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Cover: Original artwork by Ken Albrecht commissioned by the AAC and used as the Aquaculture Canada '98 logo.

Calendar

•**3rd Annual Conference on Open Ocean Aquaculture**, 10-15 May 1998, Corpus Christi, Texas, USA. Possible topics include: Liability impediments to converting offshore platforms to aquaculture sites; Legal, regulatory, and policy issues that might include development of aquaculture zones; Compatibility of producing platforms with ongoing aquaculture operations and re-design and engineering of platforms to accommodate multipurpose functions; Education of the public, environmental groups and the regulatory agencies on offshore aquaculture operations; Status and prioritization of candidate species; Current biological information about species; and Development of technology transfer programs and opportunities. Information: Dr. R. R. Stickney, Conference Chair, Texas Sea Grant College Program, 1716 Briarcrest, Suite 702, Bryan, Texas USA (fax 409 845-7525; e-mail stickney@unix.tamu. edu).

•**Aquaculture Canada '98,** 31 May–3 June 1998, 15th Annual meeting of the Aquaculture Association of Canada. Program of invited speakers, technical sessions, industry workshops and trade show. Delta Hotel, St. John's, Newfoundland. Theme: Research — An Investment in the Future. Special Sessions: seaweed aquaculture, molecular biology, aquaculture engineering, live feeds, broodstock, and production capacity. Conference information: Dr. Jay Parsons, tel 709 778-0307, fax 709 778-0535; e-mail jparsons@gill.ifmt. nf.ca or visit www.ifmt.nf.mi/aac/. Trade show information: Lynette Carey, tel 709 754-2854.

•**Atlantic Aquaculture Exposition, Conference and Fair,** 17 – 21 June 1998, St. Andrews, NB Canada. Theme: Reducing Production Costs with New Technologies. Conference information: Charlene White, tel 506 529-4578, fax 506 529-4284, e-mail aquafair@nbnet.nb.ca. Exposition information: Master Promotions, tel 506 658-0019, fax 506 658-0750.

•**Cornell 4th Annual Aquaculture Water Reuse Systems Short Course**, 23 – 27 June 1998, Cornell University. Cost $650. Course intended to give thorough coverage of the design, operation and management of water reuse systems for fin fish. Taught by members of the Cornell Aquaculture Program and outside experts. Information: Dr. Michael Timmons, Ag & Biological Engineering, Riley Robb Hall, Cornell University, Ithaca, NY 14853 (tel 607 255-2801, fax 607 255-4080, e-mail mbt3@cornell.edu).

•**2nd International Conference on Recirculating Aquaculture Systems,** 16–19 July 1998, Roanoke, Virginia. Symposiums include: Isolation and Quarantine, Small Scale Systems, Automation, International Recirculating Aquaculture Systems, Business Management and Economics, Waste Management, Fish Health, and Denitrification. Technical sessions by the Aquacultural Engineering Society and the Fresh Water Institute will also be featured. There will be a special session "Hands on computer applications". Information: Dr. George Libey, Commercial Fish and Shellfish Technology Program, Virginia Tech, Blacksburg, Virgina 24061-0418 (tel 540 231-6805; fax 540 231-9293, e-mail CFAST@vt.edu).

•**8th International Symposium on Microbial Ecology,** 9-14 August 1998, Halifax, Canada. Conference will highlight the central role of microbes in the regulation of biosystems. Registration information: ISME-8, Ardenne International Inc., Suite 444, World Trade Centre, 1800 Argyle Street, Halifax, N.S. Canada B3J 3N8 (tel 902 494-8000, fax 902 423-2143, e-mail ardenne@fox.nstn.ca)

•**Coastal Zone Canada '98 (CZC '98),** 30 August – 3 September 1998, Victoria, B.C. Theme: Coastal Communities in the 21st Century, Sharing our Experience, Building our Knowledge. Information: http://www.ios.bc.ca/ios/czc98/ (e-mail czc98@ios.bc.ca, tel 250 721-8746, fax 250 721-8774).

•**3rd International Symposium on Aquatic Animal Health**, 30 August – 3 September 1998, Renaissance Harborplace Hotel, Baltimore, Maryland. Four days of scientific session, including plenary lectures and contributed oral and poster presentations. Symposium office: Division of Comparative Medicine, Johns Hopkins University School of Medicine, 720 Rutland Avenue, Baltimore, Maryland 21205 (tel 410 955-3273; fax 410 550-5068).

Contents

Proceedings of the Contributed Papers, Aquaculture Canada '97

S. L. Waddy and M. Fréchette, editors

Aquaculture Canada '97

Salmonid Culture — Physiology and Genetics

Salmonid Culture — Nutrition, Health, and Disease

Tilapia Culture

AAC Special Publication No. 3

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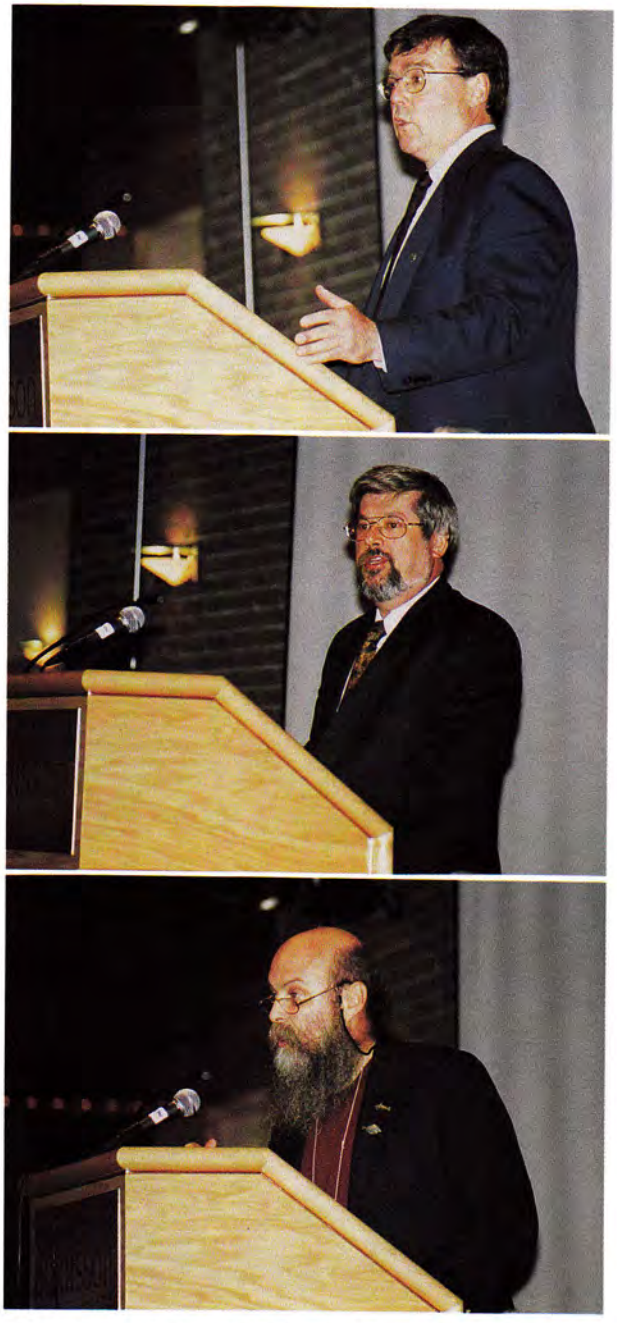
Meeting Report

The 14th Annual Meeting of the Aquaculture Association of Canada, "Aquaculture Canada '97" was held in the beautiful walled city of Québec. The venue was the Radisson Hôtel des Gouverveurs, perched on the edge of "vieux Québec" and a location well suited to ventures into the old city or the Plains of Arbraham during the evening or between breaks in the program.

The meeting was one of the more ambitious ones the association has organized, both in terms of the technical program, with its large number of plenary sessions and over 40 invited speakers, and the very full social program that culminated in a truly spectacular banquet/ theatre.

There were over 300 registrants and a total of 110 papers were presented during the 3-day meeting. Included were 42 invited overviews, 44 contributed oral papers and 24 contributed posters. Invited speakers came from throughout Canada as well as from Italy, Chile, France, the United Kingdom and the United States.

The opening session featured Mr. Louis Tousignant, the Senior Assistant Deputy Minister in the Department of Fisheries and Oceans (DFO), Mr. G. Julien, Minis-



Opening ceremonies at Aquaculture Canada '98. Top, Mr. L. Tousignant, Senior Assistant Deputy Minister, Fisheries and Oceans Canada; centre, Mr. G. Julien, Minister, Ministere Agriculture, des Pêcheries et de l'Alimentation du Québec; bottom, Dr. Joseph Brown, President of the Aquaculture Association of Canada

ter, Ministère Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ), Ms. Marlie McNeil, Vice-President of the Canadian Aquaculture Industry Alliance (CAIA) and myself as the President of the Aquaculture Association of Canada. Mr. Tousignant spoke on the federal government's role in aquaculture and on the mandate of the new Commissioner for Aquaculture Development. Mr. Julien talked about the important role of aquaculture in the economy of the country and of its increasing importance in Québec. Ms McNeil, speaking for the aquaculture industry, emphasised how important the new Commissioner for Aquaculture Development was to the industry, especially the mandate to lead regulatory reform.

Opening reception on-board the cruise ship Fleuve St-Laurent.

In addition to the technical and scientific program there was a small trade show with 24 exhibitors, which added a great deal to the meeting. Yves Bastien was the Chair of the Organizing Committee and he and his committee deserve a great deal of thanks for the excellent meeting they organized.

The program was coordinated by Yves Bastien and Bruno Myrand, ably assisted by Pierre Dubé, Tom Sephton and Andrée Leblanc. The special sessions were organized by Bruno Myrand, Pierre Dubé, Éric Gilbert, Cynthia McKenzie, Sharon Ford, Roberta Lavigne, Maureen McInerney-Northcott, André Mallet, Yves Boulanger, J. de la Noüe and myself. All of the above contributed to an excellent technical program with very informative special sessions.

The social program was organized by Yves Bastien, Jean-Yves Savaria and Diane Tremblay. I think everyone who attended the meeting will agree that the social events were outstanding, and speaking as one who "lost

his head" at the medieval dinner theatre, I congratulate them on an excellent job.

