel Johnson from the University of Northern British Columbia and Paige Ackerman, Peter Tyedmers and Carlos Gomez Galindo from the University of British Columbia. The BC Science Council sponsored the students from British Columbia universities, Nutreco co-sponsored Louise Copeman and Aqua Health co-sponsored Jennifer Ramsay. Dr John Morgan of Malaspina University College did an excellent job organizing the student travel and presentation awards



The Honourable Herb Dhaliwal, Minister of Fisheries and Oceans, at the salmon BBQ sponsored by the BC Salmon Farmers Association.

Aquaculture Canada '99

Sponsors

Student Travel and Awards

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Salmon Health Consortium
Heritage Aquaculture

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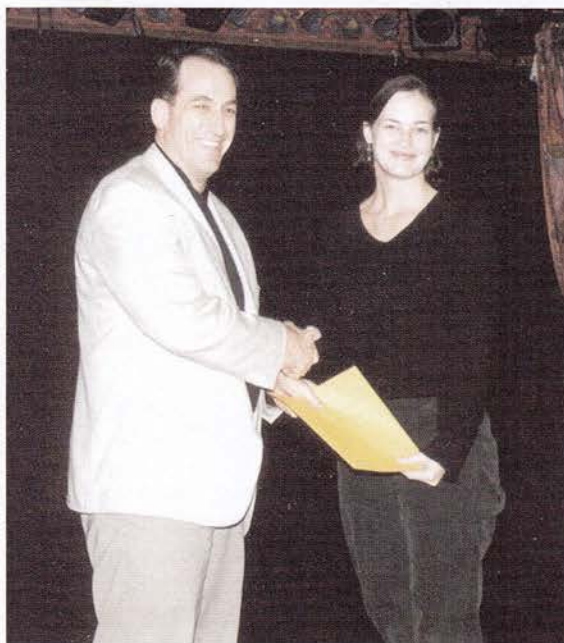
at the meeting. Two students organized and chaired sessions: Paige Ackerman was responsible for the poster session and Virginia Eccleston organized and chaired the vendor's technology session.

Student presentation award winners were Melissa Mooney for best oral presentation. Melissa is from the Marine Institute of Memorial University and her

talked was entitled *A Comparison of Feeding Physiology in Two Size Classes of Cultured and Wild Blue Mussels *Mytilus edulis* and *M. trossulus**. Paige Ackerman from the Department of Animal Science, University of British Columbia was given the award for best student poster presentation. Her poster was entitled *The Relationship Between Stress Proteins and Physiological Stress during Acute Disease Progression*.



Paige Ackerman (right) receiving the award for best student poster presentation from Jay Parsons, President of the Aquaculture Association of Canada.



Melissa Mooney (right) receiving the award for best student paper from Myron Roth, President of the Salmon Health Consortium.



The Honourable Herb Dhaliwal, Minister of Fisheries and Oceans, meeting with the AAC Board of Directors. L to r: Cyr Couturier, Linda Hiemstra, John Bonardelli, Ted White, Mr. Dhaliwal (wearing an AAC t-shirt), Jay Parsons, Marc Kielley, Shawn Robinson and Andrew Boghen.

Heritage Aquaculture sponsored an afternoon reception for the students after they had given their presentations. Mr. Bill Robertson, Director of Aquaculture for Heritage Aquaculture, spoke briefly to the 30 assembled students. Chris Hendry, chairman of the student affairs committee, also held a meeting

with the students to discuss future initiatives for the committee.

The Royal Gala was held at the BC Royal Museum of Natural History. At this popular venue, seafood stations were set up throughout the museum, so the 470 delegates who attended could eat while visiting the various exhibits. A magician performed and his impressive deft of hand left people checking for their wallets and watches at the end of the performance!

The Honourable Herb Dhaliwal, Minister of Fisheries and Oceans, was present on the last day of the conference. Mr. Dhaliwal met with the AAC board of directors, then addressed the Aquaculture Canada delegates where he outlined his support and commitment to the aquaculture sector. Mr. Dhaliwal also attended the salmon BBQ which was generously sponsored by the BC Salmon Farmers Association.

A final thank-you goes to Linda Hiemstra, chairman of the conference steering committee, and all the volunteers for organizing one of the largest and most successful Aquaculture Canada meetings.

— Jay Parsons, President



Linda Hiemstra (right), the chairman of the Aquaculture Canada '99 steering committee, with Al Castledine, the chairman of the funding committee.



Andrew Boghen, the newly installed president of the Aquaculture Association of Canada with the 9 past-presidents in attendance at the annual business meeting. L to R: Neil Bourne (1987), Al Castledine (1995), Cyr Couturier (1996), Susan Waddy (1992), Bill Pennell (1991) Yves Bastien (1993, 1999), Jay Parsons (1998, 1999), Joe Brown (1997), Andrew Boghen (2000) and Dave Aiken (1986).

Rearing of Sablefish (*Anoplopoma fimbria*) from Egg to Juvenile

W. Craig Clarke,⁽¹⁾ John O.T. Jensen,⁽¹⁾ J. Klimek,⁽²⁾ and Zbigniew Pakula⁽²⁾

The sablefish *Anoplopoma fimbria* occurs in the North Pacific Ocean and is valued for its tender, rich flesh. Commercial aquaculture of this species has been constrained by a lack of technology to produce juveniles for grow out. Juvenile sablefish were produced from eggs for the first time in 1998. Fertilized eggs were incubated in upwelling incubators at 6°C and larvae were held in the incubators during the yolk sac phase. In preparation for feeding, larvae were transferred to 2-m³ tanks greened with *Isochrysis galbana* (T-Iso strain). Enriched rotifers were offered at first and enriched *Artemia* later. The highest mortality was experienced during the first month after the time of first feeding. After notochord flexion, mortality decreased and the growth rate increased. Sablefish are readily weaned onto formulated diets and grow rapidly, reaching a weight of 700 g within their first year.

Introduction

The sablefish (*Anoplopoma fimbria*) is frequently marketed as "black cod". However, it is not a cod and in fact belongs to the order Scorpaeniformes that includes rockfishes and greenlings. In the wild, sablefish are pelagic during the juvenile phase, but as adults are found along the continental slope at depths of 300 to 1500 m. Growth is rapid in the first year and slows following the onset of sexual maturity at about 5 years of age.⁽³⁾ The sablefish is considered a candidate for aquaculture because of its choice white flesh with high oil content that is in demand for smoking. Development of aquaculture has been blocked, however, by a lack of juveniles for growing out. In this paper, we describe the results of collaborative research conducted with Northern Marine Farms Ltd. at the Pacific Biological Station to develop hatchery methods for production of juvenile sablefish from fertilized eggs.

Materials and Methods

Eggs and sperm were stripped from sablefish broodstock and fertilized in 35‰ seawater.⁽⁴⁾ The floating eggs were rinsed several times in clean 35‰ seawater and carefully placed in conical, upwelling incubators supplied with filtered sea water at 6°C.⁽⁵⁾ Egg quality was assessed 10 h after fertilization. Samples of 100 eggs were examined under a dissecting microscope for fertilization success and blastomere symmetry. Because the eggs are negatively buoyant at the ambient salinity of 28‰, flow in the incubators was adjusted to maintain eggs suspended in the water column. Light was excluded except during brief inspec-

tions. Eggs and larvae were held in the upwelling incubators for about 5 weeks (i.e., about two weeks during the egg phase and about three weeks during the yolk-sac larval phase). Larvae were transferred into 2-m³ tanks containing *Isochrysis galbana* (T-Iso strain) at 80,000 to 100,000 cells/mL and enriched rotifers *Brachionus plicatilis* at 5 to 10 /mL. Temperature in the tanks was allowed to increase to 10°C over several days. Feeding with enriched *Artemia* (1/mL) was initiated three weeks later. At five weeks from first feeding, a commercial dry diet was co-fed with the *Artemia*. Weaning was completed at 7 to 8 weeks from first feeding.

Results and Discussion

Egg fertilization rates varied widely and were often less than 50%. In egg lots with fertilization rates less than 80%, few survivors reached the time of first feeding. Larvae hatched after 14 to 15 days at 6°C and lacked pigmentation. Larvae swam actively near the surface when introduced into the start-feeding tanks. By the end of the first week, following complete reabsorption of the yolk, there was high mortality. There was another surge in mortality three weeks after first feeding at the beginning of notochord flexion. Subsequently, mortality was low. There was no increase in mortality attributable to weaning. Overall survival from time of first feeding to weaning ranged from 5 to 10%.

By 30 days from first feeding, postlarval sablefish swam actively with the aid of very large pectoral fins with pigmented margins. Linear growth was slow prior to notochord flexion but then increased abruptly

(Fig. 1). Ten weeks after start feeding, the weaned juveniles had an average weight of about 1.2 g. This represents an average growth rate of approximately 9% per day from the time feeding was initiated. Over the next 40 weeks, growth of the 1998 year-class averaged 1.9% per day, resulting in an average weight of 640 g (Fig. 2). The 1999-year class is about 6 weeks ahead of this growth curve. Earlier studies with post-larvae captured in the wild demonstrated that sablefish have a very high growth potential in captivity.^(6,7)

In earlier experiments, few larvae survived beyond

notochord flexion and none to the time of weaning.^(6,8) Various sizes and shapes of tanks have been tried in order to keep the larvae suspended in the water column. We found that the 2-m³ rearing tank was suitable for this purpose. Increasing the temperature at the time the larvae were introduced to the rearing tanks also resulted in an improved feeding response. The first 25 juveniles were produced in 1998 and more than 1600 were produced in 1999.

Recommendations

Although sablefish can be reared through the life cycle, considerable work remains to be done to refine procedures for commercial aquaculture. Particular attention needs to be directed at increasing the reproducibility of spawning in captive broodstock and improving egg quality. Egg quality has a major effect on the viability of larvae. Furthermore, large numbers of high quality eggs are required for improvement of hatchery procedures and scaling up for commercial production

We are indebted to Dr JNC Whyte for guidance on establishing the cultures of algae and rotifers and to John Blackburn, Paul Callow, Christy Falkenberg, and Melinda Jacobs for technical assistance.

Notes and References

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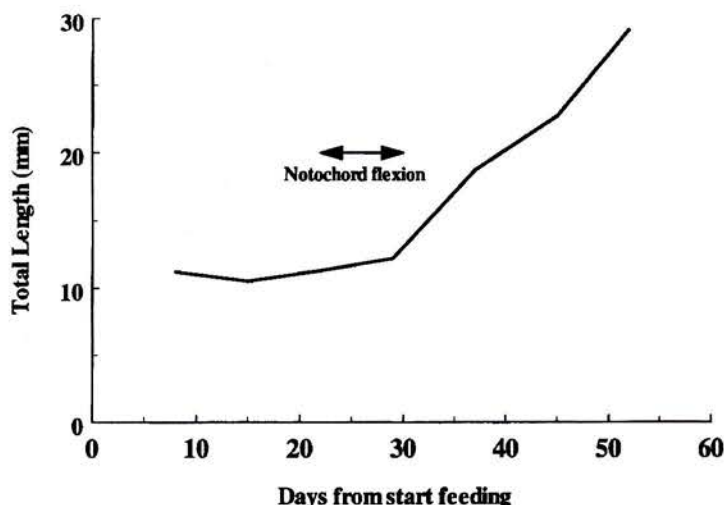


Figure 1. Linear growth of sablefish around the time of notochord flexion. The beginning of the arrow indicates the first time when some individuals had undergone flexion and the end indicates that all individuals had undergone flexion.

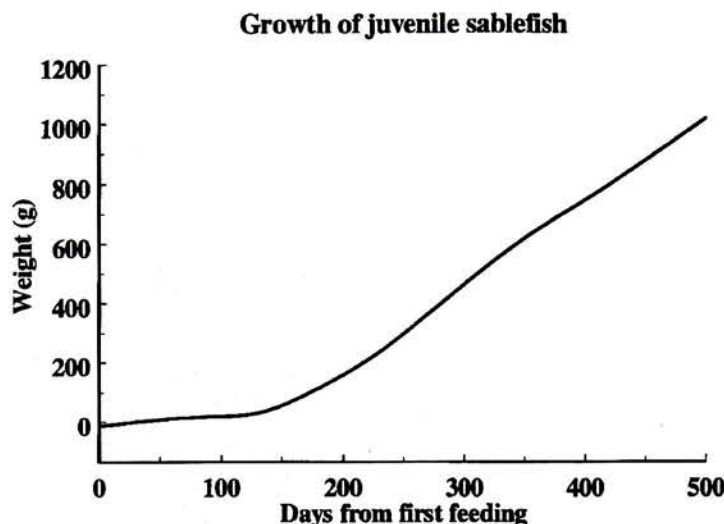


Figure 2. Growth in weight in the 1998 year-class of sablefish.

Hormonal Production of All-Female Atlantic Halibut (*Hippoglossus hippoglossus*): The Timing of Sexual Differentiation

Christopher I. Hendry,^(1,2) Deborah J. Martin-Robichaud⁽¹⁾ and Tillmann J. Benfey⁽²⁾

The Atlantic halibut (*Hippoglossus hippoglossus* L.) is a sexually dimorphic species in which females grow larger and mature later than males, making monosex (all-female) culture economically advantageous. Monosex populations can be produced by using hormones (sex steroids) to direct sexual differentiation towards the desired sex. Knowledge of the timing of sexual differentiation in fish is important in the application of hormones for production of monosex populations. We histologically determined that sexual differentiation in Atlantic halibut has occurred by the time halibut reach 37 mm fork length, which coincides with the weaned, post-metamorphic stage. Sexual differentiation is a gradual process that coincides with other organogenesis in the developing larvae. The results of these experiments are directly relevant to halibut aquaculture in Canada.

Introduction

Canada's aquaculture industry is based for the most part on Atlantic salmon.⁽³⁾ However, due to national overproduction of farmed salmon and global competition, alternate species are being sought to sustain and enhance the value of the industry.

The Atlantic halibut, the largest of the flatfishes (family Pleuronectidae), is a prime candidate for aquaculture due to its excellent flesh quality and the fact that it commands the highest price of any groundfish in Atlantic Canada.⁽⁴⁾ Furthermore, landings of wild halibut have declined in Canada from 3.7 million kg in 1986 to 0.9 million kg in 1995,⁽⁵⁾ making aquaculture of this species all the more desirable. The use of hormones to produce all-female populations is useful for aquaculture purposes when one sex has preferred characteristics such as growth performance or delayed maturation.⁽⁶⁾ Female halibut grow at a faster rate and mature later than males,⁽⁷⁾ making female monosex culture more economically sound.

For effective sex reversal, adequate doses of steroids must be administered while the gonad is undifferentiated and continued throughout the stage of gonadal differentiation.⁽⁸⁾ The timing of sexual differentiation varies in teleosts from only a few days past hatch to very late in development (Table 1) and is under the influence of both endogenous (hormonal) and exogenous (environmental) factors.⁽⁹⁾ In the species studied, various indices have been used (e.g., size, age, degree-days) to define the timing of sexual differentiation. Unfortunately, many of the values reported for time of differentiation cannot be compared due to differences

in the life cycles of the various species. It would be beneficial to be able to compare time of sexual differentiation in terms of life cycle stage with respect to temperature (e.g., yolk-sac stage, first-feeding stage, etc.). In the teleosts, sexual differentiation has been most widely studied in the salmonids;⁽¹²⁻¹⁶⁾ relatively few reports have been published on flatfish.⁽¹⁷⁻²⁰⁾

The timing of sexual differentiation is determined by histological examination of the primordial gonads and their development toward testes and/or ovaries. Development occurs in two stages: cytological and anatomical differentiation.⁽⁸⁾ The former involves the differentiation of oogonia and oocytes or spermatogonia and spermatocytes, while the latter involves structural changes into testes or ovaries. In teleosts, this is initiated by the differentiation of germ cells from the cortically-derived embryological primordium into distinct male and female gonads.⁽²¹⁾

The timing of sexual differentiation determines the method of steroid administration: immersion is used in species which sexually differentiate before start-feeding and dietary supplements are used for those that differentiate after start-feeding. The objective of our research was to determine the timing of sexual differentiation in Atlantic halibut, as a first step towards developing methods for the production of monosex halibut for aquaculture.

Methodology

A developmental time series of larvae from hatch to approximately 180 days post-hatch (dph), fixed in 10% neutral buffered formalin, was examined histo-

Table 1. Comparison of the timing of sexual differentiation among teleost species.

Common name	Scientific Name	Timing of Sexual Differentiation
Three-spined stickleback	<i>Gasterosteus aculeatus</i>	8 days post-hatch (dph) ⁽¹⁰⁾
European eel	<i>Anguilla anguilla</i>	22 to 30 cm body length ⁽¹¹⁾
Chum salmon	<i>Onchorynchus keta</i>	55 days post-hatch ⁽¹²⁾
Coho salmon	<i>Onchorynchus kisutch</i>	77 days post-fertilization (dpf) ⁽¹²⁾ 380 degree-days (d°) ⁽¹³⁾
Masu salmon	<i>Onchorynchus masou</i>	5 weeks post-hatch (wph) ⁽¹⁵⁾
Rainbow trout	<i>Onchorynchus mykiss</i>	137 days post-hatch ⁽¹⁶⁾
Barfin flounder	<i>Verasper moseri</i>	53 to 55 mm body length ⁽¹⁷⁾
Sole	<i>Solea solea</i>	5 to 10 cm body length ⁽¹⁸⁾
Japanese flounder	<i>Paralichthys olivaceus</i>	15 to 30 mm body length ^(19,20)

logically. Small whole larvae, or sections of larger larvae, were embedded in paraffin after dehydration (ethanol series) and clearing (toluene). Paraffin blocks were sectioned to a thickness of 6 µm and stained with haematoxylin and eosin Y.⁽²²⁾ Using light microscopy, the primordial and differentiated gonads

was identified to determine the timing of sexual differentiation, beginning with larger, differentiated gonads, and gradually moving back through the time series to smaller fish. Photomicrographs were taken using a Leitz Wild MPS 46 Photoautomat fitted with a

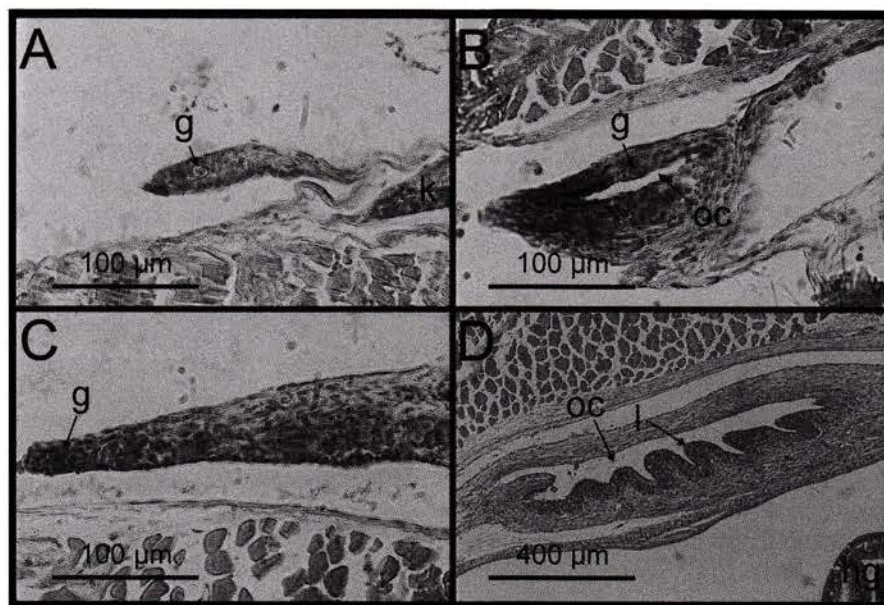


Figure 1. The indifferent gonad of a larval Atlantic halibut, 21-mm FL (A). After the onset of anatomical differentiation, an ovarian cavity (oc) has formed in a 37-mm female (B), while in a male of the same size (C), no differentiation is evident. Even as further ovarian differentiation occurs in a 70-mm female (D), with the formation of ovarian lamellae (l), the male gonad remains undifferentiated. (g = germ cell; k = kidney; hg = hindgut)

blue filter, and attached to a Leitz Laborlux S compound microscope.

Results

The gonads in the Atlantic halibut differentiate from a common, indifferent gonad (Fig. 1A), which contains a few germ cells destined for oogenesis/spermatogenesis. The gonads are paired and found on both sides of the body (ocular and blind) just ventral to the kidney, which is ventrally situated from the spine and spinal cord.

As the indifferent gonad develops, the first sign of differentiation is evident in females, with the increase in the number of germ cells and formation of the ovarian cavity (Fig. 1B). Presumptive males of the same size show no signs of sexual differentiation (Fig. 1C); their gonads remain like the indifferent gonads of smaller fish (Fig. 1A). The position of both differentiating ovaries and undifferentiated testes still remains paired and ventral to the kidney.

As the ovary further differentiates, with formation of ovarian lamellae (Fig. 1D), the testes are still undifferentiated. The specific timing of male differentiation has yet to be accurately defined.

Discussion and Conclusions

From the observed ovarian differentiation, it is evident that sexual differentiation in Atlantic halibut starts anatomically just prior to reaching 37 mm FL, which corresponds to the post-metamorphic, settling stage. Pittman et al.⁽²³⁾ denote this period in the development of halibut as "post-larvae" and, according to the timing of organogenesis reported during larval and post-larval development, the gonads are one of the last organs to develop. The developmental stage of subsequent cytological or testicular differentiation has yet to be defined. However, it has been shown in the barfin flounder (*Verasper moseri*) and the Japanese flounder (*Paralichthys olivaceus*) that differentiation of the testes follows that of the ovaries, in the latter by almost twice the size and age.^(16,18)

It is important to note that the fork lengths of Atlantic halibut described here are not exact, as measurements were taken from formalin-fixed samples. Recent research has shown there is significant shrinkage of larval fish during preservation.⁽²⁴⁾ Reporting the length of the fixed larvae does, however, ensure that subsequent treatment of halibut larvae with exogenous steroids for the purpose of sex reversal will commence prior to sexual differentiation.

The present research allows the development of effective methods for the production of all-female stocks of halibut for commercial aquaculture using hormonal manipulation. The results will be of direct

relevance to the halibut aquaculture industry, as well as providing insight into the basic mechanisms of sexual differentiation and reproductive development in this and related species.

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The Effect of Dietary Protein and Lipid on Juvenile Halibut

Tammy J. Blair,⁽¹⁾ John D. Castell,⁽¹⁾ Sarah Mercer⁽¹⁾ and Frank Powell⁽²⁾

Growth and survival of juvenile halibut fed different diets were compared. Four diets were formulated with constant protein (P) levels (50%) and varied lipid (L) amounts (13 to 22%). Five diets were isocaloric and varied in protein level (40 to 60%). The 50% diet was the same as the 13% L, so only 8 diets were used. After 10 wk, fish fed 60% P were significantly larger than those on other protein treatments. Protein level affected survival, with fish fed 60% P having significantly higher survival, followed by 55% P having significantly higher survival than diets containing 45 or 50% P. Lipid level had no significant effect on either fish weight or survival. Fish receiving the 60% P diet had an average feed conversion of 1.0 ± 0.5 ; feed conversion for the 55% P diet averaged 2.0 ± 1.5 . Fish fed the 40% P diet lost weight and the feed conversion value was negative. It appears that juvenile halibut have a high protein requirement that is not spared by increasing dietary lipid.

Introduction

Recent improvements in the growth and survival of Atlantic halibut larvae through metamorphosis suggest a promising future for commercial culture of this species in Atlantic Canada.⁽³⁾ There have been few studies on the effects of dietary composition on Atlantic halibut. These studies have used halibut of various size classes, from juveniles in the size range of 5 to 140 g⁽⁴⁻⁸⁾ to those approaching maturity at 1 kg or more.⁽⁹⁻¹⁰⁾ A few researchers have compared the effects of different protein, lipid, and carbohydrate levels on fish growth and development, but there is not clear agreement as to the optimal protein level or the protein sparing value of dietary lipid. One study concluded that juvenile halibut require at least 58% dietary protein⁽⁸⁾ while another estimated an optimal level of 74%.⁽⁴⁾ It has also been reported that for halibut weighing between 140 and 260 g, not more than 51% dietary protein is needed because lipid can supply a portion of the required energy.⁽⁶⁾

The present study was designed to evaluate the effects of varying protein and lipid levels in diets fed to juvenile Atlantic halibut. The results presented include effects on growth and survival, as well as feed conversion.

Materials and Methods

Test diets with various protein and lipid levels were formulated (Table 1). Five of the diets were used to test the effects of protein levels (% P) ranging from 40 to 60%. These diets were isocaloric, so the amounts of lipid and carbohydrate were adjusted to balance the energy values. Four of the diets formulated with 50%

protein were used to test lipid levels (% L) ranging from 13 to 22%. The 13% L diet is the same as the 50% P diet in the protein level experiment.