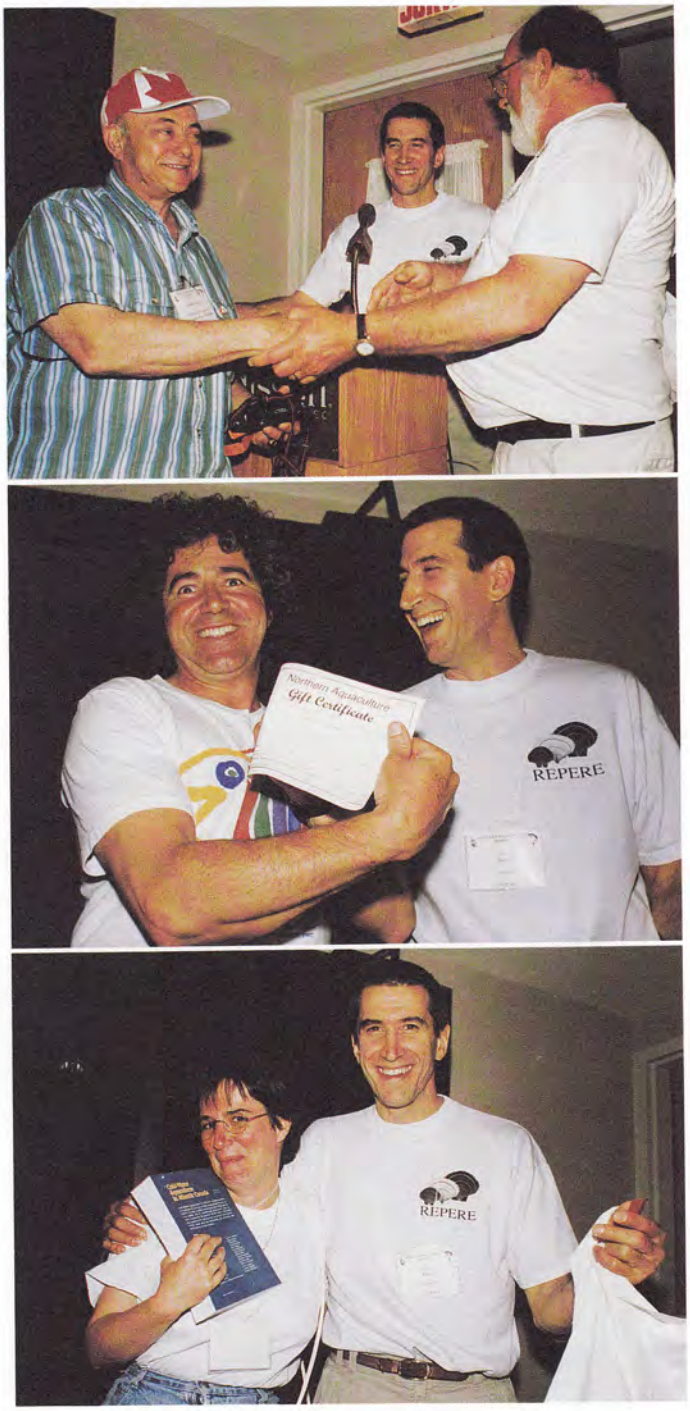
The trade show was organized by Michael Larrivée. The booths were located in the common areas between the meeting rooms and this arrangement seemed to worked well; there as much milling about the booths during refreshment breaks and between talks.

Registration was handled by Maureen McInerney-Northcott. André Dubois organized the audio visual services, and the simultaneous translations were coordinated by Yves Bastien and Céline Major. Even though the simultaneous translation added to the complexity of organizing the meeting, I think it was an excellent feature and made the talks more accessable to all those in attendance. The Department of Fisheries and Oceans deserves special thanks as they provided the funding for this service.

Student activities and student presentation awards were organized by Jean-Yves Savaria and Maureen McInerney-Northcott. Students are very important to the Aquaculture Association of Canada and to the future of aquaculture in Canada and it was gratifying to see so many students involved in t-shirt sales and planning of the the BBQ. Proceeds from these activities are used to assist students with the costs incurred in travelling to AAC meetings to present their papers.

For the second year, Connors Brothers Ltd. sponsored a Student Breakfast which was well attended by stu-

Jay Parsons, President-Elect, presenting the best student paper awards to Janice Lawrence (top) and Robyn O'Keefe (bottom). The $250 prizes were donated by the University of Guelph — Aquaculture Centre.



Yves Bastien (bottom right) and John Gracey (top right) presenting prizes to winners in the student raffle: Thomas George (top) won an airline ticket to the 1998 meeting, George Cliche (centre) won a subsrcription to *Northern Aquaculture* and Celine Audet (bottom) won a copy of *Coldwater Aquaculture in Atlantic Canada.*

dents and provided an opportunity for them to meet industry representatives and others involved in aquaculture. AAC appreciates the support Connors has given to student activities and student awards for the past several years.

The site visits were well subscribed, with the most popular being the visit to La Pisciculture des Alléghanys, a leading aquaculture production facilities in Québec that specializes in the production of rainbow trout, brook trout and Arctic charr. The tours were organized by Grant Vandenberg.

Linda Anctil handled media relations. Other members of the organizing committee not named above were Sylvain Vigneau, Jean-Pierre Réville, Lucien Maheux and Michel Bombardier. This group of people did an outstanding job and I offer a heart felt thank-you for your volunteer efforts and the great meeting you helped organize!

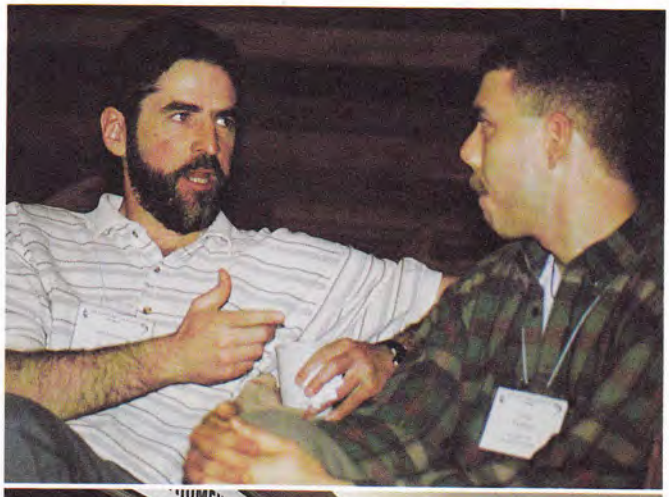
This year Janice Lawrence of Dalhousie University in Halifax won the presentation award for the best student paper for her talk "The elusive source of diarrhetic shellfish poisoning in Nova Scotia." Honourable mentions went to Craig Purchase of Memorial University, Lincoln Simons of the University of Guelph and Rejean Tremblay of Université Laval. Robyn O'Keefe of the University of New Brunswick In Fredericton won the award for best student poster for her presentation titled "Comparative growth and food consumption of diploid and triploid

brook trout *(Salvelinus fontinalis).''* Congratulations to these students and to all the students who actively participated by presenting papers or posters. Their contributions are important to the AAC and to the aquaculture industry.

I would also like to thank the sponsors of the student awards and travel burseries. Travel bursaries are awarded each year to help students defray the costs of attending the meeting. Sponsors were the University of Guelph – Aquaculture Centre, Heritage Salmon, Moore-Clarke Co. (Canada) Inc., Contact Canada, and A-F Protein. Celine Audet, Laura Brown, Elaine Cooke, Vanya Ewart, Marcel Fréchette, Stewart Johnson, Renè Lavoie, Jean-Marie Sévigny and John van de Meer judged the student papers and posters. The $250 best paper awards were donated by the University of Guelph – Aquaculture Centre. Prizes for the Honorable Mention awards were donated by Heritage Salmon, Contact Canada and Andrew Boghen.

During the BBQ a raffle a held by the students to raise money for the Student Endowment Fund. The winners were:
Thomas George won a ticket from Canadian Airlines International to next year's meeting in St. John's, Newfound; *Georges Cliche* won a 1-year subscription to *Northern Aquaculture*; Cyr Couturier won a Cape Spear t-shirt, and and Celine Audet won a a copy of *Cold-Water Aquaculture in Atlantic Canada.*

Winners in the t-shirt con-



Top: Bill Robertson (left) and Craig Purchase at the Student Breakfast sponsored by Connors Bros. Ltd. Centre: Trade Show, Bottom: Poster Session.

Medieval Dinner Theatre at Aquaculture Canada '97

test were John Bonardelli for the oldest t-shirt (from the 1989 AAC meeting in Newfoundland), Andrew Boghen for the nicest t-shirt, and Clayton Brenton for the shabbiest t-shirt.

A meeting of his sort is impossible to organize without sponsors, both those who provide in-kind services and those who provide financial support. Aquaculture Canada '97 was fortunate to have the following support:
Department of Fisheries & Oceans;
Ministère de l'Agriculture, des Pêcheries et de l'Allimentation du Québec;
Centre Specialise des Peches;
Canadian Aquaculture Industry Alliance;
Société de Recherche et de Dévelopement en Agriculture Continentale;
Canadian Centre for Fisheries Innovation;
Office du tourisme et des congres de la Communaute ubaine de Québec;
Universite Laval;
Pisciculture des Alleghanys;
Station piscicole de Tadoussac;
Association des Aquiculteurs du Québec;
Marinard Aquaculture Ltee.;
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Natural Sciences and Engineering Research Council;
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Hydro-Quebec;
Société de développement de l'industrie maricole;
Atlantic Provinces Council on the Sciences;
Northern Aquaculture;
Contact Canada;
Aquaculture Centre, University of Guelph;
Heritage Salmon;

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Restaurant Le Lapin Sauté;
Andrew Boghen; and
Claude Levasseur.
Donors of seafood were:
Coopérative Aquicole du Québec, Viamer Inc.,
Les moules de culture des Îles Inc.,
Pêcheries Gros-Cap Inc.,
and Poséidon Inc.
Thanks to all of the above for their gracious support.

I felt fortunate to have been part of this meeting as I have always thought that Québec City is a special place. The AAC annual meeting is also a "special" meeting for me because, as a scientist who works on fish, I often attend specialized meetings. The AAC meeting is always diverse, as the people who attend represent all aspects of aquaculture, from university researchers, to students, to representatives from various levels of government, regional assocciations, funding agencies, suppliers and manufactors, through to the farmers. It is this diversity which is interesting and makes aquaculture an exciting, challenging, and sometimes frustrating industry to be associated with.

I hope to see you at the 1998 meeting in St. John's!

— Joe Brown, President

"The King", Yves Bastien and Jay Parsons (left to right) at the Aquaculture Canada '97 Banquet [John Gracey photo, reprinted from Northern Aquaculture with permission].