The diets were mixed using Hobart mixing equipment and steam-pelleted in a California Pellet Mill (3-mm diameter pellets). The pellets were air-dried overnight, bagged, and stored at -40°C until use. Proximate analysis of the diets was done to verify the nutrient composition (Table 1). Analysed lipid levels were close to formulated values but protein levels were 4 to 5% higher than formulated values.

Each diet was fed to 3 replicate tanks of 32 halibut from the period April 29 to July 7, 1999. The tanks were on a flow-through, seawater system with 1- μ m filtered water supplied at a rate of 1 ± 0.2 L/min. Water level inside the 65-cm diameter dark green fibreglass tanks was maintained at 17.5 cm. The salinity of the water was 31 ± 2 ppt. The temperature was program-controlled by mixing heated and ambient filtered seawater with ambient seawater. The initial temperature (8°C) was held for 1 wk to allow the fish time to acclimatize to the new environment. After the first week, the temperature was raised by 0.5°C every third day to a final temperature of 10°C. This constant temperature was maintained until the last 2 wk, when ambient temperature rose to 13°C. The tanks were lit with standard overhead fluorescent lights (11.4 ± 2.8 lux) programmed for a 12 h L:12 h D photoperiod.

The initial mean weight of the fish was 13.6 ± 5.8 g. The halibut were hand-fed to satiation 3 times each day. Twenty minutes after feeding, uneaten food and faeces were flushed down the drain. The fish were weighed every second week, when we also noted characteristics such as tail and fin condition, pigmentation, and degree of eye migration. Feed consump-

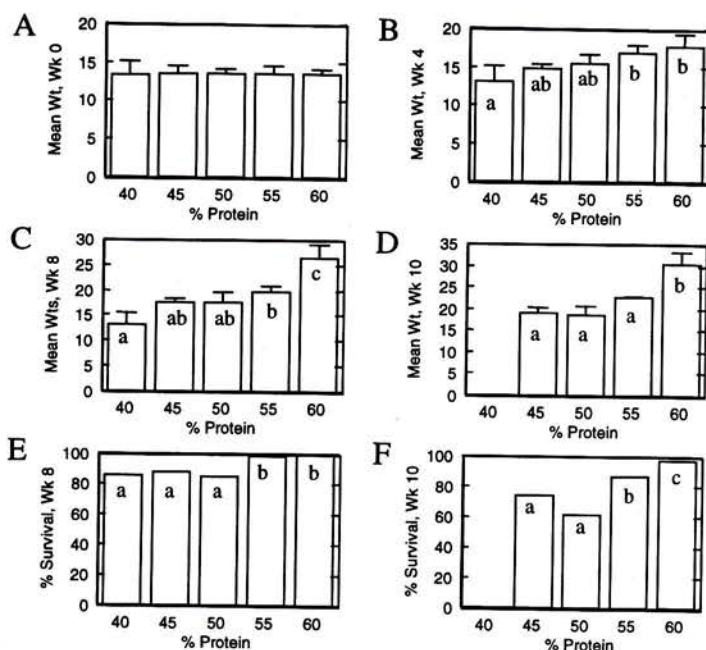


Figure 1. Growth and survival of fish fed varying amounts of protein. Mean fish weights in grams after A) 0 wks, B) 4 wks, C) 8 wk, and D) 10 wk of treatment. Percent survival after E) 8 wk and F) 10 wk of treatment. Different letters within a graph indicate significant differences when $P = 0.05$.

tion for each tank was also monitored. The feeding trial lasted for 10 wk.

The data were analysed using Systat® v. 8.0.⁽¹¹⁾ Fish weights were analysed by ANOVA with Tukey's HSD test and $P = 0.05$. Survival data was analysed using the chi-square test.

Results and Discussion

Dietary protein levels significantly affected growth. Within 4 wk of treatment (Fig. 1b) there was an increase in growth corresponding with each increase in dietary protein level. Fish receiving the lowest amount of protein did not appear to increase in size compared to their initial weights, whereas fish receiving 55 and 60% P diets grew to be significantly larger than fish fed the 40% P diet. After 8 wk of feeding (Fig. 1c), fish fed 40% P still had not grown compared to their initial weights. Fish fed 60% P diet doubled in size (mean wt 26.6 ± 2.5 g). Although the 40% P fish were not experiencing significantly higher mortality rates, their poor appetite and lack of growth indicated that they

Table 1. Formulation and proximate analysis of diets.

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8
Formulation (g/100 g feed)								
Herring meal (74% CP)	26.1	32.7	38.8	45.1	51.4	39.3	39.6	40.5
Casein (90% CP)	8.9	10.3	11.7	13.1	14.5	11.7	11.7	11.7
Wheat middlings (17% CP)	29.3	22.7	18.0	12.7	7.2	15.7	14.5	10.8
Basal mix *	16.4	16.4	16.4	16.4	16.4	16.4	16.4	16.4
Vitamin mix **	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Salt mix Bernhart-Tomarelli (modified)***	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Pre-gelatinized starch	5.7	6.6	6.5	6.7	7.0	5.3	3.2	3.0
Menhaden oil (stabilized with 0.75% ethoxyquin)	11.6	9.3	6.6	4.0	1.5	9.6	12.6	15.7
Dry Matter (g/100 g feed)	91.9	91.4	90.1	91.0	91.1	90.4	90.0	89.7
Formulated Chemical Composition (g/100 g dm)								
Protein	40	45	50	55	60	50	50	50
Lipid	17	15	13	11	9	16	19	22
Digestible energy (kJ/g dm)	17	17	17	17	17	18	19	20
Analysed Chemical Composition (g/100 g dm)								
Protein	44.9	49.3	53.8	60.0	64.6	54.5	54.0	54.2
Lipid	18.5	16.1	13.5	11.7	9.2	16.4	19.9	22.8
Ash	6.4	6.9	7.3	8.1	8.5	7.4	7.3	7.3

* Basal mix: Egg albumin, 2.2 g; Krill hydrolysate (61% CP), 2.0 g; CPSP-G (72% CP), 5.0 g; Whey (13.2% CP), 7.0 g; Choline chloride, 0.2 g.
 ** Vitamins added/kg diet: Vitamin D3, 11250 IU; Vitamin A, 9975 IU; Folic acid, 25 mg; Vitamin K, 45 mg; Riboflavin, 60 mg; Vitamin B6, 60 mg; Thiamin, 75 mg; Vitamin B12, 0.1 mg; Biotin, 1.2 mg; d-Calcium pantothenate, 180 mg; Niacin, 200 mg; myo-Inositol, 200 mg; Vitamin E, 125 IU; Vitamin C, 1000 mg; Ethoxyquin, 100 mg.

** Composition (%) of B-T salt mix: Calcium carbonate, 2.1; Calcium phosphate, 73.5; Citric acid, 0.227; Cupric citrate $\frac{1}{2}\text{H}_2\text{O}$, 0.046; Potassium diphosphate, 8.1; Ferric citrate $5\text{H}_2\text{O}$, 0.588; Magnesium oxide, 2.5; Manganese citrate, 0.835; Potassium iodide, 0.001; Potassium sulfate, 6.8; Sodium chloride, 3.06; Sodium phosphate, 2.14; Zinc citrate H_2O , 0.133.

were stressed, and the treatment was discontinued after 8 wk. After 10 wk (Fig. 1d), fish fed 45 and 50% P diets exhibited an average weight gain of 5 g. Fish receiving 55% P gained an average of 9 g. Fish receiving 60% P grew significantly larger reaching a final weight of 30.6 ± 2.5 g (average 17 g increase).

Figure 2b shows the results for the various dietary lipid levels after 10 wk of feeding. The mean fish weights in all treatments increased by 3.3 to 6.3 g, with no significant differences among treatments (in order of increasing dietary lipid content: mean weights 19.0 ± 1.7 , 20.1 ± 0.5 , 16.9 ± 1.9 , 17.1 ± 2.3 g).

The growth effects of different dietary protein and lipid levels shown in this study are comparable to those previously reported for juvenile halibut.^(4,5,7) Although one study⁽⁶⁾ using larger fish (~200 g) reported a protein-sparing effect of dietary lipid, their conclusion was based upon nitrogen and energy utilisation values rather than on differences in growth, feed intake, or feed efficiency ratios. They found that fish consuming a diet with 53.9% protein and 27.6% lipid retained the same amount of nitrogen and a higher percentage of dietary energy as fish consuming 56.9% protein and 24.5% lipid. We did not find any protein-sparing effects of increasing dietary lipid content above 13%. Though the difference was not statistically significant, the two highest lipid levels seemed to slightly reduce growth.

A chi-squared procedure was used to test for differences in survival. Although Figures 1e,f and 2c have % survival on the axis, the test was based on counts of surviving fish. After 8 wk, survival was significantly higher for the 55 and 60% P groups than for the lower

protein treatments. After 10 wk, the same trend was true although at this time the 60% treatment was greater than all others (the 10-wk survival rates in order of decreasing protein were 97.9, 87.5, 62.5, and 75.0%).

The other studies on protein and lipid levels in juvenile halibut diets did not report any significant differences in survival among their dietary treatments. In our study, the increased ambient temperature during the last 2 wk appeared to stress the fish and lead to higher mortality rates among fish fed diets formulated with 45 to 55% P. There was also a high incidence of incomplete metamorphosis and a large variation in sizes of the fish (initially, individual weights ranged from 1 to 32 g) which might partially account for higher mortality compared to other studies. Nevertheless, the initial mean weights and size distributions were the same for every treatment. We conclude that although survival was affected by a number of factors and stresses, such as the temperature increase to 13°C and the size range within tanks, differences in dietary protein level played a significant role. For the lipid diets, no significant differences in survival were found. The 13, 16, 19 and 22% L diets resulted in mean survival rates of 62.5, 64.6, 74.0 and 70.8%.

Fish fed the 60% P diet had an average biweekly feed conversion (dry weight fed/live weight gain) of 1.0 ± 0.5 . Feed conversion for the 55% P diet averaged 2.0 ± 1.5 . Fish fed the 40% P diet lost weight and the feed conversion value was negative. Taking all the results into consideration, it appears that juvenile halibut have a relatively high protein requirement, which was not spared by increasing the dietary lipid.

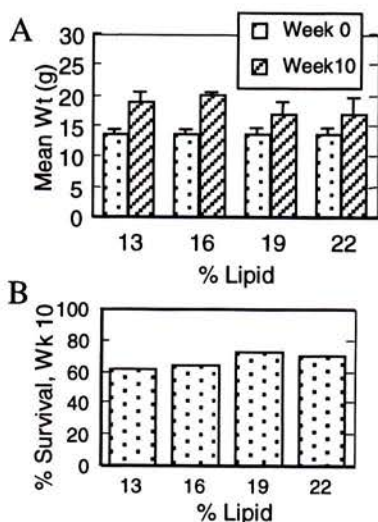


Figure 2. Growth and survival of fish fed varying amounts of lipid. A) Mean fish weights in grams after 0 or 10 wk. B) Percent survival after 10 wk of treatment. Differences were not significant when $P = 0.05$.

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Effect of Dietary Ratios of DHA, EPA and AA on Early Growth, Survival and Pigmentation of Yellowtail Flounder (*Pleuronectes ferrugineus*)

L. A. Copeman,⁽¹⁾ C. C. Parrish,⁽¹⁾ J. A. Brown,⁽¹⁾ and M. Harel⁽²⁾

Marine fish require the dietary polyunsaturated fatty acids (PUFA) docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3) and arachidonic acid (AA, 20:4n-6) for normal growth and development. However, little is known about the fatty acid requirements of yellowtail flounder (*Pleuronectes ferrugineus*) larvae. Consequently, we designed an experiment to study the role of dietary ratios of these fatty acids on their early growth, survival, lipid composition, and pigmentation. Rotifers were enriched with emulsions high in DHA (43.3% of total fatty acids), DHA+EPA (37.4% and 14.2% respectively), DHA+AA (36.0% and 8.9% respectively), or with a control (no DHA, EPA, or AA) emulsion. After four weeks, larvae fed the high DHA diet were significantly larger (9.7 mm, $P < 0.05$) and had higher survival (22%) than larvae fed the other diets, while larvae on the control diet were significantly smaller (7.3 mm, $P < 0.05$) and showed lower survival (5%). Larval lipid class and fatty acid profiles showed significant differences, with fatty acids reflecting dietary levels. The incidence of malpigmentation was higher in the high DHA+AA diet (92%) than in the other treatments (< 64%). We conclude that yellowtail larvae require diets that are highly enriched with DHA, while elevated dietary AA may exert negative effects on larval pigmentation.

Introduction

The importance of polyunsaturated fatty acids (PUFA) in fish nutrition has been extensively investigated during the past 20 years.⁽³⁾ DHA, EPA, and AA are essential fatty acids (EFA) for many marine species, as they are required for normal growth and development but cannot be synthesized from shorter chain dietary precursors.⁽⁴⁾ Both the absolute and relative amounts of these EFA have been shown to significantly affect growth, survival, neural development and pigmentation in a number of species.⁽⁵⁾ However, live-foods that are commonly used for first feeding of marine larvae, such as rotifers and *Artemia*, are naturally low in these PUFA. Therefore, enrichment of live foods with lipids rich in PUFA prior to feeding is usually necessary.⁽⁶⁾

Yellowtail flounder is a small right-eyed flounder found on the east coast of North America.⁽⁷⁾ It was chosen as a potential candidate for coldwater aquaculture due to its high fillet-to-body ratio, its relatively high market price, and a steadily declining wild stock size.^(8,9) Since 1994, research on yellowtail larviculture at the Ocean Sciences Centre has focused on the physical and biological requirements of this species.⁽¹⁰⁾ However, high mortality and malpigmenta-

tion are still challenges to the successful early culture of yellowtail. In other marine species, both of these issues have frequently been associated with the PUFA content of live food used in first feeding.⁽⁵⁾

In this paper, the role of dietary ratios of DHA, EPA, and AA on the early development and survival of yellowtail flounder has been investigated.

Methods

Three emulsions were formulated by mixing different ratios of DHA- and AA-rich triacylglycerols (TAG) from heterotrophic algae production (DHASCO and ARASCO, Martek BioSci., Columbia, MD) and a marine oil (TG 22/33 Marine Lipids, Leknes, Norway). Algal oils contained 49% DHA or 54% AA of the total fatty acids, while the marine oil contained 60% EPA and 40% DHA. A control emulsion was prepared that was high in lipid but low in these PUFA (Table 1). Rotifers were enriched at a density of 500 000 rotifers/L for 12 hours. Emulsions were added at a concentration of 0.1 g of emulsion/L of rotifer culture. Throughout the experiment, rotifers were sampled four times in triplicate for lipid class and fatty acid analysis.

Eggs from five females were pooled and incubated

in a 300-L conical upwelling tank. Hatched larvae were transferred to eight 230-L round flat-bottom tanks at a density of 60 larvae/L. Temperatures were ambient and averaged 13°C, while illumination was continuous and set at 1000 lux. Tanks were supplemented daily with a 1:1 mix of 10 L of *Isochrysis galbana* and *Nannochloropsis* spp. For four weeks post-hatch, duplicate tanks of larvae were fed rotifers enriched with one of the experimental emulsions. Larvae were fed twice per day at a density of 7000 rotifers/L which is known to be optimal for this species.⁽¹⁰⁾ Following week four, all groups were maintained on *Artemia* enriched with Algamac-2000 (Aquafauna Bio-Marine, Hawthorne, CA).

Weekly measurements of standard length, body depth, and dry weight were taken on 15 larvae per tank. Survival at the end of four weeks was estimated by counting the number of larvae in 5-L sub-samples of tank water. At this time, lipid class and fatty acid analyses were also completed on three samples of larvae per tank. Pigmentation was characterized at 13 weeks post-hatch on the ocular side of 50 fish per tank.

Results and Discussion

Following enrichment, all rotifer groups contained approximately 16% of their dry weight as lipid. Levels of fatty acids in the rotifers reflected that of enrichment emulsions. The three PUFA treatments resulted in high levels of DHA (21.5 - 28.2%) with various levels of EPA (3.2 - 11.0%) and AA (1.2 - 7.1%), while the control had low concentrations of all of these PUFA (< 2.5%). The ratio of DHA:EPA in the rotifers varied widely between groups from a high of 8.2 in the DHA treatment to a low of 0.7 in the control diet (Table 1).

After four weeks, larval standard length, body depth, and dry weight were significantly higher ($P < 0.05$) in the DHA treatment than all other groups. Larvae from the control diet were smaller in these measures ($P < 0.05$). Trends in survival reflected those seen in growth; survival was highest in the DHA group (22%) and lowest in the control group (5%, Table 2).

Lipid analysis of the larvae showed that the DHA and DHA+EPA groups had significantly higher levels of total lipid and TAG than larvae from other dietary groups. In other species, such as herring (*Clupea harengus*)¹¹ and Atlantic cod (*Gadus morhua*),⁽¹²⁾ elevated total lipid and TAG per dry weight have been associated with a relative improvement in larval condition. In this study, however, larvae from the DHA+EPA treatment had lower growth and survival than larvae from the DHA+AA group, despite significantly higher total lipid and TAG per dry weight (Table 2).

The relative amounts of DHA, EPA, and AA in larval tissues reflected dietary levels. However, in the control diet there was preferential retention of these PUFA and a decrease in 18:1n-9 compared to dietary levels. This retention indicates a relative deficiency of these fatty acids in the diet.

Positive correlations existed between the dietary DHA:EPA ratio and both survival ($R^2 = 0.87$, $P = 0.001$) and size (e.g. standard length $R^2 = 0.65$, $P = 0.02$) at the end of week 4. Improved growth,⁽¹³⁾ and survival^(14,15) with higher dietary DHA:EPA ratios have been observed in a number of other marine species. A 2:1 ratio of DHA:EPA has been suggested as an adequate dietary level for marine fish larvae.⁽⁵⁾ However, our study indicates that ratios much higher than 2:1 are required for yellowtail flounder.

High proportions of malpigmented fish were ob-

Table 1. Fatty acid composition of emulsions and rotifers after 12 hours of enrichment (mean \pm SE)

Fatty acids (%)	DHA	DHA+EPA	DHA+AA	Control
Emulsions				
18:1n-9	24.7 \pm 0.1	18.6 \pm 0.2	23.1 \pm 0.6	70.0 \pm 1.0
AA	0.7 \pm 0.0	1.6 \pm 0.0	8.9 \pm 0.2	0.0 \pm 0.0
EPA	0.1 \pm 0.1	14.2 \pm 0.2	0.1 \pm 0.0	0.0 \pm 0.0
DHA	43.3 \pm 0.4	37.4 \pm 0.3	36.0 \pm 0.4	0.5 \pm 0.1
Rotifers				
18:1n-9	26.7 \pm 1.0	20.8 \pm 0.4	26.4 \pm 0.8	52.1 \pm 1.0
AA	1.2 \pm 0.0	2.2 \pm 0.4	7.1 \pm 0.2	0.7 \pm 0.0
EPA	3.5 \pm 0.3	11.0 \pm 0.2	3.2 \pm 0.2	2.5 \pm 0.2
DHA	28.2 \pm 0.5	21.5 \pm 0.7	23.4 \pm 0.8	1.7 \pm 0.1
DHA:EPA	8.2 \pm 0.7	1.9 \pm 0.1	7.5 \pm 0.6	0.7 \pm 0.0

served in all dietary groups at the end of the experiment. However, less than 64% of the fish were malpigmented in the DHA, DHA+EPA and control groups, while 92% of the fish in the DHA+AA treatment were malpigmented (Table 2). This suggests that nutrition during the first four weeks is critical for successful pigmentation. High dietary AA during this period has a negative effect on pigmentation. Preliminary studies on turbot (*Scophthalmus maximus*) and Atlantic halibut (*Hippoglossus hippoglossus*) have also shown that high dietary AA during early larval development negatively affects pigmentation.^(5,16) These results have been related to increased eicosanoid activity with higher dietary AA and a resulting heightened state of stress in the larvae.^(5,16) Fortunately, the high levels of AA in this study are not a practical problem in aquaculture, as commercial enrichment products contain low levels of this fatty acid (< 3 %).

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Table 2. Larval growth and lipid composition after four weeks of feeding on differently enriched rotifers (mean \pm SE)

	DHA	DHA+EPA	DHA+AA	Control
Growth Parameters				
Standard Length (mm)	9.7 \pm 0.2	8.7 \pm 0.2	8.9 \pm 0.2	7.3 \pm 0.2
Body Depth (mm)	1.6 \pm 0.1	1.1 \pm 0.1	1.3 \pm 0.1	0.7 \pm 0.1
Dry Weight (mg)	1.0 \pm 0.1	0.7 \pm 0.1	0.8 \pm 0.1	0.3 \pm 0.0
Survival (%)	22.2 \pm 0.4	12.3 \pm 2.1	19.0 \pm 1.7	5.2 \pm 1.9
Pigmentation (% malpigmented)	53.0 \pm 4.2	60.0 \pm 2.8	92.1 \pm 1.4	64.0
Lipid composition				
Total lipid (μ g/mg)	125.6 \pm 16.7	123.7 \pm 4.0	87.4 \pm 7.2	88.6 \pm 13.7
Triacylglycerol (μ g/mg)	25.9 \pm 4.2	27.9 \pm 0.4	15.7 \pm 0.3	9.7 \pm 4.7
18:1n-9 (%)	21.4 \pm 0.5	18.9 \pm 0.9	18.5 \pm 0.6	26.5 \pm 0.7
AA (%)	2.2 \pm 0.0	2.6 \pm 0.2	8.0 \pm 0.2	2.8 \pm 0.1
EPA (%)	4.8 \pm 0.0	10.1 \pm 0.4	4.6 \pm 0.4	6.7 \pm 0.0
DHA (%)	27.1 \pm 1.1	18.7 \pm 1.0	22.3 \pm 1.0	7.0 \pm 0.2

Observations on Sealice in Atlantic Salmon Raised in a SEA System™ Floating Bag and a Conventional Netpen

V. Brenton-Davie⁽¹⁾ and H. Kreiberg⁽²⁾

We conducted dedicated sea lice checks on two occasions after observing apparent differences in lice burden (*Lepeophtheirus salmonis*) on Atlantic salmon (*Salmo salar*) being raised in a comparison of a SEA System™ floating bag and a conventional netpen. Two months before harvest, sea lice prevalence was 64% for fish reared in the netpen and 32% for those in the bag. Mean intensity was 1.5 lice per infected fish for the netpen and 1.25 lice per infected fish for the bag. Abundance was 0.96 lice per fish in the bag and 0.40 lice per fish in the netpen. At harvest, prevalence was 60% for the netpen fish and 8% for the bag fish. The difference in lice prevalence between the two systems was significant on both occasions. At harvest, mean intensity was 1.6 lice per infected fish for the netpen and 1.0 lice per infected fish for the bag; abundance was 0.96 lice per infected fish for the netpen and 0.08 lice for the bag. Lice infestations in both systems were low by industry standards and did not result in loss of market quality of the fish. Possible causes for the observed differences between the two rearing systems include advantageous placement of the SEA System™ intake and the influence of greater current speed and exercise levels in the fish reared in the bag.

Introduction

The SEA System™ is a floating fabric bag technology for fish culture recently developed by Future SEA (Future SEA) Technologies Inc. Its pump and adjustable intake allows selection of the incoming water for optimum growing conditions and regulation of current speed. Future SEA is conducting research in collaboration with Fisheries and Oceans Canada to assess the biological performance of the bag technology compared to a traditional netpen at their test farm located at the Pacific Biological Station's (PBS) Experimental Mariculture Facility at Nanaimo, BC, Canada.

An experiment designed to monitor growth and market quality of commercially-produced Atlantic salmon juveniles was conducted at the test farm from October 28, 1998 to July 21, 1999. During regular sampling, it appeared that Atlantic salmon from the bag carried fewer sealice (*Lepeophtheirus salmonis*) than those from the netpen. To confirm the observations, dedicated lice checks were carried out 2 months prior to harvest and again at harvest. In this paper we summarize the findings of these protocols and comment on possible underlying causes for the difference

in sealice prevalence and intensity of infestation in the two systems.

Methods and Materials

Culture units

An 875-m³ SEA System™ bag (approximately 12 m diameter x 10 m deep) was tested alongside a 324-m³ (approximately 6 m x 9 m x 6 m (LWD), 2.5-cm mesh) traditional netpen stocked on October 28, 1999 with 477-g juvenile Atlantic salmon obtained from a single netpen lot on a commercial salmon farm. The two groups were harvested 9 months later at an average size of 2.5 kg.

Lice sampling

The lice sampling protocol used a dedicated three-person crew. The Atlantic salmon from each culture unit were slowly crowded using diver-assisted seining. Once the fish were settled, the sampling was conducted as soon as possible and both groups of Atlantics were handled consistently and as similarly as possible.

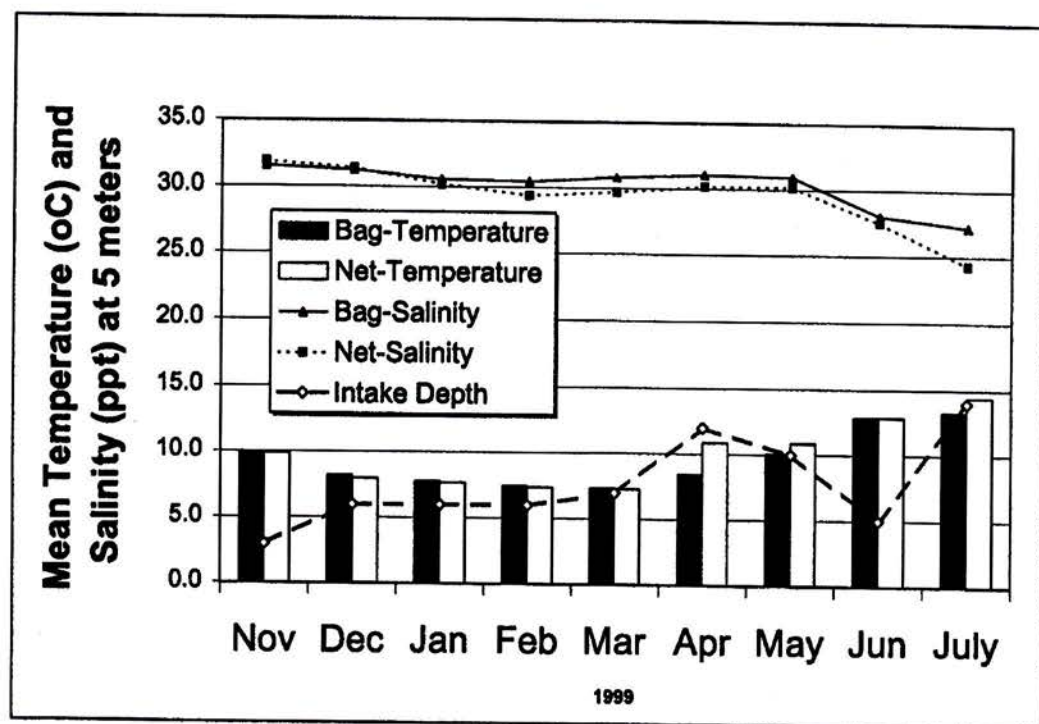


Figure 1. Monthly mean temperature and salinity at a depth of 5 m.

Table 1. Confirmed observations of sealice prevalence and intensity.^(a)

	19 May 1999		17 July 1999	
	Bag	Net	Bag	Net
Number of fish sampled	25	25	25	25
Number of fish infected	8	16	2	15
Total number of lice	10	24	2	24
Prevalence	32%	64%	8%	60%
Mean intensity	1.25	1.5	1	1.6
Range	1-2	1-2	1	1-3
Abundance	0.4	0.96	0.08	0.96
Range	0-2	0-2	0-2	0-3

Terms defined by Margolis et al.⁽⁴⁾:

Prevalence: number of infected fish divided by the number of fish;

Mean intensity (range): number of parasites divided by the total number of hosts examined.

A total of 25 fish were collected individually from each culture unit. A dipnet was used to catch one fish at a time as gently as possible. Each fish was placed singly into a 200-L anaesthetic bath (MS222, 75 ppm). Once the fish had been anesthetized it was visually assessed by 2 observers for lice burden and wound extent.⁽³⁾ Both the dipnet and the bath were checked for lice and the number of lice found was included in the tally. The dipnet was rinsed with seawater prior to obtaining the next fish. Approximately 20 adult lice were collected at each sampling date and preserved in 70% alcohol for identification.

Water quality parameters (temperature, oxygen and salinity) were recorded daily at a depth of 5 m in each of the culture systems. An OxyGuard Handy Mk III was used to measure both the temperature and oxygen and a temperature-sensitive refractometer was used to measure the salinity.

Results and Discussion

Results discussed in this section are summarized in Table 1. The difference in prevalence is statistically significant for both sampling times and confirms earlier observations that the fish in the bag had lower counts of sealice than those in the netpen despite higher stocking density in the bag (23.3 kg/m³ in the bag versus 4.8 kg/m³ in the netpen). Mean intensity and abundance data were not sufficient for statistical analysis. Lice infestations in both groups were low by industry standards and did not result in loss of market quality of the fish. Wound score results on individual fish were not higher than 20 out of a possible score of 500.

Environmental conditions (Fig. 1) for the growout trial were similar in the bag and the netpen; however, mean water temperatures in the bag were 1 to 2 degrees lower than in the netpen during April, May and July. The temperature range during the growth trial for both culture units was between 7 and 14°C. During the spring season, the study site often experiences algal blooms and fresh water runoff from Fraser River runoff. The greater variation in temperature during the spring months April, May and June may have arisen from operator adjustments to the movable intake.

The intake on the bag system was lowered more of-

ten during this period and was intentionally located between depths of 10 to 14 m to avoid harmful algae. Drawing water from this depth provided lower temperatures during this period. The average salinity for both culture units during the trial period was approximately 30 ppt. However, towards the end of the trial in June and July salinity profiles started to change. In the bag system, salinity averaged 28 ppt, while the average salinity for the netpen varied between 27 ppt to 24 ppt with a more rapid decline at the end of the growth trial in July.

Our study was not designed to identify the reasons for the difference in lice burdens that we expected to find, only to assess any difference in a reliable and objective way. Possible causes for the difference in prevalence include advantageous placement of the SEA System intake and a positive influence of the greater current speed and exercise levels in the bag.

Under a continuing collaborative agreement between Fisheries and Oceans Canada and Future SEA, further experiments are being developed to confirm the difference observed in sealice prevalence and verify possible causes.

This project was supported by a collaborative agreement between Fisheries and Oceans Canada, Pacific Biological Station, Nanaimo, BC, and Future SEA Technologies of Nanaimo, BC. The authors gratefully acknowledge their encouragement and financial support. The authors also wish to thank PBS Research Farm Manager Robert Kennedy, farm staff technician Leslie Lewington, and Co-op student Liard McMillan, and Future SEA farm staff technicians Holly Hicklin, Keir Graaten, Karina Cooke, and Gary McDonald for their assistance in collecting the data.

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Harvest Quality of Coho Salmon Raised in a SEA System™ Floating Bag

Henrik Kreiberg,⁽¹⁾ Valma Brenton-Davie⁽²⁾ and Kees Groot⁽²⁾

The SEA System™ is a floating bag technology for fish culture recently developed by Future SEA Technologies Inc. Its pump and adjustable intake allows selection of incoming water for optimum growing conditions and regulation of current speed. An 875-m³ SEA System™ was tested alongside a 324-m³ traditional netpen using commercially-produced coho salmon juveniles at the Pacific Biological Station's Experimental Mariculture Facility at Nanaimo, BC. Coho round weights at harvest averaged 2.3 kg (bag) and 1.7 kg (netpen); final density was 24.1 kg/m³ (bag) and 5.9 kg/m³ (netpen). There was no significant difference between the fish reared in these two systems in fin erosion index (1.3%), visceral fat deposit index (1.72), dressing loss (11.5% round weight), wet weight proximate composition (lipid 8.31%, water 69.3%, protein 20.7%, ash 2.28%), palatability (taste panel) or carotenoid pigment content (17.7 µg/g wet weight). Coho from the bag system had a significantly higher concentration of mucous cells per 0.25 mm skin (14.1 vs. 3.3 cells) than fish from the netpen, and chilled fillets from the fish in the bag system had a significantly lower 48-hr drip loss (1.12% vs. 1.84% wet weight) than those from the netpen. The occurrence of omega-3 fatty acids suggested that the coho from the bag system had higher levels than the netpen fish, but the results were too variable within groups for statistical proof.

Introduction

The SEA System™ bag was developed by Future SEA Technologies Inc. to create a controlled environment for the culture of aquatic animals, particularly those now raised in floating mesh-cage formats. The flexible round enclosure is supplied with pumped water that can be drawn from optimum locations to regulate temperature, salinity and water quality. Articulation of the intake line permits vertical movement through the available water depth and selection of line length permits drawing water from locations other than the actual mooring point. The use of a pump permits regulation of current speed. The bag's woven polyester fabric excludes ambient water, light and predators. An evaluation of the bag technology in comparison with traditional netpen methods was conducted in 1997 using a crop of coho salmon at the Pacific Biological Station's Experimental Mariculture Facility, Nanaimo, BC. Growth performance data from this comparison were reported earlier,⁽³⁾ and we now report on assessments of the harvest quality of the coho from this evaluation.

Materials and Methods

An 875-m³ SEA System™ bag (approx. 10 m diame-

ter) and an adjacent 324-m³ traditional netpen (approx. 6 x 9 x 6 m LWD; 2.5-cm mesh) were stocked on March 10, 1997, with 624-g juvenile coho salmon (*Oncorhynchus kisutch*) obtained from a single netpen lot produced on a commercial salmon farm. The coho in both culture systems were raised as if for commercial purposes, hand fed a commercial grower diet by the same staff, subsampled monthly for growth (fish were crowded and a sufficient number captured with a dip-net; individual fork lengths, weights and blood samples were taken under anaesthetic), and harvested on July 14, 1997. They were processed at a commercial plant and marketed. Feed rates were established from the manufacturer's guide and daily observations of feeding response. The trial was conducted in seawater and no disease treatments were applied during the trial.

Plasma lactate and glucose were determined with clinical kits (Sigma Diagnostics); cortisol was measured by radioimmunoassay. Fin erosion index for each fish was determined from the mean of 5 fin length measurements (dorsal, both pelvics, both pectorals) as percentages of fork length.⁽⁴⁾ Mucous cell counts were taken from single 1-cm square sections of skin and underlying muscle excised from a point midway between the dorsal fin and left side lateral line, stained with eosin-hematoxylin, sectioned (3 µm) and

counted under 500x magnification. Visceral fat deposit on internal organs was rated sparse (1), moderate (2) or abundant (3) by two observers. Drip loss from fresh fillets was measured on single 30- to 50-g sections of skin-on fillet stored on wire drying racks for 48 h at 4°C with a polyethylene wrap to prevent desiccation. Carotenoid and fatty acid composition of fillets was measured by HPLC and proximate composition of fillets was measured by direct lab analysis. Organoleptic quality of plain steam-cooked samples of previously frozen fillets was assessed by trained panels of tasters at an established testing facility using a multiple comparisons procedure.⁽⁵⁾ Statistical analyses were carried out using Minitab (rel. 9) software⁽⁶⁾ and included tests of assumptions of normality in all comparisons. Tests were considered significant if $P < 0.05$.

Results and Discussion

Results discussed in this section are summarized in Table 1. Coho reared in the bag grew significantly larger and converted feed better than coho grown in the netpen. Survival was high in both culture technologies. Mean levels of plasma glucose and cortisol did not differ between the systems at any time and were notably consistent in both mean and range values. Plasma lactate values in both systems rose steadily during the trial, with coho in the bag showing significantly higher levels than the netpen coho (Fig. 1). We do not suggest that our data for these metabolic parameters represent normal values in these culture technologies, as they include the response of the fish to the sampling procedure which was not designed for

accurate stress sampling. Rather, we feel that they may reflect general characteristics of the response to stress, which has been noted to vary with level of swimming exercise in several other salmonids.⁽⁷⁻⁹⁾ Ability to tolerate and clear lactate accumulations was previously seen to improve with swimming exercise in rainbow trout.⁽⁹⁾ We noted from extensive video observations of tailbeat frequencies that mean swimming speeds of coho were markedly higher in the bag than in the netpen (0.7 vs. 0.4 body lengths per second).

Fin erosion (loss of area) has been shown to increase in some fish species with increased stocking density,⁽¹⁰⁾ and can have consequences for swimming ability and market grading. Notwithstanding the nearly 5-fold difference in final fish density between the systems, we found no difference in fin erosion index. Mucous-producing cells in the skin of fish can become more abundant with an increased level of swimming exercise, which might have advantages in defense against external fish pathogens.⁽¹¹⁾ Previous work on the effect of water flow has shown that Atlantic salmon infested with sea lice reduced their lice burdens more rapidly under regimes with higher flow,⁽¹²⁾ although no direct dependency on the characteristics of the mucous layer was established. Our mucous cell counts at harvest showed that coho from the bag carried considerably greater numbers of these cells.

The deposit of solid lipid on the viscera of cultured fish may indicate diet utilization difficulties⁽¹³⁾ and is a financial loss if discarded before final marketing, inasmuch as its source is likely the feed given to the fish. Visceral fat abundance did not differ significantly between bag- and netpen-reared coho, indicating that

Table 1. Summary of performance parameters of coho salmon reared in a SEA System™ bag a traditional netpen (SD, significantly different; H, highly significantly different; N, no significant difference).

	Bag	Netpen	Conclusion
Coho mean weight, g	2292	1796	SD *
Stocking density, kg/m ³	24.1	5.0	—
Survival, %	98.4	96.6	—
Glucose, mg/dL (mean, range)	99.7 (98-102)	99.2 (92-110)	NSD
Cortisol, ng/mL (mean, range)	318.7 (278-439)	322.7 (238-471)	NSD
Fin erosion index, %	1.30	1.33	NSD
Mucous cells/ 0.25 mm skin	14.08	3.33	HSD **
Dressing loss, % (head on)	12.5	10.4	NSD
Visceral fat index	1.84	1.60	NSD
Fillet drip-loss, %	1.12	1.84	HSD **
Total carotenoid, µg/g wet	17.58	15.75	NSD
Water content, % wet	69.03	69.57	NSD
Protein content, % wet	20.79	20.56	NSD
Lipid content, % wet	8.54	8.09	NSD
Ash content, % wet	2.28	2.27	NSD

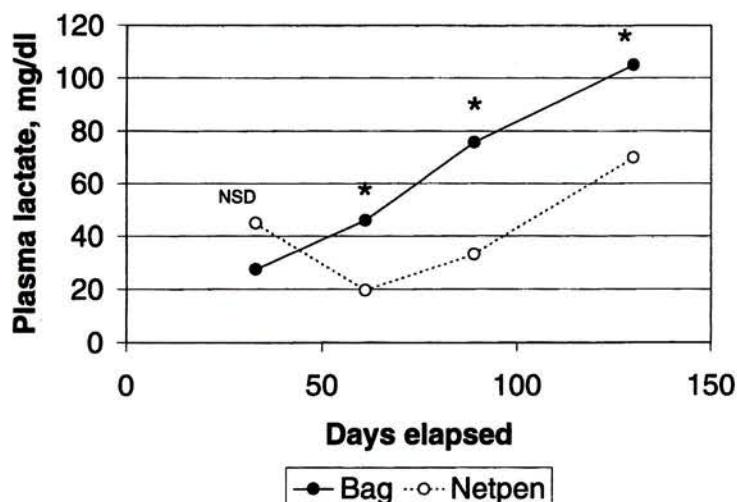


Figure 1. Plasma lactate in coho during growout in two culture technologies, March to July 1997.

fish in both systems used the energy in their diet with similar efficiency. This observation was borne out by proximate composition data, which also showed no significant difference between the culture systems for protein, water, lipid or ash constituents of the fish. Drip loss of water and soluble organic matter from carcasses is another potential cause of financial loss to the producer that has been noted in fresh products from traditional fisheries.⁽¹⁴⁾ Drip loss measured from fillets after 48 hours of refrigerated storage differed significantly, with bag coho losing less weight in storage than netpen coho. Netpen coho were estimated to have lost 30-40 grams more wet weight per fish during storage than bag fish, which on a lot of 10,000 kg market fish, could represent a farm gate difference of about CDN\$500 at current prices. Rounding out the information from the processing stage is the fact that dressing loss (head and entrails removed) did not differ between the fish reared in the two culture systems.

Carotenoid pigment content is a strong factor in consumer preferences in farmed salmon. Carotenoid content (sum of astaxanthin and canthaxanthin) did not differ between the bag and netpen coho, indicating that both groups successfully incorporated dietary-source pigments and that the availability of natural source supplemental feed, which could have been different in the bag system with its deeper intake, did not result in any discernable color difference. Omega-3 lipids (fatty acids) in marine fish are associated with improved health prospects for consumers.⁽¹⁵⁾ Coho were analysed for presence of the two main omega-3 lipids (eicosapentaenoic and docosahexaenoic acid, EPA and DHA). Results suggested that levels of both fatty acids were greater in coho fillets from fish reared

in the bag; however within-sample variability was unusually high in both groups, preventing a reliable conclusion.

An objective assessment of the table quality of coho harvested from the bag and the netpen showed that trained tasters did not detect any difference between bag and netpen coho in aroma, flavor or tenderness (texture). The test did not include samples of commercially-farmed coho. Coho harvested from the bag system were processed commercially and received favorable comments for flesh firmness, minimal scale loss and incidence of low-grade fish relative to the normal farm-raised coho handled by the processor.

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IncubWin: A New Windows® 95/98/NT Computer Program for Predicting Embryonic Stages in Pacific Salmon and Steelhead Trout

J.O.T. Jensen⁽¹⁾ and M.E. Jensen⁽²⁾

A new salmonid incubation program IncubWin was developed to run on Windows® 95/98/NT operating systems. The program is based on relationships between incubation temperature and embryonic development rates for eggs of Pacific salmon (chinook, *Oncorhynchus tshawytscha*; chum, *O. keta*; coho, *O. kisutch*; pink, *O. gorbuscha*; sockeye, *O. nerka*) and steelhead trout (*O. mykiss*). A total of 150 mathematical models for embryonic stages, hatching, and maximum alevin wet weight (MAWW), ponding, or emergence have been incorporated into the program. Hence, the user can easily determine the time (i.e., hours, days, or °C-days) it takes eggs or larvae for any of the six species to attain a specific stage of development at various temperatures. Also included in the program are digitized, colour photographs of the various developmental stages. The program can be obtained by downloading IncubWin.zip from the Pacific Biological Station's aquaculture web site (<http://www-sci.pac.dfo-mpo.gc.ca/aqua/sirp/incubwin.htm>).

Introduction

In 1988, a computer program, INCUB, written in Microsoft® QuickBASIC 4.0 for Microsoft® DOS was developed that allowed fish culturists to predict the development rates of six salmonid species during egg incubation and early larval development in response to temperature.⁽³⁾ Since that time, computer operating systems have changed considerably, with Windows® 95, 98, and NT being the most common today. Hence, a new program, IncubWin, was developed to run on Windows® 95/98/NT operating systems. This paper briefly describes the mathematical models, developmental stages, and new features of this program.

Models Used

Temperature is the main factor influencing the rate of embryonic and larval development in salmonids. As described for the original INCUB program, the thermal sums model is still the simplest and most widely used relation for predicting embryonic and larval development in salmonids and other fish species. The thermal sums model (i.e., °C-days or ATUs), such as proposed by Wallich,⁽⁴⁾ can be stated as:

$$\{i\} \quad y = \frac{a}{x} \quad \text{or} \quad a = x \times y \text{ (}^\circ\text{C} - \text{days)}$$

where y is incubation time (days) to a particular stage of development (e.g., hatching), x is water temperature (°C), and a is a constant. At low temperatures, this model predicts very poorly by greatly overestimating development time. Hence, three better models, with improved predictive power, are used in IncubWin.

First, the modified Belehradek model,⁽⁵⁾ stated as:

$$\{ii\} \quad y = \frac{a}{(x - c)^b}$$

where a , b , and c are constants, was used for predicting development time for 23 embryonic stages and for time to maximum alevin wet weight (MAWW), ponding time, or time to fry emergence for all species, except chinook eggs.

The second model, for chinook eggs only — which exhibit a wide range in egg size — was developed by Rombough.⁽⁶⁾ The model includes egg size in predicting time to MAWW. It is described by the following equation:

$$\{iii\} \quad \ln D = 5.88 - 10.152 \times T + 0.000513 \times W$$

where D is time in days, T is temperature (°C), and W is initial egg weight (mg).

The third model, the Schnute growth model,⁽⁷⁾ was

Table 1. Brief descriptions of the 25 embryonic and larval stages that are calculated in IncubWin.

Stage number	Description	Stage number	Description
1	Fertilization - bipolar differentiation	14	Epiboly and convergence: yolk plug closed
2	2-cell - 1 st cleavage	15	Trunk-tail mound raised
3	4-cell	16	Tail bud free
4	8-cell	17	Heart beat starts
5	16-cell	18	1/4 yolk vascularized
6	32-cell	19	2/3 yolk vascularized
7	Morula (many small cells)	20	Eyed; 3/4 yolk vascularized
8	Flattening of blastodisc	21	Caudal flexing
9	Epiboly and convergence: germ ring	22	Operculum covers first branchial slit
10	Epiboly and convergence: 1/3 epiboly	23	Beginning of hatch
11	Epiboly and convergence: 1/2 epiboly	24	50% hatch
12	Epiboly and convergence: 3/4 epiboly	25	MAWW, emergence, or ponding
13	Epiboly and convergence: narrowing of germ ring		

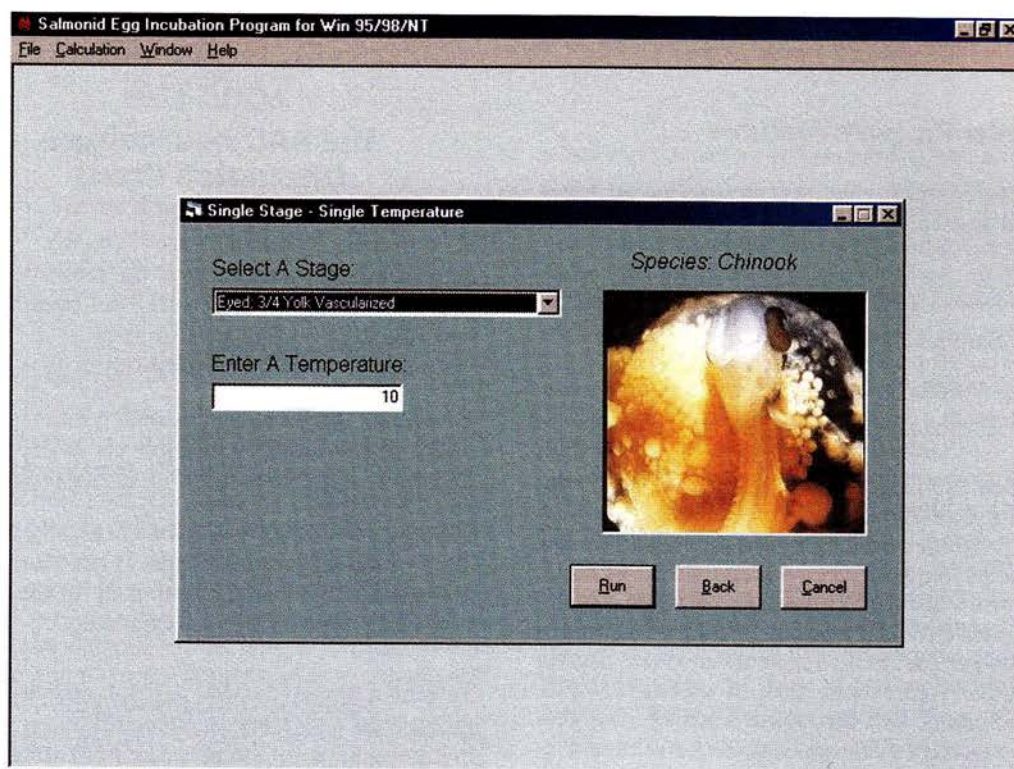


Figure 1. Captured page from IncubWin, illustrating the selection of a developmental stage with corresponding colour photograph.

used to predict time to 50% hatch. Although not specifically developed to describe the time-temperature relation of developing teleost eggs, it works well in describing embryonic and larval development rates. The model, with constants a and b not equal to zero, is described by the equation:

$$\{iv\} \quad y = \left[y_1^b + (y_2^b - y_1^b) \left[\frac{1 - e^{-a(x-x_1)}}{1 - e^{-a(x_2-x_1)}} \right] \right]^{1/b}$$

where x_1 and x_2 are the minimum and maximum incubation temperatures of 1 and 20°C, respectively, while y_1 and y_2 are the predicted development times at those temperatures.

Embryonic Development

Development staging is based on data reported by Velsen⁽⁸⁾ and McLean et al.,⁽⁹⁾ and consists of embryonic stages 1 to 23, 50% hatch (stage 24), and MAWW, ponding, or fry emergence (stage 25). In addition, a series of colour photographs by Velsen⁽¹⁰⁾ have been included in the program to illustrate embryonic development. Table 1 provides abbreviated descriptions of the 25 stages. For more detailed descriptions of the embryonic stages the reader is referred to Table 26 in Velsen.⁽⁸⁾

Computer Program Features

The program allows the user to first choose a species, and second to choose the type of calculation, namely:

1. Single Stage – Single Temperature
2. Single Stage – Range of Temperatures
3. 25 Stages – Single Temperature
4. 25 Stages – Range of Temperatures.

For calculation-type 1 and 2 above, colour photographs for corresponding stages are displayed (Fig. 1). The program then is designed to predict the time (i.e. in hours, days, or °C-days) it takes eggs or larvae for any of the six species to attain a specific stage of development (from fertilization to MAWW) at various temperatures. The results can be saved as text files for use in other applications such as spreadsheet programs, or can be sent directly to a printer. This program should have many applications for salmonid researchers, fish culturists, and students. The program can be obtained by downloading IncubWin.zip from the Pacific Biological Station Web Site (<http://www-sci.pac.dfo-mpo.gc.ca/aqua/sirp/incubwin.htm>).