Aquaculture Canada '97

We gratefully acknowledge the following supporters and sponsors
Nous remercions sincèrement tous nos supporteurs et commanditaires

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Canadian Center for Fisheries Innovation (CCFI)

Association des Aquiculteurs du Québec (AAQ)

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Association des Aquiculteurs du Québec (AAQ)

University of Guelph Aquaculture Centre

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Andrew Boghen

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Growth, maturation and spawning times in crosses of three strains of rainbow trout

L. R. McKay and I. McMillan [1]

Three diverse strains of rainbow trout were crossed as part of the development of a fast-growing, spring-spawning strain of rainbow trout, *Oncorhynchus mykiss* (Walbaum). In the crosses, growth was inherited in an additive manner with little evidence of heterosis or reciprocal effects. Non-significant deviations from additivity were present for percent precocial males, due to biological interactions with growth. Spawning dates of crossbred females were generally intermediate between parental strains. Further evaluation of the crossbred progeny will allow us to determine the optimal long-term breeding strategy.

Introduction

As part of a long-term breeding strategy to produce fast-growing, spring-spawning rainbow trout, three diverse strains of rainbow trout were crossed to determine the extent of heterosis and reciprocal effects. In addition, these initial crosses will form the basis of a synthetic population. The resulting synthetic population may be developed further into a single synthetic population combining all desired traits, or into a dam line combining adequate growth with a desired spawning time. Such a dam line would then be crossed with a fast-growing male line.

Methods

Diallel crosses of three strains of rainbow trout were made in two spawning seasons (February 10 to April 27, 1994 and November 30, 1995 to March 27, 1996). The parental strains were previously evaluated [2,3] for several traits.

Strain A was a synthetic strain, combining commercial, fall/winter strains and non-commercial, spring-spawning strains. This strain grew quickly, with low to moderate proportions of males maturing at 2 years and a late fall/early winter spawning time.

Strain C originated from the Ontario Ministry of Natural Resources (OMNR). It was a non-commercial, naturalized strain, which had been subjected to two generations of mild selection for size on a commercial fish farm. This strain exhibited poor growth, very low rates of early male maturation and a spring spawning time.

Strain D was imported from a hatchery in Washington State. Growth and spawning times for this strain were intermediate compared with Strain A or C, but a high proportion of males matured early (i.e. at 2 years of age).

On each of 5 (in 1994) and 8 (in 1996) fertilization dates, up to 5 males from each parental strain were crossed with up to 5 females from 1 or 2 of the strains. It was not possible to cross all female strains on any given fertilization date because spawning times for the 3 strains did not overlap sufficiently.

Eggs from each sire strain x dam strain combination were incubated separately. After hatching, each fertilization date x sire strain x dam strain combination was housed separately in 0.7-m square fibreglass tanks until the fish were large enough to fin-clip. After marking, groups of fish from the same spawn date were housed together in 1-m square fibreglass tanks.

Each group of fish was weighed periodically so that growth curves could be calculated for each spawn date x sire strain x dam strain combination. Growth rates to 37 weeks after first feeding were calculated by regressing the cube root of weight on age.[4]

Rates of early maturation were determined for the 1994 year class from two sources. In September, 1995, when the fish were in their second

year, excess fish were randomly culled and examined internally to determine their stage of maturation. The remaining fish were examined externally for secondary sexual characteristics and/or the presence of milt in April, 1996 (at 2 years of age). In calculating maturation rates for males, equal sex ratios were assumed.

Spawning dates for 3-year-old females from the 1994 year class were determined from weekly checks of fish from October, 1996 to May, 1997. Spawn dates were converted to integers representing the week number in which the female spawned starting with the week of November 26, 1996 as Week 1.

Growth data were analyzed with analyses of variance using a model that included effects due to year class, fertilization date within year class, strain of sire and strain of dam. Contrasts of individual strain of sire x strain of dam combinations were used to test for the presence of heterosis and reciprocal effects. Early male maturation data were transformed to logits and then analyzed as for the growth data, except that year-class effects were not included because data was available only for the 1994 year class. Similarly, spawn week was analyzed with a model that included fertilization date, strain of sire, strain of dam and the interaction of sire strain and dam strain.

Results

Analysis of variance on growth rate revealed significance for all of the effects in the model, except for the interaction of sire strain and dam strain (Table 1). In contrast, only the dam strain effect was significant for the percent of 2 year males, while only the sire strain was significant for the week of spawning.

Mean growth rates, maturation rates and spawning dates are given in Table 2. Pure strains (on the diagonal) ranked similarly to the parental strains for these traits. Crosses between any two strains were always intermediate for growth, and usually intermediate for spawning date (except for the C female crossed with the D male). Maturation rates of crosses were often intermediate between the rates of the pure strains, but crosses of Strain A and C, and the cross of D females with A males were not. Nevertheless, these deviations were not significant, since the heterosis contrasts were non-significant for all strain crosses for all traits. None of the reciprocal effects for any trait or strain

Table 1. Analyses of variance for the three traits.

Source/Contrast	Growth to 37 Weeks		% Early Males		Spawn Week	
	df	Mean Square [a]	df	Mean Square	df	Mean Square
Year class	1	523 *	—	—	—	—
Fertilization date	11	256 *	4	0.403	4	489
Sire strain	2	4681 ****	2	2.202	2	4946****
Dam strain	2	1094 ***	2	6.185*	2	483
Sire strain x Dam strain	4	80	4	0.649	4	426
heterosis (A/C)	1	309	1	2.529	1	526
heterosis (A/D)	1	56	1	0.054	1	72
heterosis (C/D)	1	15	1	0.341	1	565
reciprocal (AC/CA)	1	1	1	0.065	1	841
reciprocal (AD/DA)	1	37	1	0.044	1	93
reciprocal (CD/DC)	1	14	1	1.477	1	932
Error	29	99	9	1.023	56	281

[a] $x10^3$

[b] *, **, ***, **** indicate significant F-test with $P < 0.05, 0.01, 0.001, 0.0001$, respectively

Table 2. Least squares means for growth rates ($g^{1/3}$/day x 10^4) to 37 weeks post first feeding (upper), percent males mature at 2 years (middle) and spawn week from November 26, 1996 (bottom), for the crosses of the three parental strains, and the overall strain of sire and strain of dam.

Strain of Dam	Strain of Sire			Overall
	A	C	D	
A	140	113	122	125
	33.3	5.0	35.7	24.7
	5.0	12.0	7.3	8.1
C	114	70	84	89
	6.6	9.1	16.1	10.6
	9.4	13.0	14.0	12.1
D	117	82	93	97
	43.0	23.9	39.3	35.4
	7.4	11.2	10.5	9.7
Overall	124	88	100	104
	27.6	12.7	30.4	23.6
	7.3	12.1	10.6	10.0

combination were significant, although there was some asymmetry for some combinations.

Conclusions

Since growth rates combined in a mostly additive way (i.e. without heterosis), terminal crossing systems (which take advantage of heterosis) would not be favoured over synthetic line formation, at least with respect to this trait. Spawning time also combined in a mostly additive way. However, when both traits are considered together, a terminal crossing system, using spring-spawning females and fast-growing males would give better overall performance than the reciprocal cross. The progeny of the first cross will already have the desired spawning time of the female parent, although with poorer growth than the male parent. In practice, some synthetic line development might be desirable to improve the growth rate of the dam line, while continually selecting for desired spawning time.

The percent of males mature at 2 years in the crosses may depend on the interaction of an intermediate growth rate with an intermediate size or growth threshold for maturation. If the results of the 1996 year class follow the same pattern as those for the 1994 year class, then the cross of Strains A and C will result in a lower maturation rate than even the slow-maturing Strain C parents. Possibly these crossbreeds inherit a slower growth rate (and hence, lower maturation rate) than their Strain A parent, but a higher size threshold for maturation than their Strain C parent. The opposite effect may be occurring in the cross of Strain D females with Strain A males.

This project was funded by the Ontario Ministry of Agriculture, Food and Rural Affairs through the University of Guelph's Aquaculture Program 42. We would also like to acknowledge Cheryl Quinton and the staff of the Alma Aquaculture Research Centre.

Notes and References

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Comparative growth and food consumption of diploid and triploid brook trout (*Salvelinus fontinalis*)

R. A. O'Keefe and T. J. Benfey[1]

The production of triploids is the only economically-feasible method available for producing sterile salmonids for aquaculture. Triploids differ from diploids in cell size and number, consequently triploids may be less efficient than diploids when competing for and processing food. Comparisons of food intake and growth were made of 144 individually PIT-tagged fish held in separate and mixed ploidy groups. The fish were measured for length and weight and fed a special diet containing radio-opaque glass beads once every three weeks for a 39-week period. After eating the special diet each fish was radiographed and food intake was determined by counting the number of glass beads on the X-ray plate. Preliminary results suggest that there is no difference in growth or food consumption between diploids and triploids either cultured separately or in mixed ploidy groups.

Introduction

In order for the commercial production of triploid fish to be economically viable, triploids must be shown to have growth and food consumption rates as good as, or better than, those of diploids. Triploids have three sets of chromosomes in their somatic cells, hence they have fewer but larger cells than diploids. This difference may have an effect on the growth and degree of dominant behaviour observed between the ploidies.[2]

There is substantial evidence that dominant fish acquire the larger portion of feed when food is limited and delivered from a point source.[3,4] Growth measurements, coupled with the ability to calculate individual meal size by radiography of fish held in large groups, allows the study of inter-individual variation in food acquisition and growth of fish. This permits the indirect assessment of social structure with respect to the establishment of feeding hierarchies within groups.[5] The aim of this experiment was to determine if in fact ploidy has an effect on the dominance level and growth of diploid and triploid fish fed a limited diet and held in mixed and separate ploidy groups.

Materials and Methods

Seventy-two brook trout (*Salvelinus fontinalis*) of each ploidy were implanted with passively integrated transponder (PIT) tags. They were then subdivided into duplicate groups of 100% diploid, 100% triploid, and mixed tanks of 50% of each ploidy. The stocking density for each tank was approximately 8.3 kg/m^3 at the beginning and 44.8 kg/m^3 at the end of the experiment. The fish were fed half the feed manufacturer's (Corey Feed Limited, Fredericton N.B.) recommended ration once daily by hand from a point source. Every three weeks the fish were fed a diet labelled with lead glass beads (Jencons Limited) and two hours later the fish were anesthetized in 1% tert-amyl alcohol and radiographed.[3,6] A known amount of food was also radiographed and used to prepare a standard curve so that the amount of food that was in the gut of the fish could be determined by counting the number of glass beads on the

radiograph. The following day the fish were again anesthetized and their weight and length measured. This procedure was repeated 14 times, from September 1995 to June 1996.

The specific growth rate is a measure of fish growth that is independent of fish size. It was calculated using the formula SGR = $[(\ln wt_T - \ln wt_t)/(T-t)] \times 100$. Wt_T and wt_t are weights at times T and t respectively and $(T-t)$ is the time between weighing. Analysis of variance was used to analyze the growth data.