We gratefully acknowledge Dr. D.F. Alderdice for his ideas, invaluable contributions, and guidance in the original experimental studies on embryonic and larval development. In addition, we must also acknowledge Mr. F.P.J. Velsen for providing the macro-photographs that have been digitized and used in this program.

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Can Indicators of Disease Resistance Predict Performance in Pure and Hybrid Strains of Wild and Domestic Chinook Salmon?

R. Johnson,⁽¹⁾ C. Bryden⁽¹⁾ and D.D. Heath⁽²⁾

Disease related mortality in farmed chinook salmon (*Oncorhynchus tshawytscha*) continues to have a substantial impact on the development and financial viability of salmon aquaculture in British Columbia. The identification and evaluation of indicators of disease resistance, as potential tools to predict future fish performance, may significantly reduce stock losses when applied within a selective breeding program. The purpose of this study was to first compare disease resistance within a population of pure and hybrid families of wild/domestic crossed chinook salmon using three indicators: plasma lysozyme activity, a necropsy based health assessment and the polymerase chain reaction (PCR) based diagnosis of bacterial kidney disease (BKD). The predictive value of the indicators was then estimated by assessing their ability to predict performance, as measured by weight and mortality. Preliminary results suggest that disease resistance as measured with these indicators has a genetic basis; while no evidence of heterosis was found, wild/wild fish exhibited both higher plasma lysozyme activity levels and a greater incidence of infection than the other cross types. Of the measured indicators, plasma lysozyme activity was found to be the best indicator of performance.

Introduction

Bacterial kidney disease (BKD) is a major cause of disease related mortality for farmed chinook salmon (*Oncorhynchus tshawytscha*) in British Columbia.⁽³⁾ While the development of effective medicines and vaccines may occur sometime in the near future, the development of more resistant strains would provide many long term benefits for the farmer, the consumer and production stock. Potential benefits include i) reduced use of chemotherapeutics, ii) reduced deposition of medicinal residues (in the environment as well as in fish flesh), iii) reduced number of stressful interventions for fish, iv) reduced development of resistant bacterial strains, v) increased use of a native species, and vi) increased profit for industry. The identification and use of variables that measure the health of fish but that also can predict fish performance within an aquacultural context would lead to maximum efficiency in a program of selective breeding.

Use of the polymerase chain reaction (PCR) for the detection of pathogenic organisms in animals is extremely powerful; it is becoming a much more commonly used diagnostic technique.⁽⁴⁾ For the detection of *Renibacterium salmoninarum* (the causative agent of BKD) infection in fish, PCR has been shown to be

much more sensitive and specific as well as less time consuming than the more traditional serological or culture-based diagnostic tests.⁽⁵⁻⁸⁾ Thus, its use leads to a reduction in false diagnoses, increased detection power and reduced time to diagnosis. These are all critical parameters when evaluating the health status of an aquacultural stock especially for broodstock purposes. However, because PCR diagnosis is a proxy measure of pathogen presence and an indirect indicator of health status, it is important to validate its use with other known measures of health.⁽⁹⁾

In this study, variation in health status of a population of pure and hybrid strains of wild and domestic chinook salmon was evaluated using three variables, the presence of *R. salmoninarum* as detected by PCR, plasma lysozyme activity and necropsy assessment. Additionally, the value of the PCR diagnosis as an indicator of health status was investigated and the ability of the three variables to predict performance as measured by mortality and growth was evaluated.

Methods

The families of fish examined in this study were generated using a standard nested mating design. Fifty-two domestic chinook salmon (26 females, 26

males) from Yellow Island Aquaculture Ltd. (YIAL), Quadra Island, BC, and 52 wild chinook salmon (26 females, 26 males) from the Big Qualicum River Salmonid Enhancement Project (SEP) hatchery, Qualicum Beach, BC, were used as parents. The YIAL fish were four generations removed from fish that originated from the Robertson Creek SEP hatchery, Port Alberni, BC. Fertilization of ova took place in October, 1997. The population of fish thus created consisted of 7200 fish within 104 families and 4 cross-types (wild x wild, WW; wild x domestic, WD; domestic x wild, DW; domestic x domestic, DD). Emergent fry were ponded in January 1998 and a total of 94 families were nosetagged and transferred to a single saltwater netpen at YIAL in July 1998.

Seven hundred and forty five fish were sampled from the netpen in December, 1998. Each fish was measured for length and weight and blood samples were taken. Nose tags were retrieved and a modified necropsy assessment⁽¹⁰⁾ of 511 of the fish was performed. Kidneys of all necropsied fish were aseptically removed for PCR analysis. Each qualitative necropsy variable was translated to a quantitative health assessment index (HAI) value⁽¹¹⁾ for comparison. Plasma lysozyme activity was measured using the modified⁽¹²⁾ lysoplate technique of Osserman and Lawlor.⁽¹³⁾ DNA was extracted from kidney samples

using a standard DNA extraction protocol and the presence of *R. salmoninarum* was tested for using PCR and the primers Rs1 and Rs2.⁽¹⁴⁾

Chi square analysis was used to identify differences between the families and cross types 1) in the incidence of BKD infection and 2) in the categorical necropsy assessment parameters and HAI values. ANOVA was used to identify differences between the families and cross types in 1) plasma lysozyme activity levels and 2) the quantitative necropsy assessment parameters. To determine the value of the PCR-based detection of *R. salmoninarum* as an indicator of health, the relationships between HAI values, plasma lysozyme activity, specific quantitative necropsy parameters and the PCR-based family incidence of BKD were investigated using bivariate correlation analysis and ANOVA. To determine if the three indicators (lysozyme activity, HAI, *R. salmoninarum* presence) could be used as predictors of mortality (from Dec., 98-Oct., 99 and during a spring of 1999 vibrio outbreak [*Vibrio anguillarum*]) and growth (at age 14 months and 21 months), correlation analysis was used.

Results

The sampled fish represented 83 of the original 94 families placed into saltwater and were composed of 35% WW, 20% WD, 27% DW and 18% DD fish. While no significant differences in BKD incidence were found among either cross types or families (cross type, $\chi^2 = 6.82$ (3, n = 478) $P = 0.07$; families, $\chi^2 = 64.45$ (82, n = 480) $P = 0.92$), most fish testing positive for BKD occurred within the WW cross type (12.4 % of WW fish, 9.0% of DD fish, 7.99% of DD fish and 2.9% of WD fish tested positive for BKD). While HAI differences among families were not significant, Chi square analysis of HAI categories by cross type revealed significant differences ($\chi^2 = 16.76$ (6, n = 511) $P = 0.01$) with most fish exhibiting abnormalities within the WD cross type (WD, 16%; WW, 9%; DD, 5.4%; DW, 4%). ANOVA revealed significant differences among both families ($F(83, 432) = 3.48$ $P = 0.05$) and cross types ($F(3, 507) = 2.61$ $P = 0.05$) of fish in plasma lysozyme activity levels with WW fish exhibiting the highest activity levels (followed by DW, WD and DD fish).

The PCR based detection of *R. salmoninarum* appeared to be a good indicator of health status; BKD positive fish had significantly higher lysozyme activity levels ($F(1, 478) = 3.98$, $P = 0.04$, n = 479) and significantly lower hematocrits ($F(1, 492) = 6.61$, $P = 0.01$, n = 479) than BKD negative

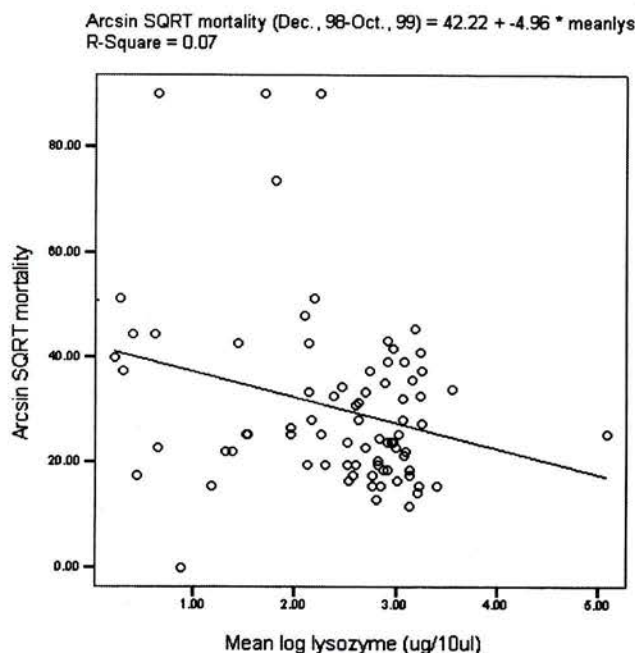


Figure 1. Correlation between family mortality (Oct 98 - Dec 98) and mean family plasma lysozyme activity for 79 families of wild x domestic chinook salmon. The regression line shown is statistically significant; $r^{*2} = 0.074$, $P < 0.001$, n=79.

fish while all fish identified as BKD positive during the necropsy assessment also tested positive for *R. salmoninarum* with the PCR. HAI scores indicated that most PCR diagnosed BKD positive fish were probably presymptomatic. When indicators were assessed for their ability to predict mortality and growth, plasma lysozyme activity was found to be the only indicator with a significant negative correlation with both mortality from Dec, 98-Oct, 99 ($r^2 = 0.074$, $P = 0.001$, $n = 79$; Fig. 1) and mortality during a vibrio outbreak ($r^2 = 0.049$, $P = 0.001$, $n = 79$; Fig. 2).

Discussion

Heterosis was not observed and no cross type performed consistently better or worse across the measured variables in this study. However, the variation present within this population of fish, at both the family and cross type levels, indicates that there is a genetic basis to the health related traits measured and that a program of selective breeding to increase disease resistance may be successful. PCR detection of *R. salmoninarum* was found to be a potentially good indicator of health status; however, the low numbers of fish found to be exhibiting clinical signs of infection during the necropsy assessment and the fact that BKD positive fish tended to have high lysozyme activity levels suggested that the PCR was detecting presymptomatic carrier fish or fish in the early stages of infection. Additionally, the significant negative correlation between mortality and plasma lysozyme activity level may indicate that fish experiencing high mortality were unable to express a pronounced nonspecific immune response, that they were in the later stages of an acute infection or that a factor that did not elicit a strong nonspecific response was causing this mortality. These results, as well as those in the literature, indicate that plasma lysozyme activity may be a fairly good but population-specific indicator of both health status and potential mortality. The failure of the HAI and PCR diagnosis of BKD to predict performance (mortality) may have been attributable to the healthy nature of this population. Disease resistance is probably a result of a large number of variables influenced by epistatic, environmental, host and pathogenic interactions. Extension of this study to include a larger number and wider range of health related variables as well as an examination of the molecular genetic basis of resistance may result in a better definition of some of these interactions.

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Arcsin SQRT mortality = $34.30 - 4.06 * \text{meanly}$
R-Square = 0.06

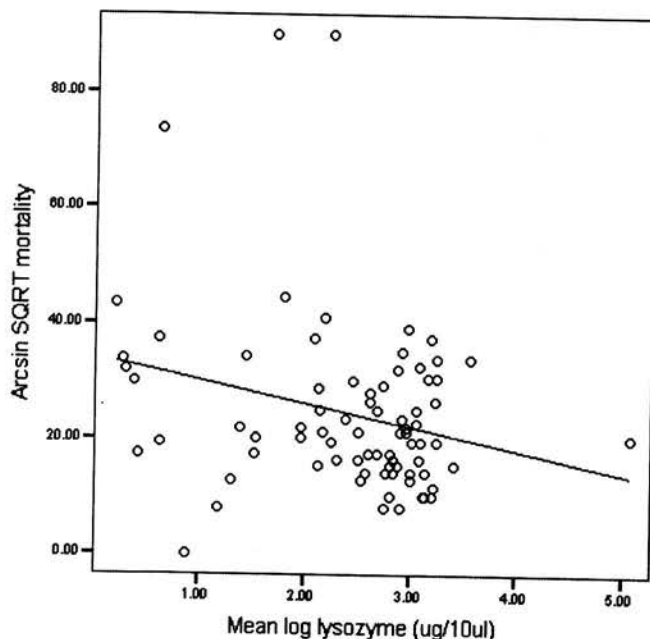


Figure 2. Correlation between family mortality during a *Vibrio* outbreak and mean family lysozyme activity for 79 families of wild x domestic chinook salmon. The regression line shown is statistically significant; $r^{**2} = 0.059$, $P < 0.001$, $n = 79$.

Utilization of Physiological Telemetry to Monitor Behavioural Responses of Rainbow Trout, *Oncorhynchus mykiss*, to Captive Culture Conditions

K.P. Chandroo,⁽¹⁾ R.D. Moccia⁽¹⁾ and R.S. McKinley⁽²⁾

Aquaculture employs a variety of husbandry methods which need to be evaluated in terms of the health and welfare of the farmed fish. The objective of this study was to validate the use of physiological telemetry as a behavioural indicator, by correlating telemetered electromyographic (EMG) signals with video-recorded swimming activity. Video-recorded observations of the "startle-response" of rainbow trout, induced by instant-on lighting, were analyzed and could be correlated with telemetric EMG signals, suggesting that telemetry data was an accurate measure of swimming behaviour. A preliminary experiment examining the behavioural responses of fish to transportation by truck was also completed. EMG recordings during this transportation indicated that vigorous and energetically expensive swimming patterns were occurring, which could lead to post-transport stress. Physiological telemetry may therefore allow for the objective quantification of both fish activity and behavioural responses to rearing methods used in aquaculture, and may thus prove to be a valuable tool to evaluate captive husbandry protocols.

Introduction

Aquaculture employs a variety of husbandry protocols which need to be evaluated in terms of the health and welfare of the fish. Behavioural tests can be used as indicators of short-term stress, as well as of the long-term recovery from stressors that occur in aquaculture facilities,⁽³⁾ but examples of this are sparse. The lack of published literature pertaining specifically to the analysis of behaviour of fish in intensive culture environments is, in part, due to the difficulty associated with measuring and quantifying animal activity under these conditions.⁽⁴⁾ With the advent of physiological telemetry technology in recent years, quantitative information reflecting the locomotory activity of free-swimming fish *in situ*, can now be obtained and recorded.⁽⁵⁾ Implantable, wireless devices capable of detecting and transmitting electromyographic (EMG) activity has also allowed researchers to monitor and characterize the swimming activity of wild fish.⁽⁶⁾ To date, EMG telemetry has not been used extensively to identify specific behaviours per se, but rather, has served as an indicator of overall muscle activity levels. The objective of this study was to validate the use of physiological telemetry as a behavioural indicator in fish, by correlating radio-transmitted EMG signals with video-recorded swimming behaviours. In addition, a trial to examine the behavioural response of fish to a transportation episode was conducted.

Materials and Methods

Hatchery reared, domestic rainbow trout were obtained from the Alma Aquaculture Research Station (Alma, ON). Fish ($n=8$) had a mean weight and fork length of 1145 ± 385 g and 422 ± 28 mm, respectively. The EMG detecting transmitters and radio receiver (SRX_400) used in this study were manufactured by Lotek Engineering Inc. (Newmarket, ON). Transmitter equipment and surgical procedures were similar to those of Beddow and McKinley.⁽⁷⁾ Trout were individually anaesthetized in an aerated, temperature controlled solution of tricaine methanesulfonate (MS 222) at a concentration of 70 mg/L and their gills were continuously irrigated with this solution throughout the procedure. A 3-cm-long incision was made in the ventral abdominal wall, just anterior to the pelvic girdle. The transmitters include a pair of Teflon covered electrodes with gold, muscle-anchoring tips and an antenna. With the aid of a custom tool, the electrodes were implanted into the red axial musculature along the right lateral flank, to a depth just below the integument. The transmitter was then gently inserted into the peritoneal cavity through the abdominal incision. The transmitter antenna was fed back through a 16-gauge hypodermic needle inserted just posterior to the incision and allowed to protrude from the body wall. The incision was then closed using 3 to 4 independent sutures (3-0 silk, Ethicon). The surgical procedure

took a maximum of 10 min to complete, at which time the fish were placed into a 2x2 m semi-square tank with fresh, well-aerated water to recover.

During recovery, transmitter output was monitored for proper functioning. An explanation of transmitter characteristics may be found in Beddow and McKinley.⁽⁸⁾ EMG measurements were standardized for each fish by expressing the EMG telemetry signals as a proportion of basal telemetry readings; this measurement is defined here as the "activity index". Standardization permits direct comparisons of telemetry data between tagged fish.

Lighting trial

In order to correlate EMG signals to fish swimming behaviour, a trial was designed in which time-synchronized EMG measurements were matched with video-recordings of the behavioural response of fish to instant-on lighting. The fish's response to this instant-on lighting (called the "light-shock" reaction) reportedly involves numerous swimming modalities⁽⁹⁾ and thus presents a diversity of body movements which can be correlated with EMG telemetry information of muscle activity. Three experimental trials were conducted, each using randomly chosen EMG tagged fish mixed with untagged fish, in a 2x2 m tank, to a bi-density of 25 kg/m³ (fish/water). Tanks were located in a windowless room which featured light-proof double-doorways, and four ceiling-mounted 150-w tungsten bulbs that generated a light intensity of 310 to 350 lumens at the tank-water surface. There were 3 independent recording events within each experimental trial. In each recording event, the fish's reaction to instant-on light activation was documented by video and telemetry recordings. Each recording event took place once daily over 3 to 4 consecutive days. Prior to the light activation for each recording event, time-synchronized EMG (for a single fish) and video recordings filming the entire tank began, and continued up to 10 min after lights-on. To avoid bias, the EMG data analysis and video transcription was done separately. Analysis of the video tapes for distinct swimming behaviours revealed three main behavioural categories. Category A was characterized by fast-start responses, burst and glide swimming,⁽¹⁰⁾ fast undulatory swimming⁽¹¹⁾ and "snout swimming";⁽¹²⁾ Category B by swimming restricted to tail-beating and slow, uniform undulations⁽¹³⁾ which allowed the fish to maintain positional equilibrium against the water flow; and Category C by undulatory swimming that was intermediate to categories A and B and independent of the water current. For each recording event, the time spent at each category was scored. EMG signals were averaged every 30 seconds, and are presented as an "activity index".

Transport trial

A preliminary experiment was also done which examined the fish's behavioural response to transportation by truck. EMG signals were recorded from fish prior to transportation (routine swimming) and again, continuously, by monitoring the EMG signals of 2 fish located in the transport container. The transport container had dimensions of 1.47x0.97x0.60 m (LxWxH). Fish were transported for 50 min along a paved, secondary highway with light to moderate traffic density. A *t*-test for unequal variances was used to determine levels of significance in EMG activity between transport and non-transport periods.

Results

The behavioural response of fish to instant-on light activation was distinguishable as three, temporally sequential categories as described in the methodology. The behavioural category A followed by B followed by C was observed in all video recorded events (*n*=8). When the mean duration of each behavioural category was expressed as a proportion of the total recording time after light activation (10 min), the fish's activity levels as measured by EMG telemetry appeared precisely correlated with the video-recorded behaviours observed (Fig. 1).

The activity levels of fish during transportation (Fig.

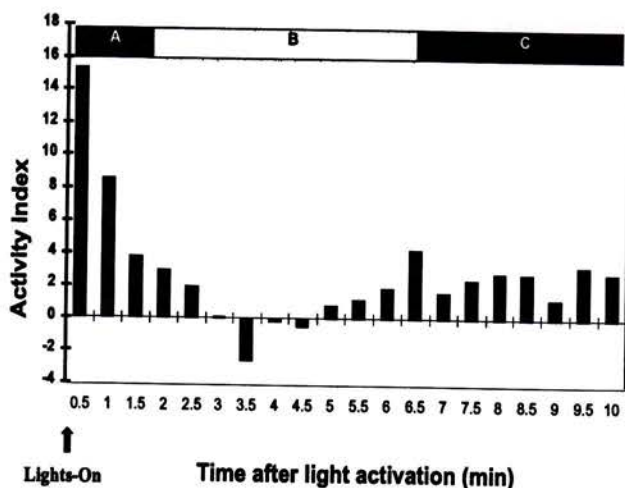


Figure 1. Swimming behaviour of rainbow trout in response to sudden light onset as recorded by EMG telemetry and video. Bars represent the mean activity level of 8 individuals. The partitioned horizontal bar at the top of the graph represents the behavioural categories denoted as A, B, and C from left to right. Each partition represents the mean duration of that category (*n*=8) expressed as a proportion of the total recording period (10 min). An activity index value of zero is equivalent to the activity level of fish 10 min prior to instant-on light activation.

2) were significantly different from activity levels measured during pre-transportation periods ($P < 0.05$). Swimming intensity during transport, at times reached 12 times the pre-transport routine levels.

Discussion

In this study, distinct, temporally sequential swimming activities were both visually observed and electromyographically quantified from fish in response to instant-on light activation. This method of assessment gave a definitive description of the swimming responses of the fish, and consequently, we concluded that EMG telemetry data could be used as an accurate measure, or proxy, of swimming behaviours. In addition, a comparison of our data with studies reporting swimming activities associated with the "light-shock" reaction,⁽¹²⁾ and startle-responses,⁽¹⁰⁾ have suggested to us that the "light-shock" reactions of rainbow trout are most likely a type of fast-start response followed by a recovery period.

The usefulness of this EMG telemetry tool was also illustrated by preliminary findings obtained from behavioural monitoring during a transportation episode. Examples of behavioural responses during transport are limited in the literature, and the results of our experiment suggest that future studies aimed at identifying periods of "stress" during transportation protocols, should take into account the possibility of vigorous, energetically expensive swimming behaviour that occurs during transportation. Based on the intensity and erratic pattern of EMG signals, it is most likely that fast swimming during transportation is manifested in bursts and turns done in reaction to the waves and

other water movements generated within the tank. Our observations may also offer further insight into the impaired swimming performance of fish after transportation.

Although the telemetry tags used in this study successfully measured changes in swimming activity, we suggest that in order to unequivocally determine specific behavioural responses electromyographically, the following protocols should be heeded. First, it is necessary to obtain a visual description of the behaviour so that definitive, quantifiable categories may be constructed, in order to be correlated to telemetric EMG signals. Second, the EMG data analysis should focus on overall levels of activity combined with a measure of the variance in activity patterns, which would indicate spontaneous activity at those overall levels. These protocols should result in more precise interpretations of behaviour from EMG telemetry data. Future work should focus on such aspects of signal analysis.

EMG telemetry may permit the evaluation of a wide range of rearing conditions with respect to fish physiology and behaviour, provided that suitable descriptive methods of analysis are incorporated. Information obtained through EMG telemetry could aid in improving the environmental conditions pertinent to the welfare of farmed fish, by offering scientific information on which to base husbandry recommendations.

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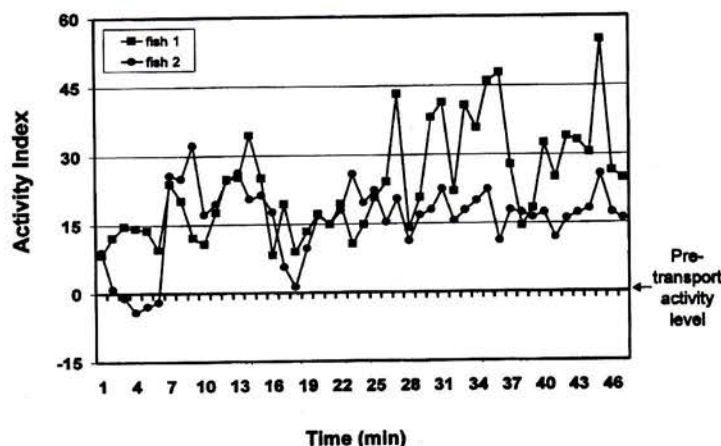


Figure 2. Activity index profiles of rainbow trout during transportation. Each data point represents EMG telemetry signals sampled during transportation, averaged over a one minute period. An activity index value of zero is equivalent to the activity levels of fish during the pre-transport period.

We acknowledge the assistance and support of the staff of the Alma Aquaculture Research Station, and Mr. Steven J. Cooke. Funding was provided by the Ontario Ministry of Agriculture, Food and Rural Affairs.

Larval Distribution of Blue Mussel (*Mytilus edulis* and *M. trossulus*) and Predatory Starfish (*Asterias vulgaris*) during a 12-hour Tidal Cycle

Miranda Pryor and G. Jay Parsons⁽¹⁾

To aid the mussel growers of Newfoundland, a larval/spatfall monitoring program was instituted in 1994. This program has achieved success in demonstrating to growers the importance of larval monitoring to ensure an adequate and consistent annual supply of spat. However, a problem arose when growers, who were conducting their plankton tows throughout the day, began obtaining results that varied with tidal height. This study examined larval size and abundance over a 12-hour tidal cycle. Vertical plankton tows were performed at two stations on two mussel culture sites every 90 minutes starting at high tide and continuing through low tide to the next high tide. At both sites, a change in numbers of both mussel and starfish larvae occurred over the tidal cycle. Changes in the abundance of such fouling organisms as saxicave clam larvae were also recorded. Environmental and hydrographic conditions (water velocity was continuously recorded) were examined in relation to larval abundance. A relationship between current speed and larval retention was observed: a greater larval abundance was recorded at the station within each site that had the higher current speed. This study demonstrated the importance of developing a standardized and accurate method for monitoring larval abundance on shellfish culture sites.