The correlation between the mean relative food intake and the coefficient of variation for the food intake of individual fish can be used to describe the social hierarchy within a fish population.[3] The effects of the different ploidy rearing groups on the inter-individual variablity in feeding were examined by the calculation of the coefficient of variation using the formula CV = (SD/mean) X 100. The food intake data were analyzed using Spearman's Rank correlation test. All results were considered significant at the $P \leq 0.05$ level.

Results

There was no statistically significant difference between the duplicate groups so the results were pooled before further analyses were carried out. Figure 1 illustrates the specific growth rates of each group of fish, which were not significantly different from each other (P = 0.548).

No significant correlation was found between the mean relative food intake and the coefficient of variation of the mean food intake between the separate (100% diploid: r = -0.088, P = 0.548; 100% triploid: r = 0.063, P = 0.66;) or mixed ploidy groups (50% diploid: r=-0.286, P=0.174 and 50% triploids: r = 0.072, P = 0.737) (Fig. 2).

Discussion

The growth rate for all the groups was good in spite of receiving only half the manufacturer's recommended ration. There was no difference in growth rate of fish between the three rearing groups. This agrees with some studies on salmonids but conflicts with others.[7] This inconsistency could be a result of a number of factors such as differences in species studied or differences in environmental conditions.

Variable coefficients of variation (CV) are often the result of strong hierarchy formation within a fish tank with the most dominant fish having the lowest CV while the subordinate fish have the highest CV.[3] This was not evident in our experiments, based on the absence of a correlation between CV and mean relative food intake. There was little variation between fish suggesting that there was no hierarchy formation between the different rearing groups.

In conclusion there was no difference in growth rate of diploid or triploid fish whether raised in separate or mixed ploidy groups, nor

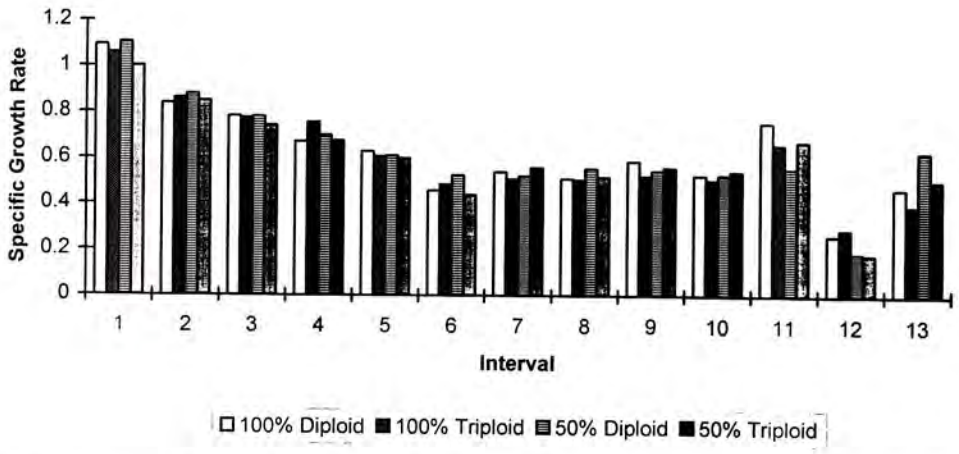

Figure 1. Specific growth rate of diploids and triploids raised in separate and mixed ploidy groups over a 39-week period.

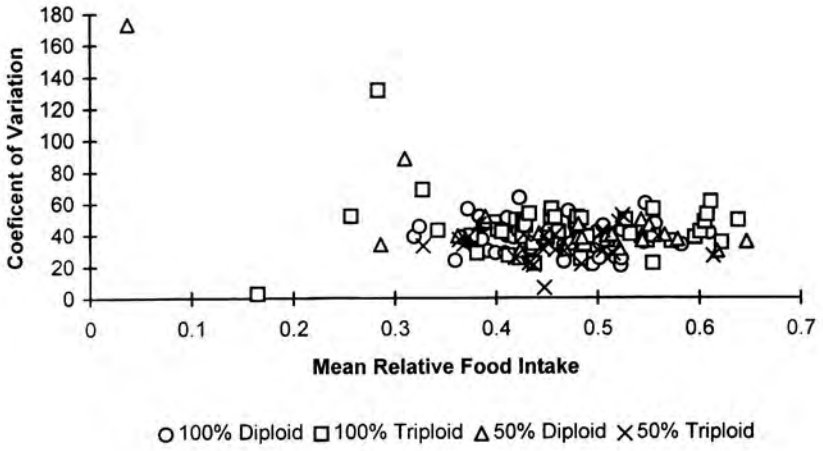

Figure 2. Inter-individual variability in mean relative feeding rate of diploids and triploids raised in separate and mixed ploidy groups.

was there formation of social hierarchies over the experimental period. This suggests that triploids perform as well as diploids under normal rearing conditions but with limited rations.

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Aquaculture Canada '97 Student Awards

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Robyn O'Keefe

Honorable mention

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Lincoln Simmons
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Microsatellites reveal no evidence for inbreeding effects but low inter-stock genetic diversity among brook charr stocks used for production in Québec

S. Martin,[1] *J-Y. Savaria,*[2] *C. Audet,*[3] *and L. Bernatchez*[1,4]

The use of a small number of breeders is assumed to cause inbreeding that may result in loss of genetic diversity in hatchery stocks and increase their susceptibility to various problems. Here, we quantified the genetic diversity among 6 domestic strains and 2 wild populations. Forty to 56 fish from each population were screened with 5 microsatellites. High genetic diversity was observed in all samples. Overall heterozygosity estimates, number of alleles and probability of unique genetic identity were highest among private stocks. An almost complete genetic homogeneity was observed among private stocks whereas both wild populations were very distinct from each other and from domestic fish. These results indicate that, while there is no evidence for inbreeding effects on genetic diversity among private stocks, brook charr production in Québec could benefit from the development of new strains from wild populations which would increase the species potential for selective breeding programs by increasing inter-stock genetic diversity.

Introduction

Brook charr (*Salvelinus fontinalis*) is the most important species for aquaculture in Québec. Because most private stocks are believed to originate from one or two domestic strains, producers relate several problems they encounter (e.g mortality, disease, early maturation) to inbreeding effects. It is also believed that the lack of genetic information and repeated crosses of a low number of breeders may accentuate such problems.[5] As in many other situations, however, inbreeding effects are more often assumed than empirically quantified.

Microsatellites are a class of hypervariable repetitive DNA being increasingly used for the assessment of genetic diversity.[6] In brook charr, it has been shown that microsatellites provide a higher resolution to characterize genetic diversity within and among closely related populations than conventional approaches.[7]

In this paper, we used microsatellites to a) test the hypothesis that domestic strains of brook charr used in Québec have lower genetic diversity than wild populations, and b) quantify the extent of genetic differentiation among domestic strains (private and public) and wild breeders currently held in captivity.

Materials and Methods

Non-invasive adipose fin clips preserved in 95% ethanol were obtained from 6 strains (sample size varying between 40 and 56). Two wild populations originated from the Rupert (James Bay) and Laval rivers (St. Lawrence River north shore), and are currently kept at the Laboratoire Régional de Sciences Aquatiques (LARSA, Université Laval), and at the Station aquicole de Pointe-au-Père (INRS—Océanologie, Rimouski), respectively. Adults of these populations exhibit late sexual maturation and reach large sizes, exceeding 70 cm in length and 5 kg in weight. We also obtained samples from the 2 governmental strains held in the province: Nashua (Pisciculture de Gaspé) and Baldwin strain (Station aquicole de Baldwin Mills). Samples were also obtained from 3 private producers: Pisciculture Bury Inc., Pisciculture des Cèdres Inc., Pisciculture du lac Williams, and one private strain held at Station piscicole de Pointe-au-Père.

Table 1. Genetic diversity parameters, with their standard deviations. Top panel: intra-strain diversity; bottom panel: inter-strain diversity.

	Wild Populations	Government Strains	Private Strains
Number of alleles (A)[a]	27.5 (1.9)	33.4 (12.2)	60.0 (4.3)
Heterozygosity	0.65 (0.03)	0.75 (0.06)	0.82 (0.020
Inbreeding coefficient (F)	0.35 (0.03)	0.25 (0.06)	0.18 (0.02)
Probability of identity (P_I)	8.8×10^{-5} (0.8×10^{-5})	9.0×10^{-6} (7.7×10^{-6})	2.4×10^{-7} (1.0×10^{-7})

	Among Private	Private vs Public	Private vs Wild
Fisher exact tests	0.031 (0.078)	0.00002 (0.00001)	0.000001 (0.000000)
Fst	0.031 (0.015)	0.078 (0.025)	0.187 (0.009)
Mean genetic difference	0.160 (0.130)	0.738 (0.168)	2.281 (1.092)
Proportion of different alleles	—	33.0%[b]	76%[c]

[a] Number of alleles is corrected for a group of 40 individuals
[b] Percentage of alleles found in public strains and absent in private strains
[c] Percentage of alleles found in wild populations and absent in private strains.

Genomic DNA was extracted following a standard protocol.[8] Four microsatellites developed for brook charr[7] and one for brown trout *Salmo trutta*[9] were used to assess genetic diversity. Polymerase chain reactions (PCR) were done in a volume of 15 µL containing about 100 ng of DNA, 0.25 units of Taq polymerase, 1.5 µL reaction buffer, 15 pmol of each primer, 75 mM of each nucleotide and 2 µCi of α-^{35}S-dATP. Radio-labelled nucleotide incorporation in amplified DNA allowed autoradiographic visualisation and discrimination of the different alleles following electrophoresis on polyacrylamide gels.[7]

Four parameters were used to quantify intra-strain genetic diversity. The total number of alleles (A) was obtained by direct counts of alleles for the 5 loci. Expected heterozygosity (He) was calculated as the mean heterozygosity for the 5 loci. Inbreeding coefficient (F) was calculated from expected heterozygosity (F = 1-He). Finally, we computed P_I, the probability of finding 2 unrelated individuals with the same genotype for the loci used.[10]

Inter-strain genetic differentiation was first quantified by assessing the heterogeneity of allele frequency distribution among all pairwise comparisons of samples by the approximation of the Fisher exact test available in the program Genepop, v. 1.2.[11] The amount of total genetic variance due to among strains differences was estimated by Fst. We used the measure of Shriver[12] as an estimate of genetic distance among strains. The resulting distance matrix was used to infer their relationships by constructing a population tree using the neighbour-joining method. Confidence estimates on branching patterns were obtained from 1000 bootstrap replicates. Finally, we computed the proportion of alleles present in public (governmental) or wild strains that are absent in private strains.

Results

Genetic diversity within strains

All 4 parameters revealed high intra-strain genetic diversity in all populations analysed (Table 1). These, however, consistently revealed higher diversity for private than for public and wild stocks (Table 1). Thus, private strains had approximately twice the total number of alleles than that observed either among public or wild stocks, resulting in a higher overall expected heterozygosity. Consequently, inbreeding coefficient (F) was smallest for private strains, intermediate for public, and lowest for wild stocks. Similarly, the probability of genetic identity (PI) was approximately 100 times smaller for private than for public strains, and about 1000 times smaller than for wild populations.

Inter-strain differentiation

High levels of genetic similarity were observed among private strains, as revealed by allelic frequencies, Fst, and genetic distance (Table 1, Fig. 1). Three loci out of 5 showed complete homogeneity in pairwise comparisons of allele frequency among private strains, whereas all loci were different in other compari-

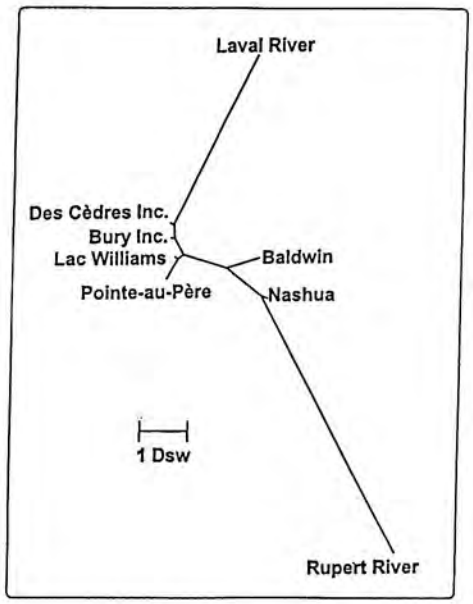

Figure 1. Neighbour-joining phenogram showing relationships among private, public and wild stocks of brook charr.

sons. Private strains were also similar to public stocks, as shown by low Fst and genetic distance estimates, and the sharing of 67% of all alleles among them (Table 1). In contrast, wild populations were distinct from all domestic strains, as shown by strikingly different allele frequencies (76.2% of wild populations alleles are not found in private strains), high Fst and genetic distances values. Both wild populations were also highly differentiated one from each other (Fig. 1).

Discussion

All intra-strain genetic parameters quantified in this study revealed higher diversity among private strains compared to both governmental and wild stocks. Consequently, these results do not support the hypothesis that management practices led to inbreeding effects and resulted in loss of genetic diversity in those populations. In contrast, similar studies in other salmonids have frequently reported reduced diversity [13-16] for fishes raised in hatcheries (but see Ferguson et al.[17]). Three possible, non-exclusive, explanations may account for the apparent discrepancies between our results and previous studies. The exact origins of private strains studied here is unknown, but given their generally higher polymorphism than both original domestic strains used in Québec, and their close relationships to these, it seems likely these are issued from a stochastic mixture of the 2 public strains. The fact that many alleles were observed only in private strains suggest that wild fish of unknown origins may also have been mixed with these.

While microsatellites did not reveal reduced intra-strain diversity, an almost complete genetic homogeneity was observed among private stocks, and very small differentiation from both governmental domestic strains. This indicates that these populations offer little potential for genetic improvement through inter-strain crosses. In contrast, both wild populations were very distinct from each other and from domestic fish. These results indicate that brook charr production in Québec could benefit from the development of new strains from wild populations which would increase the species potential for selective breeding programs by increasing inter-stock genetic diversity.

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Does gonadal development affect the level of activity of Na^+K^+ATPase, a key enzyme in osmoregulation? A preliminary study on brook charr (*Salvelinus fontinalis*) reared in estuarine conditions

N. R. Le François,[1] *P.U. Blier,*[2]
L. T. Adambounou[2] *and M. Lacroix*[3]

We altered the gonadal development of young brook charr using four levels of ionizing radiation at the hatching stage. After initial growth in freshwater, two estuarine challenge tests were conducted. The first trial was conducted during the optimal season for the species (June) and the second during a critical season (October). Gill Na^+K^+ATPase activity was measured at different times throughout the trials. The advantage in seawater tolerance conferred by sterility expressed in higher survival rates that was observed in a previous study[7] is, in the present work, associated with a higher Na^+K^+ATPase activity in sterile females compared to normally maturing fish.

Introduction

In eastern Canada, the period of growth of brook charr in estuarine conditions is seriously limited by the precocious sexual maturation of this species. Early sexual maturation reduces seawater adaptability (SWA) in several salmonid species.[2] Thorpe[3] argues that the processes of smolting and reproduction are mutually incompatible. Smolting is a commitment to life in saline waters whereas reproduction demands a freshwater habitat.[4] Sex steroids have been shown to have iono-osmoregulatory effects in several species of salmonids.[5,6] In a previous study, normal gonadal development severely impaired seawater tolerance in terms of survival of 1^+ brook charr, while sexually altered individuals (using irradiation exposure to induce sterility) displayed greatly enhanced survival in two estuarine challenge tests.[7]

Staurnes et al.[8] observed a 30% lower gill Na^+K^+ATPase activity for maturing Arctic charr (*Salvelinus alpinus*) compared to immature charr. The mode of action of sex steroids on SWA and whether their action is peculiar to the anadromous life history of salmonids is currently unknown.[9]

We present here, the results of the application of low-level ionizing exposures (0, 6.2, 7.8 and 11.4 Gy) for the alteration of gonadal growth (maturity vs sterility) on Na^+K^+ATPase activity. This enzyme complex also known as the "sodium pump" plays a central role in the salt secretory function of the gills.

Materials and Methods.

Origin of fish

A domestic strain of *Salvelinus fontinalis* was used for the induction of sterility to sub-optimal levels using increasing ionizing radiation (0, 6.2, 7.8 and 11.4 Gray). Initial growth in freshwater was followed by two estuarine challenge tests.

Experimental design

The estuarine challenge tests were designed following two seasons and two modes of transfer (**Experiment A** and **B**) to evaluate the effects of gonadal development on salinity tolerance of brook charr. **Experiment A** was conducted in the optimal period of transfer (i.e. in

spring outside the period of sexual maturation) following a direct transfer from freshwater to seawater of all 4 experimental groups.

Experiment B followed after a gradual transfer from freshwater to seawater during a critical period (i.e. during sexual maturation) of the control and 11.4 Gy groups only (in duplicate). Salinity, t° and mortality were recorded daily and sex and sterility status were evaluated at the time of death and at the last sampling in both experiments.

Sampling procedure, analysis and statistics

Fish sampled for determination of Na^+K^+ATPase activity were killed by a blow to the head. Their length, weight, white muscle water content, condition factor, HSI and GSI were determined (data not shown). Gill arches were dissected out and carefully dried with soft paper, and immediately frozen at -80°C until analyzed (**Experiment A**: prior to the experiment, a month after the transfer, and at the end of the experiment; **Experiment B**: prior to the experiment and at the end of the seawater trial). Gill Na^+K^+ATPase activity was assayed [10] (and expressed in µmole Pi/mg protein/hr). All maturing females had GSIs exceeding 5% with well developed eggs. Sterile females presented undeveloped gonads and in 80% of the sterile fish, only a thread of connective tissue with the occasional presence of a few infertile eggs was observed. Effect of sterility (i.e. gonadal growth) on enzyme activity were evaluated by one-way ANOVA (followed by a multiple comparison test when appropriate) to compare the mature and the sterile fish for significative differences ($P < 0.05$).

Results

Experiment A

Significant sterility rates were achieved with the female gender only, so males are not considered in the discussion of the results. Prior to the spring estuarine challenge tests while still in freshwater, the level of Na^+K^+ATPase activity was about 1.0 µmol Pi · (mg protein^{-1} · h^{-1} for all four experimental ionizing exposures, thereafter a gradual increase was observed. At the end of Experiment A, when gonadal development was not considered as a grouping factor, no significative difference in the level of activity of Na^+K^+ATPase was found ($P = 0.0707$) between the experimental groups. However, gonadal growth as a grouping factor, revealed a significative difference in the level of activity of Na^+K^+ATPase between mature and sterile brook charr females ($P= 0.0122$). The mature females displayed lower gill Na^+K^+ATPase activity than the sterile females.

Experiment B

Prior to the gradual transfer of the control and 11.4 Gy groups to estuarine waters, Na^+K^+ATPase activity levels were found to be similar between the pooled mature and sterile females of each group ($P = 0.0782$). At the last sampling, a slight but not significant difference was found between the mature and sterile females ($P= 0.0538$).

Discussion

Normal sexual maturation and treatment with exogenous steroids have been shown to have a negative effect on the ability of several salmonids to adapt to a hyperosmotic environment. Hormonal and/or environmental influences on the regulation of chloride cells and Na^+K^+ATPase is critical during the summer residency of brook charr in estuarine waters.

The present work presents gonadal development as the sole explanation to reduce Na^+K^+ATPase activity during the spawning season when maturing females are compared with sterile female brook charr. Triploidization has been used as a tool to study seawater performance in relation with the sterility status, but the method itself implied direct and indirect negative effects on the osmoregulatory mechanisms including Na^+K^+ATPase activity.[11,12] From previous experimental results,[7] low-level ionizing radiation exposures were proven an adequate tool to study the maturation effects on a key enzyme in osmoregulation: Na^+K^+ATPase. No positive nor negative side-effects of the ionizing treatment were found, in terms of survival, on the seawater tolerance of brook charr. A significant difference in the level of activity was observed at the end of the spring introduction. The sterile females displayed a significantly higher level of activity than the control and normally maturing fish. There was a strong indication of a positive effect of steril-

ity on Na^+K^+ATPase activity at the end of the fall estuarine trial ($P = 0.0538$). The absence of a significant difference can be possibly explained by the rapid occurrence of mortality of the normally maturing fish that could have implied an experimental bias by exerting a strong selection for the fish presenting the highest Na^+K^+ATP-ase activity within the maturing females of both experimental groups. Prolonging **Experiment B**, might have generated the expected discrepancies between mature and sterile females.

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Mixed populations of mature and immature Atlantic salmon (*Salmo salar* L.) in cages: swimming patterns and size distribution

Eric L. Boucher and Royann J. Petrell[1]

Maturing salmon are a problem for the farming industry. A non-invasive, computer-video imaging system was used to develop a tool to predict the proportion of maturing salmon in sea cages. Swimming speed, fish size, and salmon behaviour were recorded and used to discriminate between maturing and immature salmon. Unexpectedly, the salmon segregated in a cage according to swimming speed and K-factor, making it difficult to obtain a representative sample of the cage population. Because of this behaviour, it was not possible to predict the proportion of maturing salmon. A method that randomly samples the cage population, even when the fish are segregating in time and space, is required in order to proceed with the development of a predictive tool.