Introduction

The Newfoundland mussel culture industry relies on wild-caught spat for its seedstock. Since 1994, a larval/spatfall monitoring program has been in place to aid the growers. As the blue mussel industry continues to grow, there is greater pressure for a reliable supply of seed.

Geographically every shellfish site is unique, with many factors affecting the number of larvae that will pass through it on a given day. It has been demonstrated that larval abundance varies from flood to ebb tide, with large numbers of mussel larvae being present during flood tide.⁽²⁾ However, tidal level alone does not determine the retention of bivalve larvae in a site; water velocity and hydrographic conditions also play a major role.^(2,3,4) Therefore, when determining how to monitor a site it is important to consider the environmental and hydrographic conditions to ensure accurate predictions of larval abundance are made. The objective of this study was to examine the variation in larval size and abundance at two geographically distinct sites in Newfoundland over a complete 12-hour tidal cycle, so that optimization of spat collection can be achieved in the future.

Materials and Methods

Two commercial mussel sites were chosen. Site 1, Reach Run, was a "flow-through" site located on the northeast coast of Newfoundland that measured ~300 hectares in size. Site 2, Jersey Harbour, was a much smaller (~100 hectares) "dead-end" or "harboured" site located on the south coast.

Sampling dates were chosen around the spring and neap tidal cycles. At Reach Run, two 12-hour samples were obtained (spring tide on July 2, 1999; neap tide on October 5, 1999). Jersey Harbour was sampled once on July 5, 1999 (spring tide). There were two sampling stations at each site: one at the front and one at the back of the site. Sampling spanned a complete 12-hour tidal cycle (when daylight permitted) and samples were taken every 90 minutes at each station.

Each sample consisted of a vertical plankton tow to a depth of 10 m. The sample was screened through an 80- μ m mesh. Mussel, clam and starfish larvae were identified, enumerated and measured for each plankton tow. A CTD (conductivity-temperature-depth) cast was obtained at each station for each sampling period. An S4 current meter was deployed at each station to record current velocity for the entire 12-hour period.

Results

Larval abundance

Site 1 (Reach Run) July 2: The abundance of mussel larvae varied during the 12-hour sampling period at Station 2, while Station 1 displayed little change (Fig. 1a). The number of mussel larvae per liter of seawater towed was greater at Station 2 than at Station 1 for 8 of the 12 hours sampled. There was also a ten-

dency for greater numbers of mussel larvae to be collected at high tide than at low tide.

Starfish larvae (*Asterias vulgaris*) were found at both stations, with Station 2 having the greatest variation in abundance over time (2 to 8 larvae/L compared to 0 to 2 larvae/L for Station 1). In addition, there was a tendency for higher numbers of starfish larvae to be collected at high tide at Station 2 in comparison to low tide.

Site 2 (Jersey Harbour) July 5: There was a greater

number of mussel larvae in the samples from Station 1 than those from Station 2 for the entire 12-hour sampling period. There was considerable variation in the number of mussel larvae present at Station 1 over time, whereas only a minor change in larval abundance was observed at Station 2 (Fig. 1b). At Station 1 there appeared to be a correlation between the abundance of mussel larvae and the tidal cycle such that the peak in larval abundance occurred subsequent to high tide.

While no starfish larvae were observed at the site, there was a large number of saxicave clam larvae (*Hiatella* sp.). The pattern of abundance of clam larvae was similar to that of the mussels for this site. A greater number of clam larvae was found at Station 1 (90 to 250 larvae/L) than Station 2 (0 to 30 larvae/L) and there was considerable variation in the abundance of clam larvae over the 12-hour sampling period. Also, at Station 1 there appeared to be a correlation between clam larval abundance and the tidal cycle such that the peak in larval abundance occurred subsequent to high tide.

Site 1 (Reach Run) October 5:

Abundance of mussel larvae varied during the 12-hour sampling period at both Station 1 and Station 2 (Fig. 1c). The number of mussel larvae per liter was greater at Station 1 than at Station 2 for 9 of the 12 hours sampled. In addition, there appeared to be a correlation be-

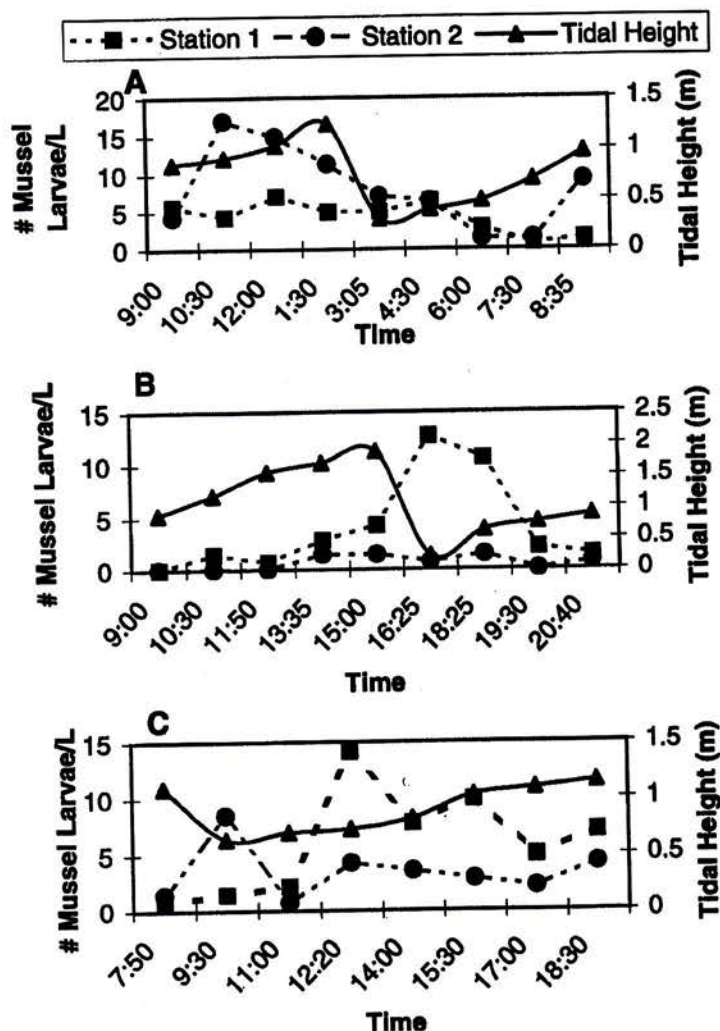


Figure 1. Abundance of mussel larvae in relation to tidal height for Station 1 and 2 at Site 1 (Reach Run) on July 2, 1999 (A), for Station 1 and 2 at Site 2 (Jersey Harbour) on July 5, 1999 (B), and for Station 1 and 2 at Site 1 (Reach Run) on October 5, 1999 (C).

tween larval abundance and the tidal cycle. As the tide was rising in the afternoon, larval abundance at both stations increased. No starfish larvae were found at Reach Run on this date.

Preliminary observations indicated there was no difference in mean larval size at either sampling site during the study.

Environmental parameters

S4 Data: For Site 1, there were no observable differences in current speed between July and October. Average current speed was higher at Station 1 (4.67 cm/s) compared to Station 2 (1.30 cm/s). For Site 2, average current speed for Station 1 was 1.39 cm/s and for Station 2 was 0.71 cm/s.

CTD: No observable changes were recorded in the values of the temperature, salinity or chlorophyll-*a* depth profiles for Site 1 or 2 during each of the three sampling periods. For Site 1, average temperature, salinity and chlorophyll-*a* values for July 2 were 16.9°C, 28.0 ppt, 3.5 µg/L and for October 5, 13.4°C, 28.8 ppt, 6.5 µg/L, respectively. For site 2, on July 5, temperature, salinity and chlorophyll-*a* values 10.4°C, 31.6 ppt and 1.9 µg/L, respectively.

Discussion

Throughout this study, at both Site 1 and Site 2, larval abundance displayed some correlation with tidal height. On most occasions, greater larval numbers were observed during high tide, in comparison to low tide. At both sites, greater concentrations of larvae were observed at the stations within each site displaying the greatest current speed. This supports the hypothesis proposed by Andrews⁽⁴⁾ that the morphometry and energy of a system play a more important role in larval distribution than do environmental factors within a system.

The geographic uniqueness of each shellfish site was also an important consideration. The Reach Run "flow-through" system had a smaller tidal range than did Jersey Harbour, the "dead-end" site. Differences observed in larval abundance varied accordingly, with the greatest change in larval abundance over the 12-hour period being observed at Jersey Harbour. Thus, knowing when to sample within the tidal cycle is as important as knowing where on a site to sample.

Furthermore, while the objective of this study was to monitor the larvae of blue mussels and predatory starfish, it became evident that many other species can be effectively monitored, such as the saxicave clam larvae (*Hydrobia* sp.), a common fouling organism along the south coast of Newfoundland. Thus, each shellfish

farm should monitor for many larval species if optimal mussel spatfall is to be achieved.

This experiment is part of an in-depth study that was initiated in 1998 to examine the spatial and temporal distribution of both the blue mussel and predatory starfish larvae (*Asterias vulgaris*) on Newfoundland mussel culture sites. The results have shown that the timing and spatial occurrence of starfish larvae and juvenile settlement coincides with settlement of mussel spat. These findings, in conjunction with the data on the correlation between larval abundance and the tidal cycle, should benefit Newfoundland mussel growers as they expand production and strive to optimize seed collection.

Conclusions

This study demonstrates the importance of a standardized and accurate method for monitoring larval abundance on shellfish culture sites throughout Newfoundland. Growers should consistently sample at the same stage of tidal height and at the same location on their site in order to achieve accurate and comparable data on larval abundance for every sampling period. Knowing *when* and *where* mussel larvae and biofouling agents such as clams and predatory starfish are passing through a shellfish site will aid in optimizing mussel spatfall on collectors.

This project would not have been successful without the generous support of the mussel growers involved. Sincere thanks are extended to Alvin and Jane Hodder of Farewell Mussel Farms (Reach Run Site) and Travis and Jeanette Mahoney of ConAqua Ltd. (Jersey Harbour Site) for providing their valuable time and assistance when needed. Funding was provided by the Aquaculture Component of the Canada/Newfoundland Economic Renewal Agreement and the Canadian Centre for Fisheries Innovation.

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Temporal Distribution of Larval and Post-set Blue Mussel (*Mytilus edulis* and *M. trossulus*) and Starfish (*Asterias vulgaris*) at Mussel Culture Sites in Newfoundland

Miranda Pryor, G. Jay Parsons and Cyr Couturier⁽¹⁾

As the blue mussel farming industry in Newfoundland grows, farmers anticipate problems from predatory starfish. Temporal and spatial patterns of planktonic larval and post-set stages of mussels and starfish were studied to determine if abundance and timing of spawning and settlement were consistent between the two organisms. Weekly larval and spat/juvenile samples were taken from May through November 1998 at four mussel sites. Mussel larvae were abundant from mid-June through late August at the three sites located on the north coast; starfish larvae were present from late July through late August. The settlement of mussel spat and starfish juveniles occurred at varying rates and the peak starfish set occurred about 1-4 weeks after peak mussel set. At the fourth site, located on the southern shore, mussel spawning was sporadic and few spat settled on the collectors. No starfish were observed on this site. The ability to predict starfish settlement in relation to mussel spat settlement will provide mussels growers with the capability of timing the deployment of collectors to reduce the potential impact of this predatory species.

Introduction

The blue mussel industry in Newfoundland has grown in recent years and is expected to double within the next two years. With the initiation of a mussel larval/spatfall monitoring program in 1994, growers began to monitor the annual appearance of mussel larvae in hopes of collecting as many settling spat as possible. In Newfoundland waters, gametogenesis progresses rapidly through the spring and early summer and spawning occurs late in July.⁽²⁾ Competent pelagic mussel larvae spend 1 to 4 weeks actively searching for a suitable substrate on which to settle (suitability depends on temperature, salinity, food and other factors).^(3,4) Interestingly, Newell et al.⁽⁵⁾ showed that larval distributions of blue mussels varied with the tidal cycle and water agitation. Therefore, when monitoring for mussel larvae many factors must be considered.

As in other regions, mussel farms in Newfoundland can be plagued by the predatory starfish *Asterias vulgaris*. Past studies have shown that, similar to mussels, starfish produce a pelagic larval stage that lasts for a period of 4 to 6 weeks.⁽⁶⁾ It has been shown that settlement of starfish in Garden Cove, NF, occurs in late autumn and that newly settled starfish have a

feeding preference for mussel spat.^(7,8) Since starfish are voracious predators of mussels,^(9,10) ways to minimize starfish settlement need to be found. The objective of this study was to determine temporal distribution patterns of mussel and starfish larvae in relation to spat/juvenile settlement and to determine the spatial patterns of mussel and starfish settlement in different regions of Newfoundland.

Materials and Methods

Four sites were chosen in three geographically distinct areas of Newfoundland: Site 1, Reach Run, is a large "flow-through" site measuring 300 ha in size. Site 2 and 3, Little Shellbird Bight and Shellbird Bight, are located in Green Bay. They are smaller, "flow-through" sites sheltered by small islands at their perimeters. Site 4, Jersey Harbour, is a more typical "dead-end" or "harboured" site of ~100 ha and is the only site located on the south coast.

Larvae and juveniles were sampled weekly from May 27 to November 13, 1998. To monitor larvae, vertical plankton tows were performed at three stations within each site. Tows were taken to a depth of 10 m. To monitor settlement, 5 rope collectors were deployed weekly on each site and were retrieved two

weeks later. Sampling of collectors overlapped from one week to the next to ensure that accurate indications of settlement patterns could be obtained. The collectors were made of 13-mm green poly rope and measured 2 m in length.

Results

Larval distribution

For Site 1, peak larval mussel abundance (18 larvae/L) occurred on July 16 and there was a smaller peak (6 larvae/L) on September 8 (Fig. 1a). Starfish larvae were present from June 24 through August 5 and the appearance of starfish larvae coincided with the first wave of mussel larvae. Peak starfish abundances (2.5 larvae/L) occurred on July 16 and August 1.

The results for Sites 2 and 3 were similar. Only one major wave of mussel and starfish larvae was observed. At both sites, mussel larvae were present from late June to early September. Peak abundance at Site 2 (325 larvae/L) was observed on July 13; Site 3 had a peak of 245 larvae/L on July 6 (Fig. 1b). Starfish larvae were recorded on both sites from mid-July

through early September. The peak in abundance of starfish larvae at Site 2 was 13 larvae/L on August 14, 4 weeks later than the peak in mussel abundance. At Site 3, the peak in starfish abundance (9 larvae/L) occurred on August 21, 6 weeks later than the peak in abundance of mussel larvae.

The occurrence of mussel larvae at Site 4 was sporadic for the entire sampling period. The peak in mussel larval abundance (6 larvae/L) was observed on August 5. No starfish larvae were observed on this site. Environmental conditions at this site were such that winds were consistently offshore for most of the spawning season.

Spat/juvenile distribution

For Site 1, two peaks of spat settlement were recorded, similar to the larval trends observed on this site. Peak spat settlement of 12,000 spat/collector was recorded on August 1, two weeks after the peak in larval abundance. A second, larger peak of 16,000 spat/collector on September 30, occurred 3 weeks after the second peak in larvae abundance. However, no starfish juveniles settled at this site.

The results for Sites 2 and 3 were similar in that one

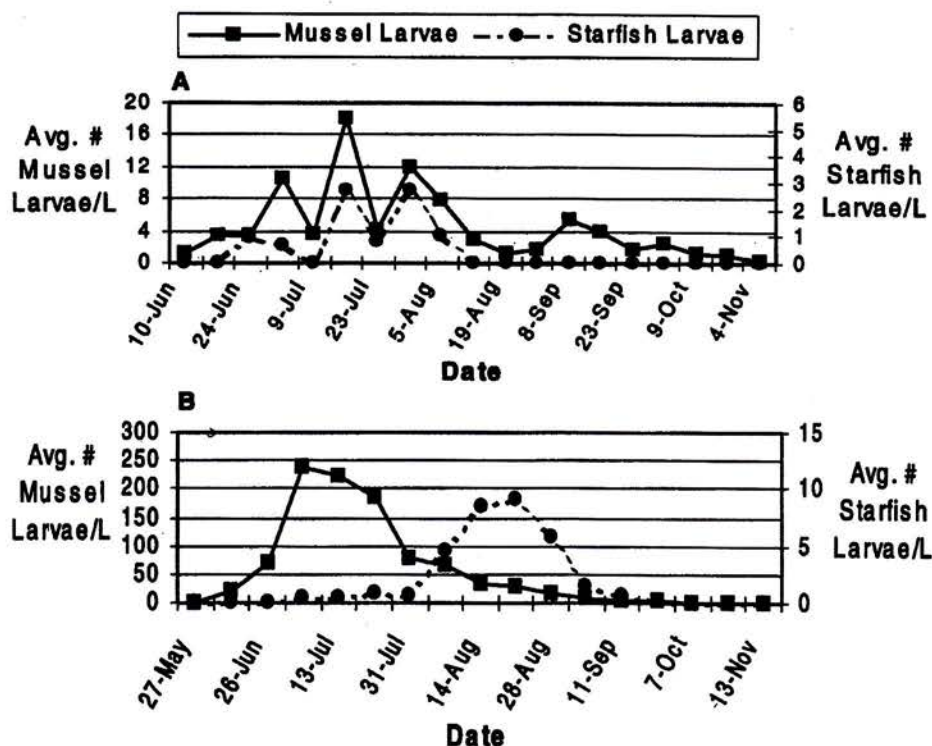


Figure 1. Abundance of mussel larvae in relation to starfish larval abundance at Site 1 (Reach Run) (A), and Site 3 (Shellbird Bight) (B) for 1998.

major wave of mussel larvae preceded one major wave of spat settlement. For Site 2, the peak abundance in mussel larvae was followed 4 weeks later by the peak in spat settlement (11 000 spat/collector on August 14). Similarly, for Site 3 the peak in mussel larvae was followed by peak spat settlement of 16,000 spat/collector 4 weeks later on August 7.

Settlement of starfish juveniles was similar at Sites 2 and 3. At Site 2, peak juvenile settlement of 3.5 juveniles/collector on August 21 was recorded 1 week later than peak larval abundance and 1 week later than peak mussel settlement. For Site 3, the peak in settlement of starfish juveniles was 9 juveniles/collector on September 4, two weeks later than the peak in abundance of starfish larvae and 4 weeks later than the peak in mussel settlement on this site.

Site 4 displayed sporadic patterns of spat settlement as it did in larval abundance. Spat counts remained very low for most of the sampling period, 1500-3000 per collector, until October 4 when a peak of 7000 spat per collector was observed. This peak coincided with the peak in abundance of mussel larvae on August 15. No starfish larvae or juveniles were observed on Site 4.

Discussion

Despite the difference in abundance from site to site, mussel larvae and spat were found at all four sites. Even with different environmental and geographic conditions, growers should anticipate some spat collection if mussel larvae are found during regular monitoring. However, while starfish larvae were observed at all three sites along the north coast of Newfoundland, juvenile settlement was observed on only the two sites in the Green Bay region. Although starfish larvae were recorded at Site 1, juvenile settlement occurred only on the grower's collectors and not on the ones used for this study. Thus for Site 1, it appears that some other factor, such as current velocity or possibly the proximity of collectors to the adult mussels, determined where the starfish preferentially settle.

In this study the appearance of starfish larvae and juveniles were recorded at the same time or directly following the peak in mussel abundance. Therefore it seems that the timing of starfish spawning occurs at the best opportunity for the newly settled juveniles to feed on their available prey. Unfortunately for mussel growers, it may not be possible to avoid starfish by delaying the deployment of collectors until after the major starfish settlement period, as the main opportunity for optimizing mussel spatfall would be lost. In turn, it appears that further research into ridding the mussel collectors of newly settled starfish using techniques such as treating the collectors with lime may be the

best and only option remaining for limiting starfish predation on most mussel farms in Newfoundland.

Implications for Mussel Farming in Newfoundland

The timing, occurrence and relationship of abundance between larvae and juveniles of mussels and starfish were site specific. For Sites 2 and 3, it was observed that *when* larvae were in the water as well as *how many* larvae were present both aided the prediction of timing and numbers of mussels settled. However, at Site 1 in contrast, the presence of larvae was only indicative of the timing of settlement, not of the number settled. When analyzing the starfish data it was evident that the timing of starfish settlement coincided with mussel settlement, so avoidance of starfish may not be possible. Since the inter-relationship between mussels and starfish is site specific, each mussel grower will have to develop an understanding of the characteristics of their individual sites in order to maximize the settlement of mussel spat on their collectors.

Sincere thanks are extended to the Newfoundland Aquaculture Industry Association Blue Mussel Larval/Spatfall Monitoring Program and the funding agencies involved: Atlantic Canada Opportunities Agency, Aquaculture Component of the Canada/Newfoundland Economic Renewal Agreement and the Canadian Centre for Fisheries Innovation. We are also deeply indebted to the mussel growers involved for their assistance and support during this study, namely Mr. Alvin Hodder, Mr. Ed Sheppard and Mr. John Carter.

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The Influence of Lubricating Oils Employed in the Sock-Knitting Process on Mussel Retention

Julie Bertrand^(1, 2) and Cyr Couturier⁽¹⁾

A new knitted mussel socking material made of flat polyethylene fibre was introduced to the industry in 1998. An unexpectedly high drop off of mussels occurred from the socks at some farms, resulting in financial losses. The present study examined the influence of lubricating oils used in the sock manufacturing process on mussel performance and drop off. Three hypotheses were evaluated: (1) the presence of lubricating oils decreases the production of byssal threads, (2) the presence of oils reduces the attachment strength of byssal threads, and (3) the minute quantities of lubricating oils used in the knitting process affected mussel behavior. Experiments were conducted to evaluate each hypothesis and all treatments were performed in triplicate. The drop off of mussels from the experimental socks exposed to the oil used in the knitting process was significantly higher than drop off from control socks (ANOVA, $P < 0.001$). Byssal attachment strength (in newtons) and byssal production rates (threads/day) in mussels exposed to lubricating oils did not vary significantly from controls (ANOVAs, $P > 0.05$). However, mussels exposed to the original lubricating oil (4 ppm) had much slower valve closure responses than mussels exposed to other oils or un-oiled treatments (ANOVA, $P < 0.001$). It is concluded that byssal production and strength are unaffected by exposure to the lubricating oil but that mussel behaviour is affected by the presence of minute quantities of oil.

Introduction

The blue mussel aquaculture industry in Newfoundland produced about 1,000 tonnes in 1998⁽³⁾ and production is expected to double annually for the next 3 to 5 years. Mussel growers experiment with various socking materials in an attempt to lower costs and increase production. A new socking material made of knitted, flat-type polyethylene fibre was introduced in 1998, but some growers found that 80 to 100 per cent of the mussel spat fell off the socks made from this fibre. Growers did not experience problems that year with three other types of mussel socking material with similar specifications. Discussions with the manufacturer and the growers suggest that food-grade lubricating oil used in the knitting process may be a factor in the poor retention of mussels by the new socking material. Interestingly, mussel growers in other areas of Canada have not observed significant mussel fall off using the same knitted material.⁽⁴⁾

The objective of this study was to determine the effects of the lubricating oil used in the sock knitting process on the production of byssal threads and retention of mussels in the socks. In addition, we were asked by the manufacturer to determine the effects of two other lubricating oils. Three hypotheses were

evaluated: (1) the presence of lubricating oils decreases the production of byssal threads, (2) the presence of lubricating oils reduce the attachment strength of byssal threads, and (3) the presence of lubricating oils affects mussel behavior.

Material and Methods

The mussels, a mixture of *Mytilus* spp., were obtained from a commercial mussel farm in Notre-Dame Bay, Newfoundland. The mussels had been previously graded in November (average length 31.1 mm). They were held in flowing ambient seawater (unfiltered, 0.6°C to 1.5°C, 30‰ to 32‰ salinity) at the Ocean Sciences Center (OSC), Memorial University of Newfoundland from 15 December 1998 to 1 February 1999.

The three oils tested were mineral oils used as food machinery lubricants:⁽⁴⁾ the *original oil* and the *low weight oil* were colorless and odorless, the *new oil* had a yellowish tint but was odorless.

Experiment A: Mussel retention in socking material

Socking materials were obtained from the manu-

facturer and were made of the same polyethylene material knitted in the same pattern. They differed only in the type of lubricating oil employed in the knitting process.