Introduction

Grilse are a major problem in the production of Atlantic salmon[2] as early maturation interferes with production schedules.[8] The ability to predict the proportion of maturing salmon in a seacage would allow farmers to develop new management strategies to help maximize yield as well as minimize stress on the fish. The purpose of this research was to develop a non-invasive technique for predicting the proportion of maturing salmon in sea cages based on the relationship between swimming speed, fish size and salmon behaviour. Data were collected using a computer-video imaging system developed by a team of researchers directed by Dr. Petrell.[7]

Methods

Equipment

The field equipment was a stereocamera system composed of two surveillance cameras (Cohu 4910 Series Rs-170) with Cosmicar 4.8 mm 1:1.8 TV lenses, an anodised aluminium stereo mount with two waterproof camera housings, a digital frame switcher (Panasonic WJ-FS 10) receiving video and genlock signals and, allowing video recording in sequence, a S-VHS video recorder (Panasonic model AG-1960) to record the sequenced video, and a black and white monitor (Panasonic TR- 930 CB).

The image analysis equipment consisted of a JVC BR-S822U S-VHS recorder, a black and white monitor (Panasonic TR- 930 CB), and a personal computer system that included a DT3155 high-accuracy monochrome PCI bus frame grabber for processing video signals from the S-VHS recorder and the specifically designed software Fish Image Capturing and Sizing System (FICASS).

Field procedures

The study was conducted on a salmon farm site, from May to July, where two stocks of Atlantic salmon (Horrex and McConnell) were being raised. The fish in the 6 selected cages (2 of each stock) were reared under similar husbandry conditions. Two recording depths were selected to represent the top and the bottom position of a swimming aggregation. The cameras were positioned 2 to 3 m from the side of the net allowing the best possible image quality for computer analysis. Filming was done at each depth before and after feeding for 20 to 30 minutes to secure enough usable footage. The

actual proportion of maturing salmon in the cages was obtained from estimates of grilse-grade fish done by an experienced member of the staff.

Image analysis procedures

The recorded tapes were analysed by FICASS. This program allows the determination of size (length and height), mass, and swimming speed of a targeted fish. The condition factor K was calculated as follows: K = mass (g) x 100 / [length (cm)]^3. An average of 100 fish were measured at each depth. Z-tests were used to detect any significant differences between means for positional and temporal pattern. Homogeneity of variance using Bartlett's test was then performed. Two-way analysis of variance (2-way ANOVA) was also done for positional and temporal patterns within cages. For all the tests, the significance level (α) was set at 0.05.

Results and Discussion

The success of this experiment was based on three premises:

1. The trade-off between swimming activity, oxygen consumption and growth of the gonads[6] is significant for Atlantic salmon;
2. The salmon population in a cage is well mixed (i.e., there is no segregation related to maturity status);
3. Frequency distributions for swimming speed and K-factor would be bi-modal due to premises 1 and 2 above.

We expected that the two stocks used in this experiment would behave differently, making it possible to determine the effect of maturation on swimming activity. However, the data indicated there was no significant difference in the proportion of grilse in the two stocks. In addition, the expected bimodal distribution pattern was not displayed by any of the measured parameters. The absence of the second mode made it impossible to detect the maturing salmon. The absence of bimodality suggests that the first two premises were not fulfilled.

The differences in swimming speed and fish condition factor between fish at the top and the bottom position of the aggregation (Fig. 1 and 2) cannot be explained by differences in environmental or physical factors. These differences in the behavior of the fish may be due to segregation according to the maturity level. If the salmon from this experiment displayed behaviour similar to that reported by Kadri et al.,[6] maturing fish would dominate the feeding area. Since the feed pellets are supplied from the surface, it is probable that the maturing salmon would concentrate in the upper section (top) of the aggregation. Because of this, and since there is a significant trade-off between swimming speed and gonadal growth, there was a higher probability of recording fish in the top position

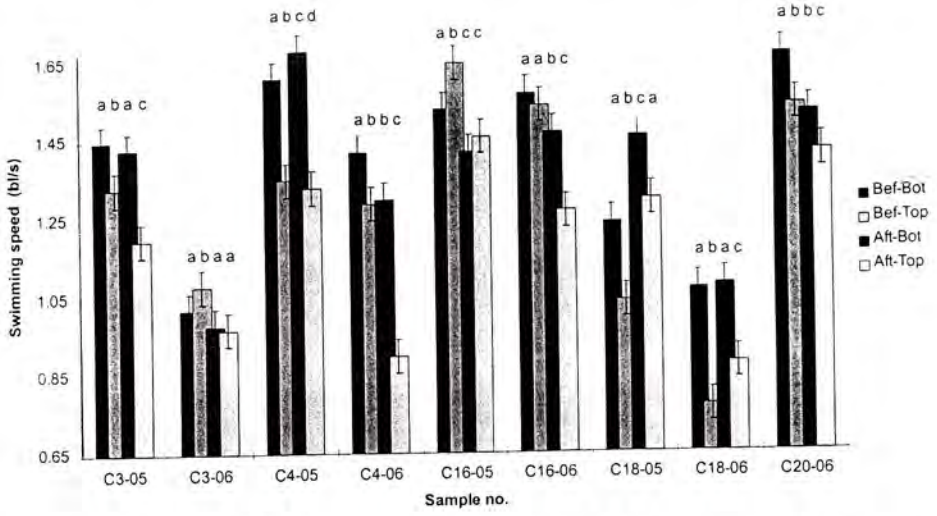

Figure 1. Average fish swimming speed at different position for each cages of the experiment. (Sample no. — cage # and month; a,b,c,d — significance of difference between averages)

with a lower swimming speed and a greater K-factor than recording comparable behaviour and fish size in the bottom position.

The above reasoning might also explain the differences obtained over time (Fig. 1, 2). The absence of maturing salmon on the videotapes before feeding might be explained by a preference by maturing salmon for the centre of the cage. It would be more likely to find dominant salmon in the centre of the cage, behind the camera, where the feed would be scattered. After feeding, these dominant fish would move to the side of the cage. Such behaviour would explain the higher swimming speed and the lower K factor before feeding. The decrease in swimming activity can also be partially explained by a reduction of activity during the postprandial recording; similar swimming patterns have previously been reported.[3,4]

Conclusions

The objective of developing a method for determining the proportion of mature Atlantic salmon was not achieved. There is evidence that the salmon were segregating as a function of maturity status. Swimming speed and condition factor distributions were correlated, suggesting that maturing salmon swim closer to the surface and at a lower speed than immature salmon. The maturing salmon might have concentrated in the centre of the cage before feeding occurred, dominating the feeding area. This behaviour caused a positional difference in swimming speed and the absence of the expected bimodal distribution of the two populations (maturing and immature salmon). A method that randomly samples the cage population even when the fish are segregating in time and space is required to achieve the ultimate goal.

We acknowledge financial and technical support from Natural Sciences abd Engineering Research Council and Heritage Salmon.

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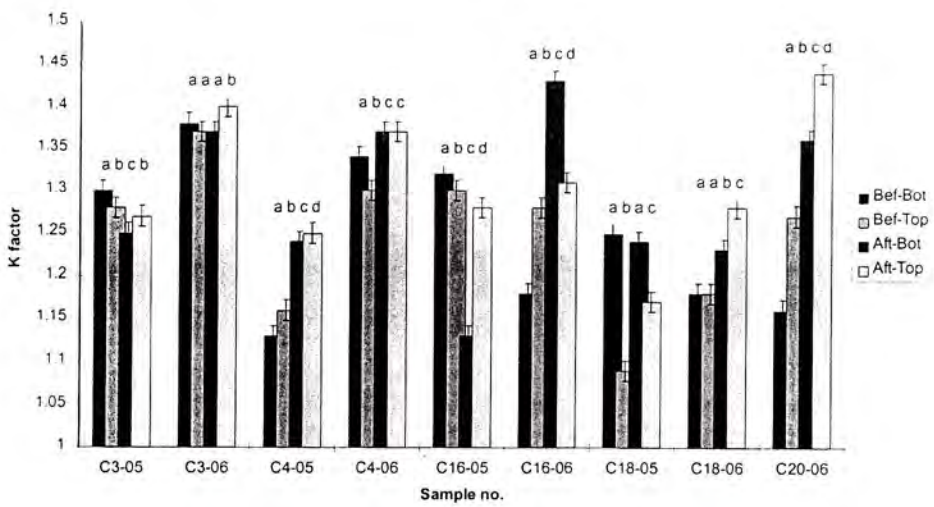

Figure 2. Average fish condition (K-factor) at different position for each cage of the experiment (Sample no. — cage # and month; a,b,c,d — significance of difference between averages)

Interspecific variation in sea lice infection intensity between Atlantic salmon and Arctic charr

A. Mustafa and B. M. MacKinnon [1]

Atlantic salmon and Arctic charr were compared for differences in infection intensities with sea lice, *Caligus elongatus*. Various host parameters that might affect infection intensity were also investigated. Highly significant differences occurred in infection intensities between the two species when they were allowed to acquire sea lice infections naturally. There were no major differences in the protein profiles between the two species, and cytology of epidermal tissues from salmon and charr, from both *C. elongatus* infected and non-infected sites, did not show any major difference. However, some evidence indicated that charr may be more susceptible to osmotic damage to the epidermis perhaps as a result of holding in sea water.

Introduction

Intensity of infection with any given parasite can vary greatly between individual hosts and individual species. Underlying factors that determine whether a fish or a group of fish resists a parasite are complex and may be based on several host characteristics. With respect to sea lice infections, intensities vary considerably even among fish with the same genetic background in the same sea pen. Casual observations by the fish farmers in the lower Bay of Fundy, have also indicated interspecific differences in infection with sea lice between Arctic charr and Atlantic salmon, with charr being heavily infected. These observations were made when charr and salmon were being raised in the same sea pen in the St. Croix estuary. The first objective of the present study was to quantify the intensity of infection with *C. elongatus* on salmon and charr.

Although charr thrive in intensive fresh water culture and some wild charr populations have a seaward migration each spring and a returning migration to fresh water each autumn, they perform less well when kept for prolonged periods of time in sea water because of their low salinity tolerance and poor osmoregulation.[2,3] This suggests that charr may be more stressed than salmon in estuarine sea pens since, unlike salmon, charr do not undergo true smoltification,[4] and naturally do not remain in seawater as adults for a prolonged period of time.

The second objective was to investigate the host factors that may, in part, affect the differences in infection intensity with *C. elongatus* between salmon and charr. Differences in infection intensity between salmon and charr may be due to the effects of stress, to innate differences in fish behaviour or skin characteristics, or a combination of these and other factors. The skin was chosen as the obvious tissue for comparison since the parasite feeds on the skin mucus and epidermal cells.

Materials and Methods

Two-year-old salmon smolts and charr were obtained from Huntsman Marine Science Centre, St. Andrews, and divided into two groups. The fish were first kept in fresh water for 14 weeks and then transferred to a sea pen and kept there for 14 weeks and allowed to acquire sea lice infections naturally. The experimental fish

were used to investigate infection intensity, mucus protein profiles, and cytology of epidermal tissues.

At the end of the experiment, fish were collected from the sea pen and placed in individual bags, killed, and kept frozen for further analysis of sea lice infections. The number of sea lice were counted for prevalence, abundance, and intensity following the definitions of Margolis et al.[5]

At the end of acclimation to seawater, mucus samples were collected from 10 representative fish of each species and analysed for protein profiles using a standard polyacrylamide gel electrophoresis method.[6]

Epidermal tissues from both uninfected and infected fish were excised from 10 representative freshly-killed fish of each species. Samples were prepared for transmission electron microscopy using Karnovsky's fixative, washed in sodium cacodylate buffer, and post-fixed in 1% osmium-tetraoxide. Following post-fixation, samples were dehydrated in acetone and embedded in Epon Araldite. Thick, 1.0-µm and ultra thin, 60- to 70-nm sections were cut using an LKB ultramicrotome. Thick sections were stained with toludine blue and examined under a light microscope, and thin sections were stained with uranyl acetate and lead citrate and examined under a transmission electron microscope.

Results

The mean intensity of infection of sea lice, *C. elongatus,* on Atlantic salmon and Arctic charr were 20.3 ± 3.3 and 32.0 ± 3.5 respectively. The difference of infection between these two species was statistically significant ($P < 0.001$).

Skin mucous protein profiles for salmon and charr did not show any major difference, except that salmon samples in the 67- to 94-kDa range showed more protein bands than did samples from charr. Salmon also had an additional strongly-staining band between 30 and 42 kDa.

Light and electron micrographs of uninfected salmon epidermis indicated an intact layer of outer squamous epithelial cells bearing microridges. Distal malpighian cells were interspersed with mucous cells. More proximal malpighian cells were characterized by large nuclei and a cytoplasm having a few vacuoles and limited endoplasmic reticulum. Several intercellular junctions were also present. In areas where chalimus larvae were attached, extensive abrasion of the epidermis was evident. Epidermal cells were absent down to the basement membrane. These holes were surrounded by ripped and dying cells. Picnotic cells were present next to the excavated hole and the normal epithelial cells bearing microridges were absent. Normal malpighian cells were located near the damaged area and there was no evidence of white blood cell infiltration, excessive mucous cells or melanocytes.

Light and electron micrographs of uninfected charr epidermis indicated an intact layer of squamous cells with microridges. Mucous cells were numerous and were seen opening to the external surface. Malpighian cells were irregular in outline and contained large electron-dense nuclei. They also contained many electron-lucent inclusion bodies similar to the mucopolysaccharide inclusions evident in mucous cells. Some poorly-fixed specimens showed extensive vesicle-like areas, in the malpighian cells, which had been leached by chemical treatments. Few intercellular junctions were evident but the outer cell membrane appeared elaborately folded, interdigitating with adjacent cells. There was some evidence of loss of cell contact in more distal areas since gaps were evident between some cells. The epidermis of charr in areas where chalimus larvae were attached showed excavated holes down to the basement membrane. Picnotic cells at the edges of the holes were evident but not as obvious as those in salmon. There was evidence of inflammation-like osmotic damage to adjacent areas but white blood cells were not evident in the area. No hyperplastic areas or melanocytes were evident.

Discussion

Sea lice, *Caligus elongatus,* are known to infect over 80 different species of hosts including salmonids.[7] Even though *C. elongatus* has a wide host range, some hosts may be more easily colonized than others. In the present study, the levels of infection with *C. elongatus* on charr and salmon were significantly different. Although charr harboured more sea lice than salmon, it is a relatively unimportant host in the life cycle and population dynamics of *C. elongatus*, since charr are not found naturally and are seldom cultured in Passamaquoddy Bay.

Lepeophtheirus salmonis, a larger and now common sea louse in eastern Canada, also infects various salmonid hosts at markedly different infection levels. In Pacific waters, Nagasawa et al.[8] showed a marked difference in infection levels by *L. salmonis* on six different host species of Pacific salmon. These differences in the infection level can be attributed to many characteristics of the host species.

This study has investigated a few of the many parameters that may explain the higher resettlement or higher survival of *C. elongatus* on charr than salmon. Differences in epidermal structure and function in different salmonid species, may be a factor contributing to the differences in infection intensity on different salmonids.[8] *Caligus elongatus* feed on the mucus and epidermal cells of their hosts. PAGE (polyacrylamide gel electrophosis) analysis of both salmon and charr skin mucus indicated that there are no major differences in the protein composition of the two species. In general, the electrophoretic patterns for the two species were reasonably similar although salmon had a few extra protein bands in the 30- to 42-kDa and 67- to 94-kDa range. It is possible that the appearance of these protein bands were due to density differences or due to artifact. It is also possible that these proteins do differ between salmon and charr. These may be important to the nutrition of *C. elongatus* and may result in adults remaining to feed longer on charr before leaving their host.

The cytology of epidermal tissues from uninfected and infected charr and salmon indicated that in both species, chalimus only caused small lesions as a result of the feeding and attachment. There was little evidence of inflammatory response and no infiltration of white blood cells. In both infected and uninfected charr, there appeared to be gaps between the distal malpighian cells that were not seen in salmon. Break down of intercellular connections is one of the first symptoms of infections associated with the skin[9] and may also result from osmotic stress. This may be an indication that charr lost some epidermal integrity as a result of stress from being kept in seawater for a prolonged period. Charr epithelial cells were interconnected by interdigitation of the cell membranes whereas salmon cells were connected by intercellular junctions. These findings may indicate that charr epidermis is more easily disrupted by a feeding sea louse. While few differences in the ultrastructure of infected sites were recorded between infected salmon and infected charr in this study, differences in histopathology have been recorded as a result of *Lepeophtheirus salmonis* infections on Atlantic, chinook and coho salmon.[10]

Charr are less adapted to withstanding long periods of time in seawater than are salmon, and are more stressed in net-pens in water with high salinity. It was therefore, hypothesized that charr, being more stressed, harbour more parasites.

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Behavioural and physical monitoring to determine the health level of salmonids in aquaculture enclosures

Valma Brenton-Davie and Kees Groot [1]

Success in raising fish in aquaculture enclosures is dependent on their health and welfare. To determine the health condition and stress level of salmonids in their culture environments, we monitored behavioural and physiological parameters, physical characteristics, and water and weather conditions. In the hatchery, spatial distribution, activity level, and social behaviour of rainbow trout were monitored at 2-wk intervals during the fry to smolt period. After introduction into saltwater enclosures, the behaviour of rainbow trout (*Oncorhynchus mykiss*), chinook salmon (*O. tshawytscha*) and coho salmon (*O. kisutch*) were observed at monthly intervals using visual, hydroacoustic, and underwater video techniques. With all species, regular physical examinations of the fish were conducted to determine body condition and pathogen load. Water and weather parameters were recorded throughout the observation periods using manual and automatic data logging methods. In this paper we discuss our findings and conclusions, and present suggestions on how to maintain low stress and high health levels of salmonids under aquaculture conditions.

Introduction

Success in raising fish in aquaculture enclosures is dependent on their health and welfare. Many factors come into play in this process, including both physical and biological parameters. To assess the health condition and stress level of salmonids in their culture environments, we monitored behavioural, physiological and morphological characteristics, and water and weather parameters.

In this paper we describe the biological and physical monitoring programs, and discuss some of our findings and conclusions.

The monitoring programs are being conducted at a commercial production facility and a R&D test site with production fish rather than under research conditions with experimental and control groups. Therefore, we have taken the pragmatic approach of "learn as we play".

Materials and Methods

Test sites and salmonids

The test sites were located on the west coast of British Columbia. They included: Seaspring Salmon Farms Ltd., a hatchery located in Duncan, B.C., with rainbow trout (*Oncorhynchus mykiss*) from fry to smolt stage from December 1995 to July 1996; Future SEA Farms Inc. (FSF) SEA Sys-

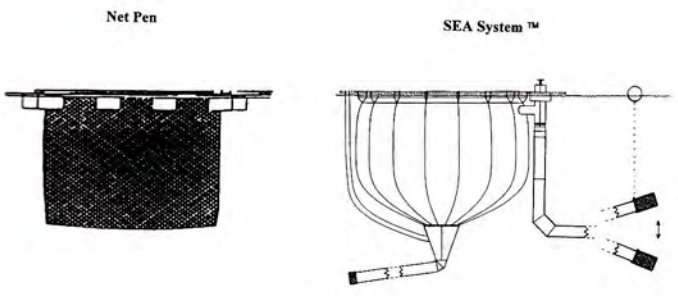

Figure 1. Culture rearing environments.

tem™ testing site in Departure Bay at the Pacific Biological Station (PBS) in Nanaimo with coho salmon (*O. kisutch)* from March 1997 to June 1997; and Phillips Arm Seafarms Ltd. (PAS) with chinook salmon (*O. tshawytscha*) and rainbow trout from July 1996 to June 1997.

Both sea site facilities utilize net pen enclosures and are experimenting with a new culture rearing environment called the SEA System™ (Fig. 1). This system provides a controlled rearing environment using floating, water-tight, flexible enclosures. Each enclosure, or bag, isolates the fish from the surrounding external environment. Water is continuously pumped into the bag from adjustable predetermined depths, providing water of a composition and temperature that approximates the fish's natural habitat.

Environmental assessment

Water and weather parameters were recorded throughout the observation periods using manual and automatic data logging methods. Changes in water temperature, salinity and dissolved oxygen, both inside and outside the culture containers, were recorded at regular intervals. At the PAS site, observations were made at monthly intervals. At the FSF Departure Bay site, water parameters were recorded daily at the surface of both enclosures and weekly at depths of 5 m in the bag enclosures and 2.5 m and 5 m in the net pens. Surface to 25 m ambient water conditions were recorded weekly. A 500-mL water sample from each depth was examined immediately for the fragile species *Heterosigma carterae* and then preserved with Lugol's solution for further plankton identification and enumeration. Daily observations were made if a plankton bloom was evident. Weather conditions, including air temperature, barometric pressure, precipitation, wind direction and force, sun and moon visibility, and cloud cover were recorded a number of times each day.

Fish behaviour assessment

Rainbow trout in the hatchery were moved from fiberglass tanks into an aquarium and spatial distribution, activity level, and social behaviour were monitored during the fry to smolt period at 2-wk intervals.