Five treatments were allocated to 1 m³ tanks as follows: Tank 1—*original oil-washed socks and no oil #1 socks*, Tank 2—*low weight oil socks and original oil socks*, Tank 3—*control 4.5 socks* (Irish square TMM 4.5-cm diameter) and Tank 4—*control 6.5 socks* (Irish square TMM 6.5-cm diameter) and *no oil #2 socks*. The Irish square is a standard socking material used in the mussel industry. The 4.5-cm and 6.5-cm Irish socks were smaller and larger in diameter than the knitted socking material, respectively, resulting in the need to include both in the trial.

All socks had a filled length of 60 cm and were attached to a support beam and suspended in the water. Three replicates of each socking material were used. A 4.5-L container was placed underneath each sock to collect the mussels that fell off. Initial densities (number of mussels per sock) were carefully determined at the beginning of the experiment.

The water temperature was increased gradually from 0.6° to 10°C ± 1°C over a period of 4 days and was maintained at 10°C ± 2°C by replacing about 20% of the tank water daily with unfiltered seawater. This temperature regime was representative of local conditions during periods of peak drop off on mussel farms. Salinity ranged between 30‰ and 32‰ during the experiment and oxygen levels remained above 80%.

The experiment lasted 30 days. Individual socks were weighed on day 1, 15 and 30. Mussel drop off was recorded daily for each sock.

Experiment A: Strength of byssal attachment

Byssal attachment strength was measured at the end

of the experiment (day 30) on mussels that remained attached to the socking material. Nylon twine was fixed to individual mussels with duct tape and cyanoacrylate glue. The force (in newtons (N)) required to detach each mussel was measured with a spring balance.

Experiment B: Byssal thread production

The second experiment (B) was conducted in 3-L clear plastic Sterilite® containers placed in a wet bench. Each container was provided with gentle aeration. The three oil types provided by the manufacturer, *low weight oil*, *original oil* and *new oil*, were vigorously mixed with seawater (30‰ to 32‰) to create a 4 ppm final concentration. Each treatment consisted of 3 replicates (containers) with 10 mussels per replicate. The control treatment consisted of ambient salinity seawater in triplicate containers (10 mussels per container). Treatment replicates were assigned randomly to the wet bench and maintained in these positions throughout the experiment. Temperature increased from 3.0°C to 10.6°C over the 7-day trial period.

The number of byssal threads produced by each mussel was measured daily by counting threads, cutting them, and recounting threads 24 hours later.

Experiment B: Closure time

At the end of the 7-day exposure period the remaining mussels were assessed for valve closure responses. The mussels were gently prodded with a blunt instrument and the time from stimulus to complete valve closure was measured using a stop watch (closure time in seconds).

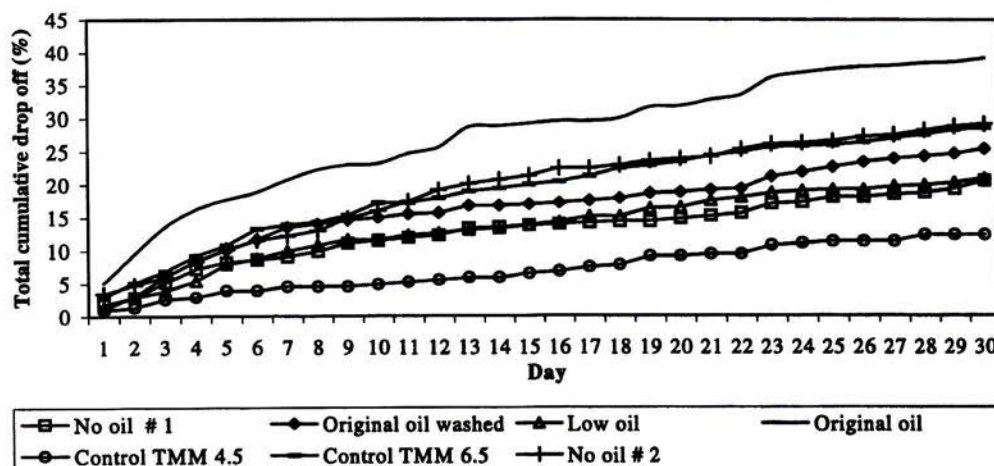


Figure 1. Cumulative drop off of mussels held in 1-m³ static tanks. Each point represents the average of three replicates.

Results and Discussion

Experiment A

A two-way ANOVA showed highly significant drop off of mussels from the socks for the factors *day* and *sock type* ($P < 0.001$) (Fig. 1). Cumulative drop off ranged from 12.3% for the control 4.5 sock (average 0.4% per day) to 39.2% for the original oil sock (1.3% per day).

The drop off from the original oil sock was approximately 10 times faster than earlier observations on drop off from commercial socks.⁽⁵⁾ This suggests there is something about the socking material that affects the mussels, or the physical properties of the socking material (e.g., mesh dynamics) are causing mussels to fall off more easily. The size range of mussels used in these trials was well within the technical specifications of the manufacturer, suggesting the mussels are reacting to some property of the socking material. Casual observations suggest that the knitted mesh can easily stretch beyond the nominal mesh size specifications of the manufacturer. Very few mussels, however, were observed to fall out of the socking material during the trial.

In commercial situations, the fall off of dead mussels may lead to slippage of living mussels from a sock.⁽⁵⁾ Mussel mortality was less than 2% in any of the treatments in our experiments, making it unlikely that this is a cause of the mussel fall off noted here.

Average byssal attachment strength varied slightly from 0.1 N to 0.2 N but these differences were not significant (one-way ANOVA, $P > 0.05$).

Experiment B

Rates of byssal thread production varied from 1.65 threads per mussel per day (*new oil*) to 2.92 threads per mussel per day (*original oil*). There were no significant treatment effects for byssal production rates (one-way ANOVA, $P=0.215$). The rapid fall off rates observed in the knitted socking in Experiment A might suggest fewer byssal threads are produced by mussels on these materials, but this does not appear to be the case. Interestingly, Carr and Reish⁽⁶⁾ observed an enhanced rate of byssal thread production in mussels exposed to Sta-lube outboard motor oil after 96 hours of exposure at concentrations below those which elicited an inhibitory effect. A similar phenomenon may have occurred in our experiments but was not evaluated.

Average mussel valve closure response times varied from 2 to 36 seconds, with the mussels exposed to the *original oil* showing the slowest response to mechanical stimuli and the *control* mussels showing the most rapid response. These treatment effects were significant (one-way ANOVA, $F=15.92$; $df=3, 11$; $P <$

0.001). A slower behavioural response in mussels exposed to low levels of various oils has been observed many times previously.⁽⁷⁻⁹⁾

Conclusions

Our findings generally agree with those in the literature that very low concentrations of pure oil or sub-components such as benzene, naphthalene, lubricating outboard motor oil, heating oils, or any oil in the hydrocarbon family will have adverse effects on mussels.

The relatively high fall off rates observed in the new knitted socking material were not associated with decreased byssal thread production rates or attachment strength to the socking material. Instead, exposure of mussels to low concentrations of the lubricating oils appeared to cause a narcotic effect on the mussels, lessening their ability to respond to mechanical stimuli. These findings support hypothesis 3 outlined in the Introduction. Thus, mussel behaviour is altered by the presence of lubricating oils employed in the sock knitting process and this may provide an explanation for the rapid fall off of mussels experienced by mussel growers with the new socking material.

Further research is warranted to better understand the high drop offs with the new socking material experienced by the Newfoundland mussel growers. A closer look at the mesh dynamics of the socking material and an evaluation of the effects of long-term exposure to lubricating oils is suggested.

We thank Dr. Jay Parsons, Ray Fitzgerald, Danny Au, Damian Whitten and Jerry Ennis for assistance during this project.

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A Comparison of Feeding Physiology in the Blue Mussels *Mytilus edulis* and *M. trossulus*

Melissa Mooney, G. Jay Parsons and Cyr Couturier⁽¹⁾

The physiology of feeding demand was examined in two size classes of cultured and wild *Mytilus edulis* and *M. trossulus*. Monthly samples of small and large (shell length ≤ 25 or ≥ 50 mm, respectively) cultured and wild blue mussels were obtained from a commercial cultivation site over a 16-month period. Mussels were fed low, medium or high algal rations (3500, 5000-7500 or 9000 cells/mL, respectively). Weight-specific rates of clearance (L/h/g), ingestion (cells/h/g), filtration (g/h/g) and oxygen consumption (mL O₂/h/g) were measured. No significant differences were observed between cultured and wild mussels. Smaller mussels had significantly higher weight-specific rates for each of the investigated variables (ANOVAs, $P < 0.05$). *M. trossulus* had significantly higher rates of clearance, ingestion and oxygen consumption than *M. edulis* (ANOVAs, $P < 0.01$). Seasonal patterns were observed in all variables (ANOVAs, $P < 0.01$). The size of the mussels, seasonal variation in feeding, and species proportions will affect the food demand of a stock and should be incorporated into models used to estimate carrying capacity.

Introduction

Rapid expansion of the blue mussel cultivation industry has stimulated interest in problems associated with estimating carrying capacity. Additional research is needed to determine the most cost-effective and time-efficient method of achieving rapid growth and high quality meats. In particular, better estimates of feeding demand (clearance, ingestion and filtration) and oxygen consumption are needed for incorporation into production models.

Molluscs are ideal candidates for physiological studies because there are many documented techniques for quantifying food supply, food uptake and metabolic loss.⁽²⁾ An experimental approach to measuring the effects of environmental variability on organisms will not only provide insight on distribution and abundance but will also assist in the management of aquaculture stocks. It is equally important to compare the physiological responses of different cohorts of blue mussels. In Newfoundland, blue mussel populations consist of cultured and wild *Mytilus edulis* and *M. trossulus*.

In addition to seasonal variability, size and species proportions may affect the overall food demand of a particular stock. This has direct implications for stocking density and stock performance. Continued research in this field is crucial to the maintenance and

expansion of the Newfoundland mussel aquaculture industry. The objective of this project is to contribute to the development of improved models for estimating carrying capacity on mussel sites in Newfoundland by providing values for clearance, ingestion, filtration and oxygen consumption, and comparing these processes over time in two size classes of cultured and wild *M. edulis* and *M. trossulus*.

Methods

Unprocessed cultured and wild blue mussels were obtained monthly from a commercial aquaculture site in Newfoundland (Reach Run) from April 1998 until August 1999. Mussels were transported to the Ocean Sciences Centre, Memorial University, and within 12 hours were placed in a flow-through system supplied with coarsely filtered ambient seawater. Mussels were maintained on a batch-fed diet of cultured algae. Daily ration was approximately 3% of the dry weight of the soft tissue. The diet consisted of two species of microalgae: *Chaetoceros muelleri* (CHAGRA) and *Isochrysis galbana* (clone T-ISO).

A standard octopus set-up was used (i.e., a header tank with 8 individual feeding chambers). Monthly sampling periods consisted of 7 to 10 trials with each trial lasting 3 days. For each sampling period, small and large (shell length ≤ 25 or ≥ 50 mm, respectively)

cultured and wild mussels (*M. edulis* and *M. trossulus*) were used. Mussels were exposed to low (~2000-3500 cells/mL), medium (~5000-7500 cells/mL) or high (> 9000 cells/mL) concentrations of a 1:1 mixture of CHAGRA and T-ISO.

On day one, 7 cultured and wild (small and large) mussels were selected and cleaned. Once the feeding pumps were activated, the mussels were placed in the octopus set-up and left to acclimate to the holding conditions and diet for approximately 24 hours.

On day two, outflow water samples were collected for the calculation of clearance (L/h),⁽³⁾ ingestion (cells/h) and filtration (g/h).⁽⁴⁾ Samples for clearance and ingestion were quantified using a Model II Coulter Multisizer fitted with a 100- μ m aperture tube. Filtration samples (4 L) were vacuum filtered onto pre-weighed and ashed GF/C filters. Filters were rinsed with 10 mL of 3% (isotonic) ammonium formate, dried at 60°C, combusted at 450°C for approximately 12 hours, and re-weighed.

On day three, mussels were placed in respiration chambers filled with filtered, saturated, ambient seawater. Submersible stirrers circulated the water in the chambers. Temperature was maintained within 0.5°C of ambient temperature using a Neslab unit. The chambers were sealed with an oxygen electrode, and the decline in partial pressure of oxygen was measured with an OM2000 oxygen meter.

Finally, a biopsy was taken from the adductor muscle or mantle of each mussel and preserved in 95% ethanol for allozyme and DNA analysis to be used in species identification. Remaining tissues were dried at 60°C. The effects of body size were removed by correcting the rates to a standard body size using an al-

lometric equation. Individual trial weight exponents were used to correct physiological rates to 1 gram.

Statistics were performed using the General Linear Model procedure of SPSS 9.0 for Windows. One-way analyses of variance (ANOVAs) were conducted to determine the significance of the individual factors (origin, size, species, season, food concentration) on the clearance, ingestion, filtration and oxygen consumption of the dependent variables.

Results

One-way ANOVAs demonstrated no significant differences in the weight-specific rates of clearance (3.4 L/h/g), ingestion (23,963.0 cells/h/g), filtration (0.0041 g/h/g) or oxygen consumption (3.2 mL/h/g) for cultured mussels compared to the rates of clearance (3.4 L/h/g), ingestion (20,701.0 cells/h/g), filtration (0.0056 g/h/g) or oxygen consumption (3.5 mL/h/g) for wild mussels ($P > 0.05$).

One-way ANOVAs ($P < 0.05$) showed that small mussels had significantly higher weight-specific rates of clearance (4.4 L/h/g), ingestion (29,192.4 cells/h/g), filtration (0.0067 g/h/g) and oxygen consumption (1.8 mL/h/g) compared to the rates of clearance (2.4 L/h/g), ingestion (15,440.2 cells/h/g), filtration (0.0026 g/h/g) and oxygen consumption (0.45 mL/h/g) for larger mussels. One-way ANOVAs ($P < 0.05$) also showed that *M. trossulus* displayed significantly higher weight-specific rates of clearance (4.0 L/h/g) (Fig. 1), ingestion (24,237.1 cells/h/g) and oxygen consumption (4.8 mL/h/g) compared to the rates of clearance (2.8 L/h/g) (Fig. 1), ingestion (16,579.6 cells/h/g) and oxygen consumption (3.3

mL/h/g) for *M. edulis*. Filtration was not significantly different between the two species ($P > 0.05$).

Finally, one-way ANOVAs demonstrated that food availability significantly affected rates of feeding ($P < 0.01$). Feeding rate increased with an increase in food availability (Fig. 2). Oxygen consumption was not affected by food availability ($P > 0.05$). In addition, a significant seasonal pattern was associated with all the variables investigated ($P < 0.01$).

Discussion

The results imply that cultured and wild mussels will demonstrate similar feeding patterns if they are maintained in similar conditions. Although higher growth rates and meat yields have been observed in cultured blue mussels than in wild mussels,⁽⁵⁾ this may be an envi-

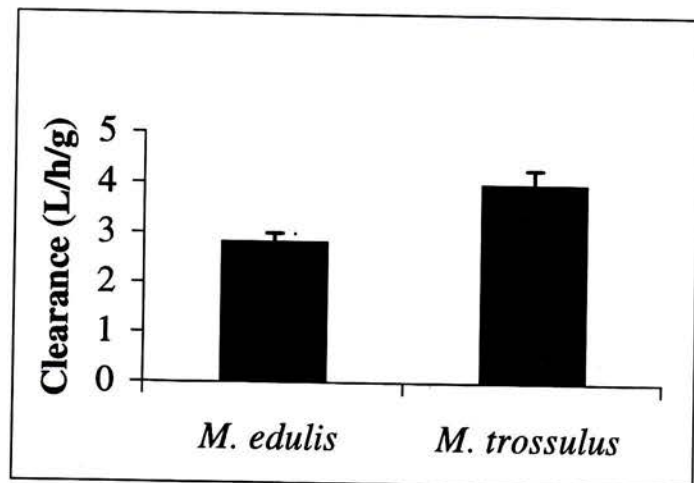


Figure 1. A comparison of clearance rate (L/h/g) in *Mytilus edulis* and *M. trossulus* ($P < 0.001$).

ronmental influence. Wild mussels are subjected to an increased risk of predation in the subtidal and intertidal zones. As well, the intertidal zone is a high energy environment. As a response to predation and high energy, wild mussels direct more energy towards survival than meat growth.

Cultivated blue mussels have been observed to allocate less than half the energy to reproduction than wild blue mussels.⁽⁶⁾ Again, this may be an environmental influence, but it is possible that cultured and wild blue mussels demonstrate different patterns of energy allocation for gametogenesis.

Recent findings in the literature support our observations that smaller mussels display significantly higher weight-specific rates of clearance, ingestion, filtration and oxygen consumption. Similar size-specific differences have been reported for *Cerastoderma edule*⁽⁷⁾ and *Argopecten irradians concentricus*.⁽⁸⁾ The results have implications for socking and stocking density: smaller mussels should be socked at lower densities. Stocking beyond optimal density increases the risk of mussel loss due to competition for food and oxygen.⁽⁹⁾ For mussels to reach market size in the least amount of time, smaller size classes must receive maximal food supply. Alternatively, deliberate overcrowding can be used to slow growth rates for specific purposes such as to maintain a continuous supply of market-sized mussels.⁽⁵⁾

Recent observations in the literature assert that different species display different physiological and morphological adaptations to different environments in order to maximize survival.⁽¹⁰⁾ Our study suggests that *M. edulis* and *M. trossulus* are physiologically distinct, employing different biological strategies. Specifically, *M. trossulus* demonstrates significantly

higher rates of clearance, ingestion and oxygen consumption. These results have implications similar to the differences seen between small and large mussels.

Also, the results show that rates of clearance, ingestion and filtration increase with increasing food availability and all variables had an associated seasonal pattern. The influence of temperature and food on the growth of bivalves is well documented in the literature, especially for mytilids.⁽¹¹⁾

Factors affecting physiological responses should be incorporated into blue mussel production models. Differential physiological adaptations may affect site carrying capacity. Seasonal variations in temperature and food availability, in addition to the size and species proportions of a stock, significantly contribute to the food demand of a stock, and therefore the carrying capacity. Size and species proportions of a mussel stock should be determined, and stocking density adjusted accordingly, in order to achieve optimal carrying capacity.

We thank the Canadian Centre for Fisheries Innovation and the Aquaculture Component of the Economic Renewal Agreement for funding, as well as the Newfoundland mussel growers for their participation. Thanks also to Miranda Pryor and Tony Zokvic for their assistance in delivering the mussels.

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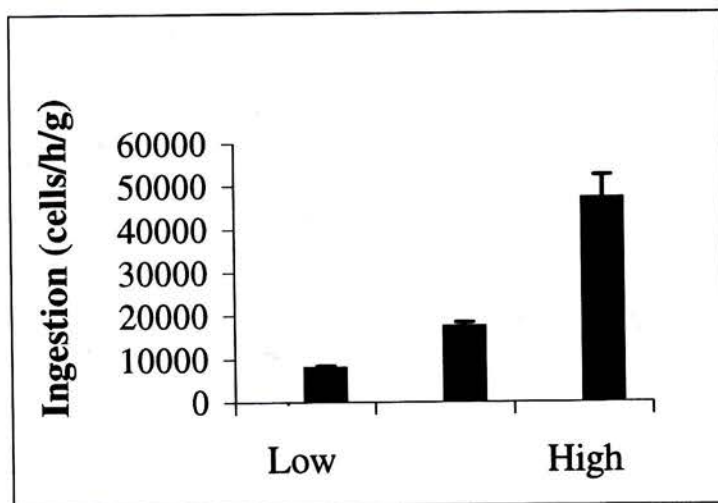


Figure 2: A comparison of ingestion (cells/h/g) at low, medium and high food concentrations in blue mussels ($P < 0.001$).

Influence of Simulated Fouling on Current Velocities in Pearl Nets

Nancy Moulard and G. Jay Parsons⁽¹⁾

Fouling and current velocity are two major factors to consider when selecting a site for scallop grow-out since they have a major impact on growth and survival. In this experiment, 6-mm-mesh pearl nets were used to examine three factors and their corresponding effect on current velocity inside the nets: external current velocities, ranging from 9-90 cm/s; percent coverage of fouling (25%, 50%, and 75%); and simulated fouling, consisting of mussels (empty mussel shells), macroalgae (flagging tape) and sea squirts (bottle caps). All three factors had a significant effect ($P < 0.05$) on the internal current velocity. Two patterns were evident from the data: 1) as external velocity increased, velocity within the pearl nets increased, and 2) as the percent coverage of all fouling types increased, the internal current velocity decreased. However, no consistent pattern was observed among internal current velocities for the different fouling agents at the same percent coverage. The restriction of water flow within pearl nets will hinder the advancement of scallop culture in areas of high fouling. Further research is required into alternative culture techniques (e.g., off-bottom techniques) or fouling-resistant gear to maximize water and food availability to cultured scallops.

Introduction

As with many marine populations, over exploitation has greatly reduced the number of scallops in several stocks over the years. Their rapid growth rates and high market value has sparked interest among aquaculturists. Canadian aquaculture production statistics indicate that in 1996 a total of 178 tonnes of scallops were produced. Of that, the Atlantic Provinces contributed approximately 36 tonnes, while British Columbia produced 143 tonnes.⁽²⁾

A common off-bottom technique used in the aquaculture industry is the suspension of bivalves in mesh baskets called pearl nets. Growth and survival of scallops reared in pearl nets are dependent on a number of factors including fouling, current velocity and mesh size. Fouling on pearl nets causes serious maintenance and operational problems, as well as a reduction in water flow through the nets, which decreases the amount of food available to the bivalves.⁽³⁾ The reduction in water flow is inversely related to the mesh size of the pearl nets.^(4,5) As a result, several net systems used in large commercial aquaculture operations have failed.⁽⁶⁾ For this reason, it is essential that further research be conducted to understand the mechanism of food limitation.

The objective of this study was to examine the effect of different amounts and types of fouling on current

velocities inside pearl nets over a wide range of external current velocities. Based on previous reports in the literature, it is hypothesized that nets with the greatest amount of fouling will have the highest reduction in internal current.

Materials and Methods

In this experiment, 6-mm-mesh pearl nets were used to examine the effect of three factors and their corresponding treatments. The factors were: external current velocities ranging from 9-90 cm/s at 7 cm/s increments with internal current velocity being the variable that was measured; percent coverage of fouling at 25%, 50%, and 75% of the surface area; and simulated fouling consisting of mussels (empty mussel shells), macroalgae (flagging tape), and sea squirts (bottle caps). The pearl net trials were set up as follows: individual pearl nets had either simulated mussels, algae or sea squirts covering 25%, 50%, or 75% of the net. Three replicates of each treatment were tested, for a total of 27 nets with fouling. Another three pearl nets without fouling were used as the control nets.

The experiment was carried out in the model flume tank at the Marine Institute of Memorial University. Each net was individually suspended in the flume tank from a carriage spanning the width of the tank. Current velocity inside the pearl nets was measured

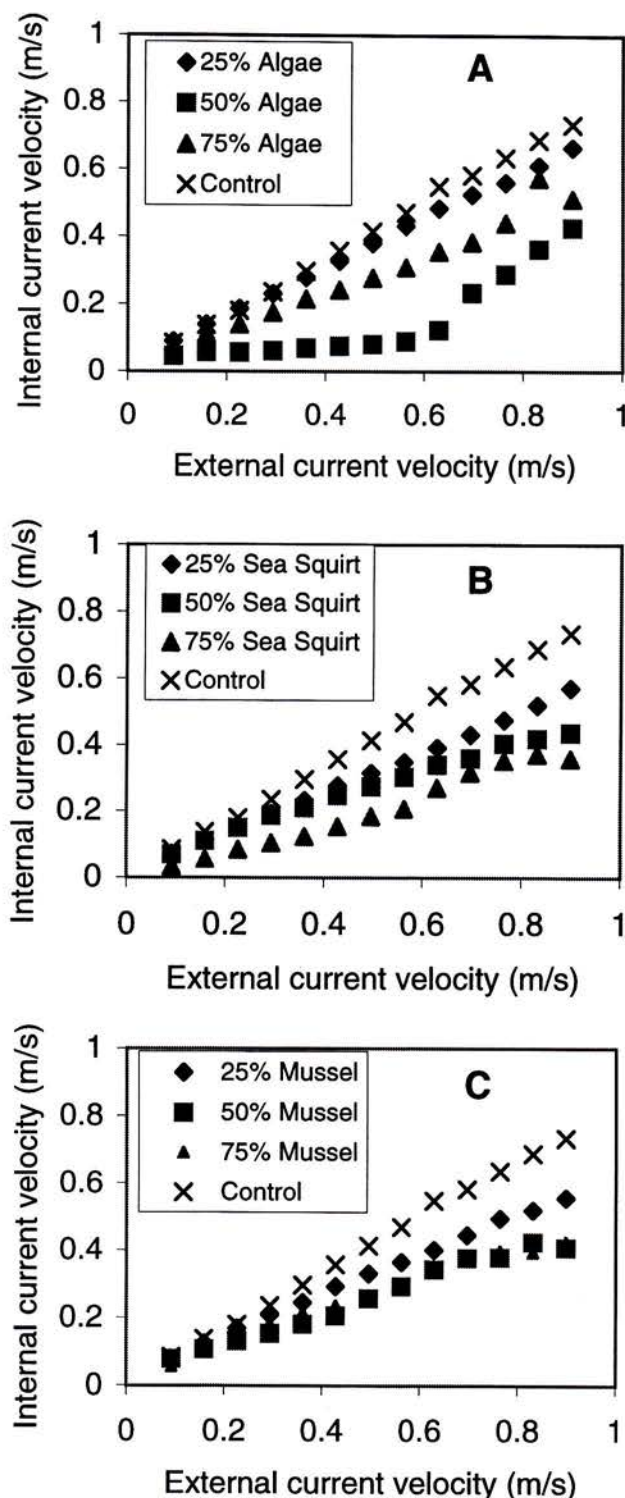


Figure 1: Average internal current velocity for pearl nets with 25%, 50%, and 75% coverage by simulated algae (A), sea squirts (B) and mussels (C).

using a current meter with an impeller (Geneq Inc.). The impeller, attached to the end of a supporting rod, was placed inside and positioned in the center of each net. Mechanically-controlled current velocities, ranging from 9-90 cm/s, were created in the flume tank. Statistical analyses were performed using the SPSS statistical package and Type I error was set at $\alpha=0.05$.

Results

A three-way ANOVA was used to determine if there were significant differences among the three factors that were tested. Based on this analysis, there was a significant difference in the internal current velocity for all three factors (speed: $F=149.2$, $P < 0.001$; fouling: $F=3.3$, $P = 0.037$; percent coverage: $F=120.5$, $P < 0.001$). Two-way ANOVAs were performed on fouling and coverage to test for differences between fouled and nonfouled (control) nets at the difference current velocities. There was a significant difference in internal current velocity for each of these two factors (speed: $F=1.6$, $P < 0.001$; fouling: $F=5.3$, $P=0.032$ and speed: $F=1.8$, $P < 0.001$; percent coverage: $F=1.6$, $P < 0.001$).

In comparing nets with the same type of fouling at different levels of coverage, several patterns were evident. Nets covered with 25% algae experienced the highest internal velocities while those with 75% algae had much lower inside velocities (Fig. 1a). Of the nets covered with sea squirts, those with 25% coverage displayed higher internal velocities at an external speed of 36 cm/s or higher. Those nets with 75% coverage experienced the lowest internal velocities (Fig. 1b). Of the pearl nets fouled with mussels, those with 25% coverage had the highest internal current velocities, while those with 50% and 75% coverage with mussels experienced similar, lower inside velocities (Fig. 1c). Again, the control pearl nets had the highest internal current velocities in all cases. There was no consistent pattern in flow reduction when nets with sea squirts and algae were compared. However, nets with

mussels had intermediate values when compared to nets with sea squirts or algae.

Discussion

The data obtained in this experiment indicate that the presence of any level of fouling (25 to 75% coverage) on the pearl nets reduces the amount of water flow reaching the inside of the nets. As was expected, the greater the amount of fouling on the nets, the greater the reduction in internal current velocity. Similarly, Devaraj and Parsons⁽⁵⁾ found that fouled pearl nets containing simulated algae experienced lower internal current velocity than control nets.

In most cases, scallop aquaculture sites are located in environments where current speeds are at optimal levels. In areas with optimal currents, the presence of heavy fouling organisms would be detrimental to the scallops and ultimately the grower. A number of studies have shown that fouling and the subsequent reduction to flow and food levels has negative effects on the growth and survival rates of scallops.⁽³⁾ Thus, it is essential that both current velocities and fouling be considered when choosing the most appropriate culture site and method.

Off-bottom culture, despite its greater costs, is more economically efficient than rearing scallops on bottom since growth and survival rates are enhanced considerably and the grow-out period may be reduced by two or more years. In terms of suspension methods, however, pearl nets may not offer the best return on investment per unit of scallop. Although they are relatively inexpensive and readily available, the cost related to handling the nets and the overall performance reduces their value to the aquaculture industry⁽⁷⁾ unless ways can be found to minimize the effects of fouling.

Summary and Conclusions

Fouling has been one of the major husbandry concerns associated with pearl nets. Fouling causes a restriction in water flow, which reduces the amount of food available to the scallops. Fouling organisms also directly compete with scallops for food. Ultimately, growth and survival rates may be reduced, thus affecting production levels. To minimize the problems associated with fouling, nets should be placed in deeper areas of the ocean and should be cleaned on a regular basis. As well, site selection criteria based on optimal currents inside the nets should be considered. Finally, the economic efficiency of alternative grow-out methods should be explored.

We thank Ray Fitzgerald and George Legge for their technical assistance.

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Questionnaire for Student Members

A questionnaire for students has been posted in the student affairs section of the AAC web page (www.mi.mun.ca/mi/aac). The results will be used to influence the Association's decisions on matters relating to student members. Please take the time to respond to this questionnaire, if you have not already done so. It will be worth your time!

— Chris Hendry, Chairman, Student Affairs Committee

Somatic Growth Trials for Juvenile Green Sea Urchins Fed Prepared and Natural Diets

Eddy J. Kennedy,^(1,2) Shawn M.C. Robinson,⁽¹⁾
G. Jay Parsons,⁽²⁾ and John Castell⁽¹⁾

An important aspect of the development of any culture industry is the maximization of juvenile somatic growth to reduce production time and increase the size of the final product. This study on the green sea urchin (*Strongylocentrotus droebachiensis*) investigated the effect of three factors, protein source, protein concentration, and juvenile size, on somatic growth rates over 280 d. A kelp diet of *Laminaria longicruris* was used as a control. The specific growth rates (SGRs) for all the protein treatments in the small cohort were significantly lower than the kelp treatment ($P < 0.05$). For the large cohort only two protein diet treatments (95% soybean : 5% fishmeal at 20% and 30% dry mass) had significantly lower SGRs than the kelp treatment. For both cohorts, there were no significant differences in SGR ($P > 0.05$) among sea urchins fed the prepared diets, indicating that sea urchins do not require animal protein for boosted growth. This has major impacts for feed manufacturers since cheaper plant proteins can be utilized in juvenile sea urchin feed production. More research is required to identify the nutritional components of the kelp that may be deficient in the prepared diets.

Introduction

The green sea urchin (*Strongylocentrotus droebachiensis*) has gained attention within the aquaculture industry due to the increasing demand for roe ("uni") in the Asian and European markets and the market price of high quality product (US\$75-\$85/kg).^(3,4) The demand for sea urchins has enticed many fishermen in coastal areas to overexploit the natural stocks. Thus, new sources of sea urchins are essential if the industry is to continue to grow. Complete intensive aquaculture, which involves the raising of sea urchins through their complete life cycle, is one method of prolonging the sea urchin industry.⁽⁵⁾ Most research on sea urchin culture has been on controlling the quality (size, taste, color, texture) of the roe in adults through nutrition and feed type.⁽⁵⁾ The next step is to understand the nutritional requirements of juveniles and develop a diet that optimizes somatic growth. It is important to reduce the time required for sea urchins to reach market size as this will increase the economic potential for the aquaculturist.

Protein has been shown to affect echinoid production.^(6,7) In this experiment, the effects of protein source and concentration on juvenile somatic growth were studied. Plant proteins are less expensive than animal sources of proteins, so it would be advantageous for feed manufacturers to be able to increase the proportion of plant protein in the diet. The objective of

this study was to examine the effect of varying levels and proportions of two protein sources on the somatic growth of juvenile green sea urchins.

Materials and Methods

Experiments were carried out at the Biological Station in St. Andrews, NB. The holding system for the juvenile sea urchins consisted of 30 rearing tanks erected in 10 columns standing three units high. Each tank contained four perforated baskets, thus allowing 120 treatment units. Two size classes (4-8 and 12-20 mm test diameter) of juvenile sea urchins were collected by SCUBA from a wild sea urchin bed off Tongue Shoal in Passamaquoddy Bay, NB. They were acclimated to laboratory conditions for 1 wk. The two cohorts were used to test the effect of size on the dietary requirements of juveniles.

Diet treatments consisted of three proportions of two protein sources (100% soybean protein (SBP), 95% SBP : 5% fish meal protein (FMP), and 50% SBP : 50% FMP) and four protein concentrations (20%, 30%, 40%, and 50% dry mass). A natural kelp diet (*Laminaria longicruris*) was used as a control. Shur-Gain, Maple Leaf Foods Inc. supplied all feed ingredients and the diets were prepared in the form of a moist extruded pellet (2 x 5 mm). A treatment sample consisted of 30 sea urchins and each treatment had four replicates (total of 120 sea urchins). At the sixth

sampling period (150 days), two replicates of each treatment were terminated. Individual tanks were allocated a specific dietary treatment based on a randomized block design and each tank had its own flow-through system of filtered seawater at ambient temperature to prevent cross-contamination. The sea urchins were fed to excess daily and tanks were cleaned weekly.

The experiment continued from December 1998 to September 1999 and the test diameters of all sea urchins were measured monthly. Sea urchins in each treatment were individually video-taped and three measurements of test diameter were recorded for each sea urchin using image analysis software (Optimas™). Specific growth rates were calculated using the formula $SGR = ((\ln(x_f) - \ln(x_i)) / (t_f - t_i))$, where x_f = final diameter in (mm), x_i = initial diameter (mm), $t_f - t_i$ = period of growth in days. Analysis of variance (ANOVA), along with Tukey's multiple comparison test ($P=0.05$), were used to analyze for growth differences among treatments using the statistical packages SYSTAT™ and SPSS™.

Results

The kelp diet produced a greater increase in test diameter in both cohorts (Fig. 1). For cohort 1, the average test diameter (all diet treatments) increased from 6.9 mm to 16.0 mm. The kelp diet yielded an increase from 6.4 mm to 17.2 mm. For cohort 2, the test diameter increased from 14.7 mm to 23.5 mm, with kelp providing an increase from 16.0 mm to 26.8 mm (Fig. 1). For both cohorts, the increase in test diameter was slow during the first 5 sampling periods, but increased over the remainder of the experiment. The change in growth rates corresponded to the increase in water temperature during the spring and summer (Fig. 1).

The initial measures of test diameter for both cohorts were significantly different ($P < 0.05$, ANOVA) even though they were randomly assigned. The significant differences in size that occurred throughout the experiment could not be separated as either an effect of the diet treatments or initial difference in test diameter. Consequently, specific growth rates were calculated to provide an estimate of

growth somewhat independent of the initial differences in test diameter.

For both cohorts, the SGR was initially high and then dropped suddenly in all diet treatments (Fig. 2). Cohort 1 had a higher SGR than cohort 2; therefore SGR was dependent upon the initial size of the sea urchins. The highest SGR in both cohorts was in the kelp-fed sea urchins, indicating that the kelp diet provided the factors essential for enhanced juvenile somatic growth. For cohort 1, the kelp diet had a significantly higher SGR than any of the prepared diets ($P < 0.05$), and there were no significant differences among any of the prepared protein diets ($P > 0.05$) (Fig. 2a). For cohort 2, the SGR for the kelp diet was not significantly different from the protein diets ($P > 0.05$), except for the 95% SBP : 5% FMP @ 20% and 30% protein treatments ($P=0.015$ and $P=0.009$, respectively), where kelp was higher. There were no significant differences in SGR among any of the prepared protein diets ($P > 0.05$) (Fig. 2b). Survival rate was high (93-95%) in all treatments for the duration of the experiment, except for one treatment replicate (100% SBP @ 50% concentration) in which the water flow became blocked and all the sea urchins died.

Discussion

The prepared protein diets supported sea urchin growth over the course of the experiment. Thus, formulated feed appears to be a potential alternative to natural diets for sea urchin aquaculture. Similar to other studies, this experiment showed that all the prepared protein diets supported the same somatic growth regardless of protein source and/or concentra-

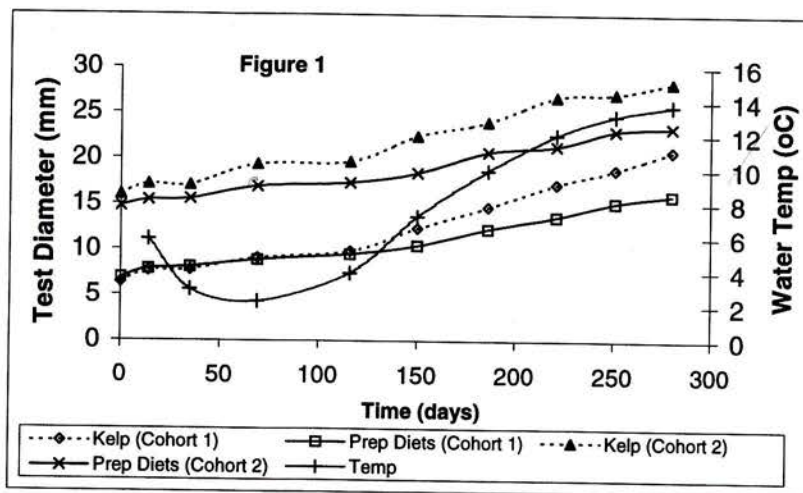


Figure 1. Test diameter for two cohorts of juvenile green sea urchins fed prepared protein diets and kelp (*Laminaria longicurris*) over 9 months at ambient seawater temperature.

tion.^(5,8-10) This has consequences for feed manufacturers, since the same growth can be achieved using plant protein at low concentrations in the diet as with animal protein at high concentrations. This will reduce feed production costs, as more inexpensive sources of protein can be used at lower concentrations.

However, the prepared grain-based diets did not yield superior growth compared to the kelp diet,^(9,11) suggesting kelp (*Laminaria longicruris*) may contain other factors essential for growth that were limiting in the prepared diets. Kelp generally has a protein concentration of 12–17% dry mass,^(12,13) so the optimal protein concentration for growth of juvenile sea urchins may be below the lowest concentration tested. This conclusion, however, is not supported by other studies. McBride et al.⁽¹⁴⁾ and Tollini et al.⁽¹⁵⁾ suggested a 40% protein concentration yielded the best growth rates in sea urchins. The lower growth rates from the protein diets may also be a reflection of diet form or leaching of nutrients.

In conclusion, there appears to be nutritional factors that are available in kelp that are limiting in the prepared diets that support somatic growth in juvenile

green sea urchins. As well, stress on the sea urchins due to increased diet manipulation and poorer water quality may be potential factors for reduced somatic growth compared to the kelp diet. Further studies are required on juvenile sea urchin nutrition to identify and refine the dietary requirements essential for optimal somatic growth. Such advancements will ultimately produce mature sea urchins faster, thus improving the economic feasibility of the sea urchin aquaculture industry.

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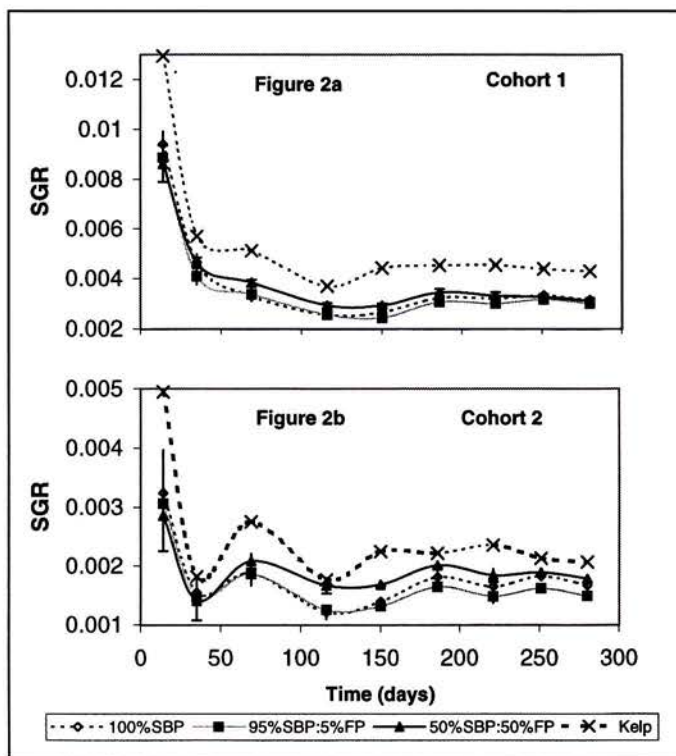


Figure 2. Specific growth rate of two cohorts of juvenile green sea urchins fed prepared protein diets and kelp (*Laminaria longicruris*) diet over 9 months (bars represent 1 standard error of the mean).

Chemical and Physical Spawning Inducers of the Green Sea Urchin *Strongylocentrotus droebachiensis*

Nicole Caron and G. Jay Parsons⁽¹⁾

This study investigated chemical and physical spawning inducers of ripe adult sea urchins *Strongylocentrotus droebachiensis*. Potassium chloride and acetylcholine chloride induced the greatest spawning activity, sustained the longest duration of spawning, resulted in the shortest elapsed time to gamete release and stimulated the release of the largest number of gametes. Of the physical/environmental inducers, the presence of phytoplankton induced the greatest amount of spawning activity and resulted in the shortest elapsed time to gamete release. Aeration also induced spawning; it resulted in a longer spawning duration than phytoplankton and a greater number of released gametes. Temperature shock did not induce spawning, except in one sea urchin.

Introduction

Investigations of potential spawning inducers and techniques for sea urchin broodstock management are essential for the commercial production of seedlings. The green sea urchin, *Strongylocentrotus droebachiensis*, is considered a potential aquaculture species, especially for sea ranching and land-based culture in Atlantic Canada.⁽²⁻⁴⁾ Wild seed are difficult to collect and abundance varies seasonally and annually due to variations in spawning times and quantities of gametes released. The long-term success of the sea urchin culture industry will probably depend on the availability of a reliable source of hatchery-produced seed.

Several factors, including phytoplankton,⁽⁵⁻⁷⁾ gamma aminobutyric acid (GABA),⁽⁸⁾ temperature shock,^(9,10) acetylcholine,⁽¹¹⁾ and serotonin (5-hydroxytryptamine, 5-HT),⁽¹²⁾ have been used to induce spawning in other invertebrates. Selected serotonin reuptake inhibitors such as fluoxetine (Prozac) also induce spawning in some invertebrates.⁽¹³⁾ Physical/environmental inducers such as thermal shocking and/or water agitation (aeration) have also been used.

The objective of this study was to determine if gamete release in the green sea urchin, *Strongylocentrotus droebachiensis*, could be manipulated through the use of chemical and physical/environmental stimuli.

Methods

Experiments were conducted at the Aquaculture Facility of the Marine Institute of Memorial University, St. John's, NF. Sea urchins were collected from the wild and acclimated in raceways with recirculated seawater for 7 d prior to the experiment. A series of

experiments were carried out examining chemical and physical inducers. For each treatment, a minimum of 5 animals was used. Sea urchins were sexed after the trials.

Experiment #1 Chemical spawning inducers

Potassium chloride, acetylcholine chloride, serotonin hydrochloride, serotonin creatinine sulphate, gamma aminobutyric acid, fluoxetine and St. John's wort were examined for their ability to induce spawning in the sea urchin (Table 1). Predetermined volumes of each inducer were mixed with filtered seawater and injected into the peristomal membrane of the animal. As a control group, 5 animals were injected with seawater filtered to 1 µm.

After injection, the elapsed time for gamete release and spawning duration were recorded. If no spawning activity was observed after 2 h, the sea urchin was classified as a non-spawner. Gamete counts were done after 2 h had elapsed. Sub-samples of eggs were counted under a dissecting microscope and sperm samples fixed with 10% formalin were counted using a hemocytometer.

Experiment #2 Physical/environmental spawning inducers

The effectiveness of physical spawning inducers was examined using water temperature (shock), aeration and phytoplankton concentration (the diatom *Chaetoceros muelleri* (CHGRA)) (Table 1). The animals were held in 5-L buckets with 1 L of seawater and the physical/environmental stimulant was administered to test the effectiveness of each inducer. The

Table 1. Chemical and physical/environmental inducers and inducer levels tested on the green sea urchin for spawning success.

Inducer	Inducer Level			
	1	2	3	4
Chemical Treatment				
Potassium chloride	0.25 M	0.35 M	0.45 M	0.5 M
Acetylcholine chloride	0.025 M	0.050 M	0.100 M	—
Serotonin hydrochloride	0.0001 M	0.001 M	0.01 M	—
Serotonin creatinine sulphate	0.0001 M	0.001 M	0.01 M	—
Gamma amino-butyric acid (GABA)	0.0001 M	0.001 M	0.01 M	—
Fluoxetine (Prozac)	0.00001 M	0.0001 M	0.001 M	—
St. John's wort (herbal supplement)	0.250 M	0.500 M	1.50 M	—
Control — seawater injection	—	—	—	—
Physical Treatment				
Temperature (°C)	0	5	15	—
Aeration (air stones)	—	—	—	—
Phytoplankton (cells/L)	2 x 10 ⁷	10 x 10 ⁷	—	—
Control — seawater bath (10°C)	—	—	—	—

sea urchins were monitored over a 2-h period for spawning activity. Variables (time of release, duration of spawning and gamete counts) were monitored following the same procedures as in Experiment 1.

Results

Chemical inducers

Chemical inducers, except for GABA and the control treatment, were effective at inducing spawning in 40–100% of the sea urchins. The effect of potassium chloride, acetylcholine chloride, St. John's wort and fluoxetine was statistically similar and sea urchins receiving these treatments had the shortest elapsed time to gamete release (Fig. 1a). Responses to fluoxetine, serotonin creatinine and serotonin hydrochloride were similar and these chemicals resulted in the longest elapsed time to gamete release. Time of release was significantly different between male and females, but it did not differ with inducer level ($P < 0.01$).

Acetylcholine chloride and potassium chloride resulted in the longest duration of spawning activity (Fig. 1b), followed by serotonin creatinine, serotonin hydrochloride, St. John's wort and fluoxetine. Duration of spawning differed significantly with the type of inducer ($P < 0.01$), but did not differ significantly between sexes ($P > 0.05$).

There was a significant difference among males in numbers of gametes released ($P < 0.01$). The greatest number of gametes released was with acetylcholine chloride and potassium chloride. For female sea urchins, the chemical inducers had no significant effect on the number of gametes released ($P > 0.05$).

Physical inducers

All of the physical inducers were effective at induc-

ing spawning, except for the control treatment and the temperature change (shock), which were ineffective in inducing spawning in almost all sea urchins. The phytoplankton group had the shortest elapsed time to gamete release followed by the aeration group (Fig. 2a). Only one sea urchin spawned in response to temperature shock, but it had the shortest elapsed time to spawning. Aeration stimulated the longest period of

spawning activity of the physical inducers, while the phytoplankton group had the shortest period of spawning activity (Fig. 2b). Counts of released gametes in the groups of sea urchins exposed to physical inducers indicated that aeration had stimulated the release of a large quantity of gametes, while phytoplankton stimulated a release of a lower quantity of gametes.

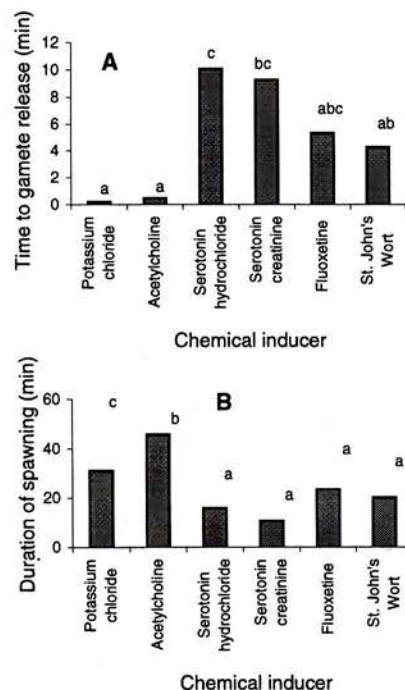


Figure 1. Mean elapsed time to gamete release (A) and mean spawning duration (B) for the various chemical inducers. Common letter denotes significant difference (Tukey's B). Bars represent standard error.

Discussion

Chemical inducers

Potassium chloride and acetylcholine chloride induced the longest period of spawning. This effect is attributed to stimulation of the nervous system which in turn induces gamete release. Cholinergic mechanisms are involved in the regulation of sea urchin spawning and acetylcholine is one of the regulators of gamete shedding in mature sea urchins.⁽¹¹⁾ Using potassium chloride and acetylcholine as spawning inducers produces a sustained spawning response.

GABA induces gamete shedding in *S. intermedius*⁽⁸⁾ and may act as an excitatory transmitter at synapse and/or neuromuscular junctions. However, no spawning activity was induced in *S. droebachiensis*, suggesting GABA may be species specific.

The other chemical inducers, known to be effective in marine bivalves,⁽¹³⁾ also induced spawning but the duration of spawning was short and number of gametes released was small.

Physical inducers

The effect of the physical/environmental inducers on spawning was expected, except for the temperature shock.^(9,10) The phytoplankton trial agreed with the findings of Starr et al.^(5,6) The coupling of spawning activity with phytoplankton concentration results in spawning synchronization, enhanced fertili-

zation success^(6,7) and optimal conditions for larval development.

The spawning activity due to aeration was not expected. However, Desrosiers et al.⁽¹⁴⁾ found that flowing seawater induced spawning in the giant sea scallop *Placopecten magellanicus*. Low levels of turbulence affected fertilization success and increased dispersion of the gametes throughout the water column.⁽¹⁵⁾

Conclusions and Summary

Of the chemical and physical/environmental inducers tested, most were effective. Among the chemical inducers tested, potassium chloride and acetylcholine chloride were the most effective. Aeration and phytoplankton were the most effective of the physical/environmental inducers. However, considerations regarding the choice of chemical or environmental inducer will depend on the quantity and quality of gametes, the cost of the chemicals and time constraints.

By producing sea urchins in hatcheries, harvesting seasons, gonad quality and yields can be improved through the manipulation of sexual maturation by regulating photoperiod and temperature.⁽¹⁶⁾ And with the establishment of systematic broodstock selection and breeding programs, it will be possible to develop improved roe yields and growth rates.

Thanks to Ray Fitzgerald for technical support.

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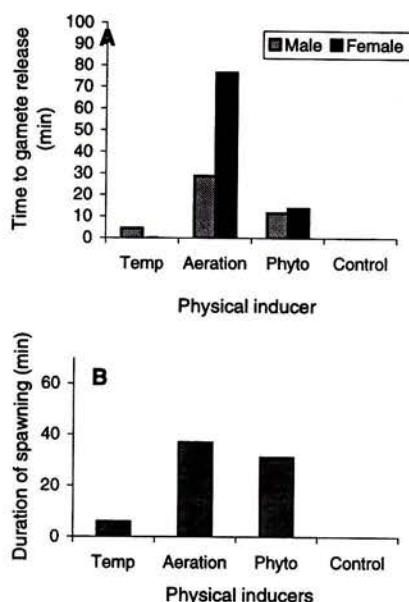


Figure 2. Mean elapsed time to gamete release (A) and mean spawning duration (B) for the various physical spawning inducers between sexes. Bars represent standard error.

Environmental Management Systems (ISO 14001) in Aquaculture

I. D. Cuthbert⁽¹⁾

Salmon aquaculture has significant environmental aspects and potential environmental impacts, and the industry is subject to a high degree of stakeholder scrutiny, and complex, rapidly evolving legislation and regulations. Environmental management systems (EMS) based on ISO 14001 provide a powerful technology for aquaculture producers wishing to achieve and demonstrate sound environmental management. Having an EMS in place reduces the risk of environmental degradation, and associated penalties and market restrictions. EMS benefits can also include improved operating efficiencies, better relations with regulators, consumers and the public, and enhanced market access. This paper provides an overview of ISO 14001 and its application and benefits in salmon aquaculture.

Introduction

Salmon aquaculture is conducted in the coastal marine environment, where overlapping and sometimes conflicting uses and interests occur, including high natural resource values, recreational activities, and commercial interests. It is also an area of overlap between government jurisdictions and foreshore property ownership. Salmon aquaculture has significant environmental aspects and potential environmental impacts, and the industry is subject to a high degree of stakeholder scrutiny, and complex, rapidly evolving legislation and regulations. Environmental management systems (EMS) based on ISO 14001 provide a powerful technology for aquaculture producers wishing to achieve and demonstrate sound environmental management.

The approaches of most organizations to environmental management can generally be grouped into the following three broad categories:

1. Reactive: an organization has not established the necessary structure and resources for sound environmental management. It takes action only in response to an incident or demand. This approach generally fails to prevent incidents or anticipate needs for change. The result is high risk, and potentially high cost. This approach is not recommended.

2. Ad Hoc: an organization implements environmental management measures on an opportunistic basis. This may include, for example, the occasional purchase of equipment, training of staff, or modification of operational procedures; how-

ever, this is not done on an ongoing basis.

While this is better than the reactive approach, there remains an unknown degree of risk of negative environmental impacts. Also, in the absence of an environmental management system, environmental initiatives are likely to have a relatively poor return on investment in terms of environmental performance or other benefits.

3. Systematic: an organization has an EMS to manage its environmental aspects in a logical, comprehensive and systematic basis. The risk of negative environmental impacts is low, and the return on investment for environmental management and related programs is high relative to other approaches to environmental management.

Overview of ISO 14001

The International Organization for Standardization (ISO) has established ISO 14001, the International Standard for environmental management systems⁽²⁾ (EMS). This Standard enables an organization to establish, assess, and demonstrate the effectiveness of a voluntary EMS designed to achieve pollution prevention, regulatory compliance and continual improvement of environmental management. Building upon an organization's existing management system, and with the commitment and involvement of senior management, the systematic process of

plan — implement — check — review enables an organization to set and achieve environmental policy, objectives and targets. ISO 14001 provides the flexibility for an organization to determine the operational boundaries of its EMS, and to decide

whether to register the EMS using an accredited registrar (auditor) or make a self-declaration of conformance to the Standard.

The cost of developing and registering an EMS will vary depending on the size of an organization, its environmental aspects, the scope of its activities, and the degree to which components of the EMS already exist within the organization. The key steps in the development and registration of an EMS are outlined briefly below.

Develop and adopt an environmental policy. The first step is to establish the organization's environmental policy, which is set by top management and must lay the foundation for the EMS. It must include commitments to: (i) comply with relevant legislation; (ii) continual improvement; and, (iii) the prevention of pollution. The policy must be appropriate to the nature, scale and environmental impacts of the organization's activities, products or services; must be documented, implemented, maintained, and communicated to all employees; and must be available to the public. Perhaps most importantly, the policy must provide the framework for setting and reviewing environmental objectives and targets.

Identify significant environmental aspects. These are elements of the organization's activities, products or services that can have a significant environmental impact. These can be identified through a preparatory environmental review. A variety of subjective criteria, including risk or environmental impact assessments, past incidences and legislative requirements can be employed to assess significance. The EMS must include procedures or programs to monitor and/or control significant environmental aspects.

Set environmental objectives and targets. Objectives (overall goals) and targets (detailed performance requirements) must be established, documented and maintained at each relevant function and level of the organization. Generally performance oriented, these should be driven by the environmental policy, relate to the significant environmental aspects, and be embedded within the EMS program. Examples within the industry may include objectives to attain specific criteria for ambient sediment or water quality, or to reduce energy consumption or packaging during transport and processing. Targets may include specific criteria for waste effluent, feed conversion rates (considering life cycle environmental aspects), or development of escape recovery plans or research and development of controls to manage salmon predators.

Establish and implement environmental management programs. This includes the designation of responsibility, authority and processes for measuring

and achieving targeted performance at each relevant function and level of the organization. As with all aspects of the EMS, programs and procedures must be documented and communicated to all relevant parties, and an EMS manual and/or computer program should be developed as the central document for the management system (ISO 14001 specifies document control and record keeping requirements). The programs must specify procedures for monitoring, checking (inspection), and corrective action. Programs must be in place to ensure that the organization keeps abreast of legal requirements, and to ensure that employees have adequate training and awareness of potential environmental impacts. Emergency preparedness measures must be included.

Establish an audit program and conduct management reviews. The system must include an adequate and appropriate EMS audit program. Periodic audits must determine whether the EMS conforms to planned arrangements for environmental management and to the International Standard. Audit results are reported to senior management, who must periodically review the EMS to ensure its continued suitability, adequacy and effectiveness. Management review is also required to determine the need for changes to policy, objectives, or other elements of the EMS to achieve continual improvement.

Make a declaration or prepare for registration. An organization may self declare conformance with the International Standard. However, assurance of conformance with the Standard is best achieved through registration, which should be undertaken after the organization has developed and audited its EMS. The time to prepare for registration for EMS will vary among organizations, generally requiring a minimum 3 to 6 months enlisting appropriate environmental expertise.

Pre-registration audit. Registration of the ISO 14001 EMS is ultimately under the control of the International Accreditation Forum (IAF). In Canada, the IAF is represented by the Standards Council of Canada (SCC), which accredits and oversees the registrar. The audit consists of two stages, each containing the four phases of planning, preparation, performance and reporting. The first stage, the pre-registration audit simulates the final audit and prepares both the registrar and the auditee for the registration audit. This step is required to ensure that the EMS is in place and that the registrar has the appropriate planning and resources to conduct the registration audit.

Registration audit and certification. The registration audit is conducted by the registrar and an audit team consisting of an accredited EMS lead auditor and

other, qualified environmental auditors and technical experts as required. The EMS audit is a systematic and documented verification process of objectively obtaining and evaluating evidence to determine whether the organization's EMS conforms to the International Standard. If there are no major non-conformance issues, and any minor issues have been closed out, the registrar may issue a registration certificate. Following registration, the registrar must return periodically to conduct surveillance audits. If the EMS has not been maintained, or if the organization has not responded adequately to any requests for major corrective action, the registration certificate will be canceled.

"Eco-certification" and Aquaculture

An EMS will help to ensure the public that the firm has a sound environmental management system and open policy in place that is made available to those who are interested or concerned. An effective, independently verified EMS conforming to ISO 14001 may provide a degree of protection or possibly afford a competitive advantage during periods when the public's attention is focused on the industry's environmental aspects.

Salmon aquaculture has been targeted by various environmental organizations actively promoting public boycotts of farmed fish. While such activists often exaggerate the potential environmental impacts of salmon aquaculture and misinform the public, their campaigns make it necessary for aquaculture producers to take action to assure consumers and stakeholders that they are operating responsibly.

In recent years, public pressure to demonstrate sound environmental management in fisheries, forestry and mining has created a demand for products that are associated with "eco-certification". While much of the public pressure and resulting "eco-labeling" are based more upon perceptions than fact, the implications for businesses are very real. Several industries are currently turning to ISO 14001 as a standardized and recognized solution to the complexities of environmental management. For example, recent events in the British Columbia forest industry demonstrate the breadth of legislative changes and market forces that can be brought to bear through the public's perception of environmental issues. In response (and to maintain access to markets and customers), forest companies are moving swiftly to develop and register environmental management systems (ISO 14001), and to adopt and demonstrate sustainable practices. A similar trend to the forest industry is evident in the mining industry. International trade already favors ISO accredited organizations, and firms in Asia, Africa, and South America are expected to adopt the 14000 series to facilitate business with the European

Community, the United States and among themselves.

Overview of EMS Benefits

Any organization whose operations occur within valued natural resource areas and whose activities have the potential for significant environmental impacts will benefit from an efficient and effective EMS. Clearly, such organizations include salmon farmers.

There is a perception that environmental management results in a net cost to an organization and can decrease profitability. In fact, any good management system should improve overall operational efficiency and enable cost savings. This is especially true when environmental objectives and targets are aligned with overall corporate and financial targets. Improving feed conversion, minimizing waste, preventing fuel spills or fish escapes, minimizing interactions with predators, and compliance with legislation are all examples of environmental objectives which are aligned with overall corporate objectives.

An EMS is a powerful tool for managing environmental affairs and reducing potential risk, liabilities and associated costs for any operations with the potential for significant environmental impacts. An EMS builds on the existing corporate structure to centralize and manage all environmental programs, information and legislative requirements into one efficient and effective system. There are presently more than 12,000 organizations that have their environmental management system registered to the ISO 14001 standard. The benefits of a sound, verified EMS include:

- reduced environmental risk and reduced potential to incur costs through fines, stop work orders, and environmental impact mitigation or remediation;
- reduced exposure to liability through sound environmental management and proven due diligence;
- improved relations with regulators, the public, and other stakeholders;
- improved operating efficiencies realized through a continually improving management system, and the alignment of environmental and corporate objectives;
- control and tracking of environmental programs, permits, licenses, incidences;
- enhanced market access.

Notes and References

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Elsevier's Dictionary of Fisheries

PK Eapen. Amsterdam : Elsevier, 1999
ISBN: 0-444-50308-0, 350 pp. US\$ 139.50.

Available in the United States and Canada
from Elsevier Science Inc., P.O. Box 945,
Madison Square Station, New York, NY
10160-0757 (<http://www.elsevier.com>).

Elsevier's Dictionary of Fisheries is part of a series of dictionaries dealing with specialized sci-tech vocabularies (see, for example, the multilingual *Elsevier's Dictionary of Aquaculture*, 1991, and *Elsevier's Dictionary of Fishery, Processing, Fish and Shellfish Names of the World in Five Languages*, 1990). This fisheries dictionary contains a large number of technical terms (7,050 in total), with their meanings expressed in concise and simple language. Definitions are of primarily English terms, and no pronunciation key is given except for particularly tricky words such as *coelacanth*. According to the publisher, the dictionary covers everything from marine biology to oceanography, aquaculture and fishing. Only the most frequently used terms or the ones thought to be helpful to different kinds of users have been chosen for inclusion.

A dictionary's success as a useful reference tool depends upon factors such as its accuracy, scope, relevancy, currency, authority and presentation. Unfortunately, this dictionary fails in several key regards.

Even a superficial examination of the text reveals a careless lack of editing. For example, alphabetical order is crucial to finding information in a dictionary. However, in this dictionary, we encounter entries such as these:

On page 85 are consecutive entries for *Danish seine*, *Danish seine trawl* and *Danish trawl*. On the next page after six other entries (dan leno assembly, danleno spreader, dan leno stick, dan leno triangle, dan line, dan wire) is the entry for *Danish seining*.

On page 3 there is an entry for *Acid pickle*, also see *Marinate*. However, when one turns to page 191, there is no entry for *Marinate*, although there is one for *Marinade*.

The sloppy editing even extends to including spell-

ing errors (on page 165 there is an entry for *International ice patrol*, on page 159 an entry for *ICBN*, *International Congress of Botanical Nomenclature*). This is really inexcusable in a dictionary.

The professional affiliations or credentials of PK Eapen to compile this reference text are not disclosed. A search of ASFA, the international aquatic and fisheries science database, does not turn up any evidence of Eapen's publishing in the fisheries field. In contrast, the soon to be published Wiley's *Encyclopedia of Aquaculture* is edited by the well-known RR Stickney and contains contributions from over 100 internationally-renowned experts.

One of the great dangers in tackling a huge subject such as this is that the same terms and phrases may have different meanings in different parts of the world. Eapen's work gets into trouble when defining common names for species that have alternate meanings elsewhere. Examples are *rock crabs*, *black fish*, *lady fish*, etc.

On a positive note, the dictionary is a useful source for its explanation of many common abbreviations and acronyms, such as NAVSAT (Navigation satellite) and NMFS (National Marine Fisheries Service). Canadian content is scarce, however, with common Canadian fishery organizations such as DFO, FRCC and CSAS not included.

As a tool for quick reference, this dictionary fills a need, especially for students in fisheries and aquaculture programs; it will also be of limited use to professionals in these areas for basic reference and terminology questions. However, with a hefty price tag of approximately \$200 Canadian, the book will have a very limited market of libraries and institutions.

I hope the publisher will issue a corrected edition of this work, one that could be recommended with more confidence and one that would stand as a valuable and much needed addition to the professional literature.

Marilynn Rudi, Librarian
Department of Fisheries and Oceans
Biological Station, St. Andrews, NB

Association News

Changes to Bylaws

The AAC was incorporated in 1984 and since then has operated as a non-profit, registered charitable organization. The Corporation's objectives and articles of incorporation (Letters Patent) may be viewed at the AAC web site: <http://www.mi.mun.ca/mi/aac/>.

The Bylaws and Policies of the Association have been circulated to members on several occasions previously, most recently in the *Bulletin* in 1995. They are also maintained on the web page. The AAC has made only minor changes to the Bylaws in the past 17 years, and these have been communicated to the membership at the annual general meetings. However, it was felt that the time had come to undertake an extensive review of the existing Bylaws and Policies and this was performed in 1999. The Board of Directors has the authority to change the Bylaws and these changes have been filed with Industry Canada, in accordance with the Canada Corporations Act. Perhaps not surprisingly, you will note very little change in the Bylaws since 1984. The changes to the Bylaws noted below are minor changes in operating procedures of the Association to better serve the membership and more accurately reflect present day realities of this non-profit organization. Many of the changes made below were made 5 to 10 years ago or more. Minor changes in language were also made, including correction of over 40 typographical errors, in order to simplify the bylaws and make them less ambiguous. Those changes are not included in the list below. The objectives of the AAC have remained exactly the same.

We hope you find this information useful, and we look forward to any comments you may have on this or any matters pertaining to the AAC.

— Cyr Couturier
Chairman, Rules Committee

Changes to Bylaws since 1984

Article Two — Membership and dues

Institutional and *Affiliated* membership categories removed. *Corporate*, *Sustaining*, *Senior* and *Honorary* membership categories added.

Article Three — Meetings, Voting, Elections

Section 3.03 changed so that all membership categories are eligible to vote.

Section 3.04 changed so that the directors are elected by the membership but that officers are elected by the Board of Directors.

Article Five — Duties of Officers

Section 5.03 duties of the vice-president include serving as chair of the awards committee.

Article Six — Board of Directors

Section 6.03 amended so that any director not a member in good standing of the Association forfeits directorship.

Section 6.08 amended so that the board of directors may remove directors from office by majority vote.

Section 6.09 added stating that the operating procedures of the board of directors follow Robert's Rules of Order.

Section 6.10 added to enable the board of directors to conduct business by teleconference, videoconference or via fax.

Section 6.11 added to deal with vacancies in director's positions.

Section 6.12 added which provides the board of directors the authority to prescribe operating rules and policies, which are ratified by the membership at the annual general meeting.

Article Seven — Committees

Three committees established as standing committees: Awards, Publications and Student Affairs.

Article Nine — Association with the World Aquaculture Society

Created two sections, one dealing with the purpose of the association with WAS and the other dealing with the terms of association with WAS.

Modifications apportées aux statuts

L'Association aquacole du Canada a récemment révisé ses règlements administratifs. Incorporée en 1984, l'AAC est un organisme de bienfaisance reconnu à but non lucratif. Vous pouvez visionner ses Lettres patentes (ou statuts constitutifs), ses statuts (ou règlements) et ses politiques à l'adresse suivante : <http://www.mi.mun.ca/mi/aac/>.

Les statuts et les politiques de l'association ont été distribués plusieurs fois aux membres, la dernière diffusion remontant au Bulletin 1995 de l'AAC, et ils sont affichés en permanence à notre page Web. En 17 ans, seuls des changements mineurs y ont été apportés et ils ont été communiqués lors de l'assemblée générale annuelle appropriée. Une vérification approfondie s'imposait et nous l'avons effectuée en 1999. Le conseil d'administration est investi de l'autorité nécessaire pour modifier les règlements et les modifications ont été déposées auprès d'Industrie Canada conformément à la Loi sur les corporations canadiennes. Vous remarquerez sans doute, et probablement sans étonnement, que les statuts demeurent très semblables à ceux qui ont été adoptés en 1984. Les réformes mineures énumérées ci-dessous concernent le fonctionnement de l'association et visent de meilleurs services ainsi qu'une modernisation de nos procédures. Plusieurs d'entre elles ont vu le jour il y a cinq ou 10 ans (ou même avant). Nous avons simplifié le langage pour éliminer les ambiguïtés et corrigé plus de 40 fautes typographiques. Ces dernières rectifications ne figurent toutefois pas dans la liste ci-dessous. Les objets de l'AAC sont en tous points identiques à ceux de la demande de constitution.

Nous espérons que cette information vous sera utile. N'hésitez pas à nous envoyer vos commentaires sur tout ce qui touche l'AAC.

— Cyr Couturier
Président du comité des règlements

Modifications apportées aux statuts depuis 1984

Article 2 — Adhésion et cotisation

Les catégories de membres *Institutional* et *Affiliated* disparaissent pour être remplacées par les nouvelles *Corporate*, *Sustaining*, *Senior* et *Honourary*.

Article 3 — Assemblées, votes et élections

Dans la section 3.03, toutes les catégories de membres ont maintenant le droit de vote.

Dans la section 3.04, les administrateurs (*Directors*) sont élus par les membres et les dirigeants (*Officers*) par le conseil d'administration.

Article 5 — Fonctions des dirigeants

Section 5.03 : le vice-président doit assumer la présidence du Comité des distinctions honorifiques.

Article 6 — Conseil d'administration

Dans la section 6.03, tout administrateur qui n'est pas membre en règle de l'association devient déchu de son poste.

Dans la section 6.08, les administrateurs peuvent destituer un des leurs par vote majoritaire.

Nous ajoutons la section 6.09 selon laquelle les règles du *Robert's Rules of Order* sont adoptées pour le mode de fonctionnement du conseil d'administration.

Dans la nouvelle section 6.10, le conseil est autorisé à communiquer par télécopieur et à organiser des audioconférences ou des vidéoconférences pour régler les affaires de la société.

La nouvelle section 6.11 porte sur la procédure à suivre dans les cas de vacances au sein de l'administration.

Grâce à la nouvelle section 6.12, le conseil est habilité à établir les règles de fonctionnement et les politiques de l'association qui sont ensuite ratifiées par les membres à l'assemblée générale annuelle.

Article 7 — Comités

Trois comités permanents sont formés : Distinctions honorifiques, Publications et Affaires étudiantes.

Article 9 — Relations avec la World Aquaculture Society

Deux sections sont créées, l'une concernant les objectifs, l'autre les modalités d'une association avec cette société.



Calendar

conferences, workshops, courses and trade shows

• **3rd International Conference on Recirculating Aquaculture**, 20-23 July 2000, Roanoke, Virginia. Information: <http://www.conted.vt.edu/recirc/aqua.htm>.

• **International Congress on the Biology of Fish**, 23 - 26 July 2000, Aberdeen, Scotland. Information on the meeting is available at the website www.fishbiologycongress.org. Information: Don MacKinlay (tel 604 666-3520, fax 604 666-6894, mackinlay@pac.dfo-mpo.gc.ca).

• **BioFuture 2000 Conference and Exhibition**, 11-13 September 2000, Telus Convention Centre, Calgary. Information: www.gwexpo.com/biofuture.

• **Coastal Zone Canada 2000**, 17 - 22 September 2000, Saint John, NB. Conference will focus on aboriginal practices, community-based actions, coastal health and oceans governance. Information: Coastal Zone Canada 2000 Secretariat (tel 506 453-2253, fax 506 453-5210, czc2000@gov.nb.ca, www.gov.nb.ca/dfa/czc-zcc2000.htm).

• **Salmon Summit 2000**, 21-22 September 2000, Copenhagen, Denmark. Information: Martin Gill tel +45 35467193, fax +45 35467181, e-mail martin.gill@eastfish.org.

• **Pacific Exchange**, 26-27 October 2000, Campbell River, BC. Information: Master Promotions tel 888 454-7469, fax 506 668-0750, e-mail show@nbnet.nb.ca.

• **Third World Fisheries Congress**, 31 October - 3 November 2000, Beijing, P.R. China. Secretariat: China Society of Fisheries, Bldg 22, Maizidian Street, Chadyang District 100026, Beijing, P.R. China (tel 86 10 64194233, fax 86 10 64194231, csfish@agri.gov.cn).

• **East Coast Live: The Business of Marketing Live Aquatic Products 2000**, 1-4 November 2000, Annapolis, Maryland. Information: Don Webster, Maryland Cooperative Extension, Wye Research and Extension Center (tel 410 827-8056, fax 410 827-9039, e-mail dw16@umail.umd.edu).

Report from the Student Affairs Committee

Aquaculture Canada '99 in Victoria was attended by a large number of students. Thanks are extended to the students who assisted with the organization of the conference.

The annual Heritage Aquaculture Student Reception was held at the Victoria Conference Centre. Students had the opportunity to chat with Mr. Bill Robertson of Heritage Aquaculture and sample numerous beverages and aquaculture products. As well, the students provided input to the Student Affairs Committee which will influence Board decisions on future student activities.

The annual Student BBQ was held at Legends Pub in the Strathcona Hotel. Proceeds from this event, as well as those from a raffle held at the BBQ, are being used to provide travel bursaries to students wishing to attend the AAC conference in Moncton.

As mentioned in the meeting report (page 7), several travel bursaries were awarded in 1999, and as always, it is hoped that even more support will be available to students in the future. The efforts of Dr. John Morgan, Paige Ackerman, and Virginia Eccleston are appreciated in organizing the travel

awards, best-paper prizes, and conference sessions.

There were 12 student presentations, all of which were of high quality. For the first time, the student presentations were grouped into a special student session. Opinions varied as to whether student papers should be presented together in one session or whether they should be presented in the contributed paper sessions devoted to a particular topic. Congratulations are extended to Melissa Mooney (MUN), winner of the best student oral presentation, and Paige Ackerman (UBC), winner of the best student poster presentation.

The city of Victoria offered venues for relaxation outside of the conference, which I am sure was appreciated by all students.

A student affairs section has recently been added to the AAC web page (www.mi.mun.ca/mi/aac/sa.html). It contains announcements and discussions about students activities.

The 2000 meeting in Moncton, NB, promises to be yet another well attended and exciting conference for the student members of AAC.

*Chris Hendry
Chairman, Student Affairs Committee*