Assessment of behaviour of fish in large enclosures presents problems because fish are not usually visible from the surface. We made hydroacoustic observations using 5 permanently-fixed stationary transducers distributed over the surface in the bag and net pen at PBS and one moveable transducer on the end of a long pole at PAS. Observations were generally carried out at 4-h intervals during a 24-h period at weekly intervals and gave information on horizontal and vertical distribution and swimming pattern (random or circular).

Underwater videography was used to assess the swimming pattern and speed of the fish, the level of social interaction including threat, attack, escape and feeding behaviour. This observation method could only be used during daylight hours. At night we relied heavily on the hydroacoustic observations.

Fish health assessment

Ten fish from the bag and the net pen were captured by seine at monthly intervals to assess body condition. Immediately after capture, blood samples were collected for hematocrit readings and blood smear analysis. External observations of gill tissue, scale loss and fin condition were also made. The carcasses were transported on ice to Malaspina University-College, Nanaimo, where a necropsy procedure, involving external and internal examination of tissues and organs[2,3] was used to quantify the condition of the fish. Slow swimmers and dead fish were also checked for physical abnormalities.

Growth monitoring

At monthly intervals, 150 fish were captured from both the bag and the net pen enclosures and anesthetized with MS222. Length and weight were measured to provide information on growth rates and feed conversion efficiency.

Physiological assessment

Under a collaborative agreement with the Department of Fisheries and Oceans (DFO), Pacific Biological Station, Nanaimo, determination of the saltwater acclimation of the rainbow trout (salinity tolerance test) was carried out.

Concluding Remarks

The growth cycle for the three species of salmonids in this study is not complete and

monitoring programs are on-going. All species will be harvested between mid July and the end of August 1997.

Water temperatures in the net pen followed ambient conditions, whereas in the bag the temperature remained consistent and depended on the depth of the intake pipe. Hydroacoustic measurements indicated that the net pen fish do not utilize the whole water column whereas the bag fish were evenly distributed throughout the water column.

We found that the body condition was a sensitive indicator of health. Specifically, gill color and texture can be used as an warning sign for deteriorating health levels and were used in a number of cases to take preventative action. For instance, early detection of costiasis *(Ichthyobodo necator)* in juvenile rainbow trout allowed early treatment and may have prevented heavy mortalities.

Coho were introduced simultaneously into the net pen and bag environments and after 4 months the bag fish had grown 27% more and had 30% greater feed conversion rate than the netpen fish (Fig. 2). Density and conversion figures for this data were indirectly obtained, and must be confirmed at harvest and with direct methods. The difference between the two rearing environments was further emphasized by mortality rates, which were 1.0 % in the bag and 2.5 % in the net pen.

Our behavioural and physical condition monitoring program suggests that salmonids in aquaculture enclosures are under stress at all times and this impacts growth, health, and mortality rate. Suggestions for maintaining low stress and high health levels of salmonids under culture conditions include providing low temperature and high levels of dissolved oxygen, controlling predators, avoiding toxic algae, and providing a current to stimulate swimming activity.

We gratefully acknowledge the assistance of H. Kreiberg and Dr. C. Clarke, Dept. Fisheries and Oceans, Pacific Biological Station, Nanaimo, Fabian Forgeron and staff from Phillips Arm Seafarms Ltd., and farm technicians, Helen Tozer, Phillip Rowe and Todd Sanderson from the Future SEA Farms test site at the Pacific Biological Station.

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PBS Coho Growth

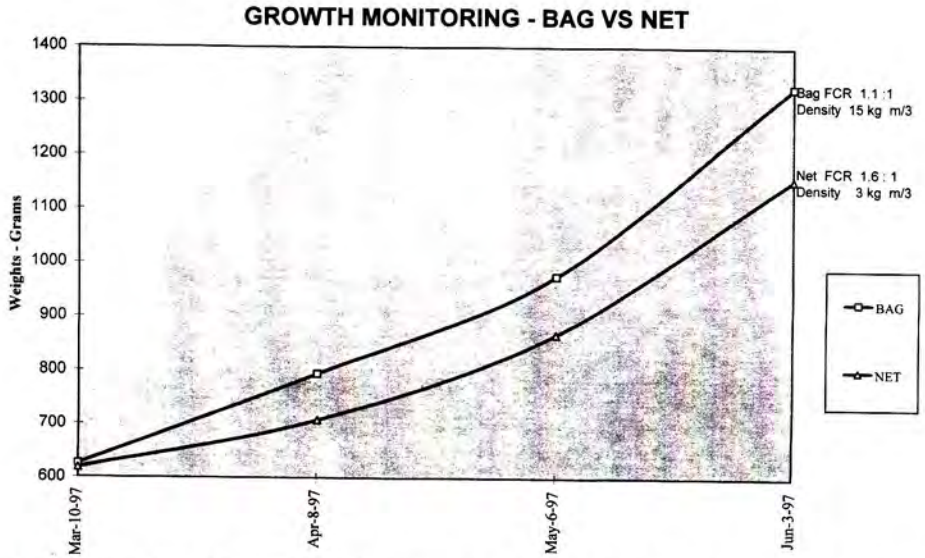

Figure 2. Comparison of growth, feed conversion and density of coho in bag versus net pen enclosures for the period 10 March 1997 until 3 June 1997.

Dietary methionine requirement of juvenile Arctic charr *(Salvelinus alpinus)*

L. Simmons and R. D. Moccia[1]

The methionine requirement of juvenile Arctic charr was studied by feeding a 40% protein basal diet, supplemented with six graded levels of DL-methionine for 16 weeks at 12°C. As evaluated by growth responses, the methionine requirement for optimal growth was estimated to be 1.8% of dietary protein. Lenses from the eyes of charr fed these diets were examined using a scanning laser monitor. With this equipment, focal length variability data indicated that a methionine level of 2.3% of dietary protein is necessary to prevent the development of nutritionally-induced lens cataracts.

Introduction

Food is a principal factor contributing to the high unit cost of production of carnivorous fish such as trout, salmon and Arctic charr. The current high price of fish meal, in concert with projected future shortages, underscores the need to find alternative sources of protein with which to produce fish feeds. Use of potentially cheaper ingredients, such as plant proteins or rendered animal by-products, requires a thorough understanding of the precise amino acid requirements of a given species of cultured fish in order to avoid nutritional deficiencies which reduce growth performance of the target fish. Arctic charr is an emerging product on the Canadian and world aquaculture scene and to date little published work is available concerning the nutritional requirements for this species. With regard to these facts, the dietary methionine requirement of juvenile Arctic charr *(Salvelinus alpinus)* was determined.

Materials and Methods

Juvenile Arctic charr originating from the fall-spawning Labrador strain raised at the Alma Aquaculture Research Station in Alma, Ontario, Canada were used in this study. Thirty charr, averaging 20.5 grams, were randomly distributed into each of 28 covered square tanks (ca. 56-L capacity). Flowing water was supplied at 12°C, at a rate of 1 L/min. A photoperiod cycle of 14 h light:10 h dark was provided with shielded fluorescent lighting and O_2 was maintained above 90% saturation.

Except for methionine content, isoenergetic test diets were formulated by combining intact protein sources (herring meal, soybean meal and wheat middlings) with crystalline amino acids to simulate the "ideal" profile of essential amino acids found in Arctic charr. The six test diets contained 40% crude protein, 17.5 MJ/kg of diet, 1.0% L-cystine, and graded levels of DL-methionine, substituted for L-glycine at 0.9 (0.36), 1.2 (0.48), 1.5 (0.60), 1.8 (0.72), 2.1 (0.84) and 2.4 (0.96) % as measured of dietary protein (dry diet). Quadruplicate tanks of fish were fed to apparent satiation three times daily at approximately 0900, 1200 and 1600 h and once per day on weekends.

Growth performance and feed efficiency were recorded every 28 days and the methionine requirement value was estimated using both broken line and quadratic regression statistical models for data analysis. Prevalence of lens cataracts was investigated both by visual inspection of fish and with a scanning laser monitor which measures the focal length of individual lenses. Focal length variability data were fit to a quadratic regression model and used to estimate the dietary methionine level that resulted in the same optical quality as that observed in the control fish. Plasma methionine

levels were also investigated at the conclusion of the experiment in an attempt to further refine the determination of methionine requirement.

Results

Growth

Growth of Arctic charr responded in a curvilinear fashion to increasing dietary methionine levels (Fig. 1). Weight gains in the order of 80g/fish were observed at high dietary levels while feed efficiencies better than 90% were achieved. Fish fed diet 1 (methionine at 0.9% of protein) had a feed efficiency of 54% and gained only 15 grams. The response in weight gain of Arctic charr to increasing dietary methionine levels can be described by the equation $y = -47.17x^2 + 198.06x - 101.61$. The methionine concentration which results in 95% of the maximum response is estimated to be 1.8% of dietary protein. The broken line model (given as: $y = -80.04 + 128.67x + (151.14)d + (-114.34)(dx)$) predicts a lower requirement value for methionine of 1.3 percent.

Lens pathology

A strong correlation existed between dietary methionine intake and the optical quality of the Arctic charr lens (Table 1). Visual inspection of fish fed a control diet indicated that a partial cataract existed in only one of the 16 fish examined. Conversely, 15 of the 16 fish examined from diet 1 had nuclear cataracts while the remaining fish had a partial or "ring" cataract in the cortical region of the lens. The only treatment group that was similar to the control with respect to the number of visually apparent cataracts was diet 6 (methionine at 2.4% of protein), indicating that a methionine level greater than that required for optimal growth may be necessary to prevent the development of lenticular cataracts in farmed Arctic charr.

Scanning results indicate that focal length variability decreased with increasing methionine concentration according to the equation $y = -0.007x^2 - 0.221x + 0.831$. From this relationship it was determined that a methionine intake of 2.3 grams per 100 grams of protein is required to maintain the same optical quality as that measured in the control group of charr. This value is in agreement with the visual observations made as to the prevalence of lens cataracts and is higher than the 1.8% required for optimal growth.

Plasma methionine concentrations

The plasma methionine levels responded to increasing dietary methionine supply in a linear fashion over the range of values tested in this experiment ($y = 0.19053x - 0.09567$) and showed no threshold with which to determine a requirement value.

Discussion

The growth response of fish in this experiment indicate that the methionine requirement of Arctic charr is similar to estimates of the methionine requirements determined for several other fish species.[2-4] The broken line estimate represents a considerable reduction in the requirement level. The broken line model is a common tool used in the determination of nutritional requirements of fish from dose response data as it provides an objective estimate

Figure 1. Weight gain of 20 gram arctic charr fed graded levels of DL-methionine for 112 days

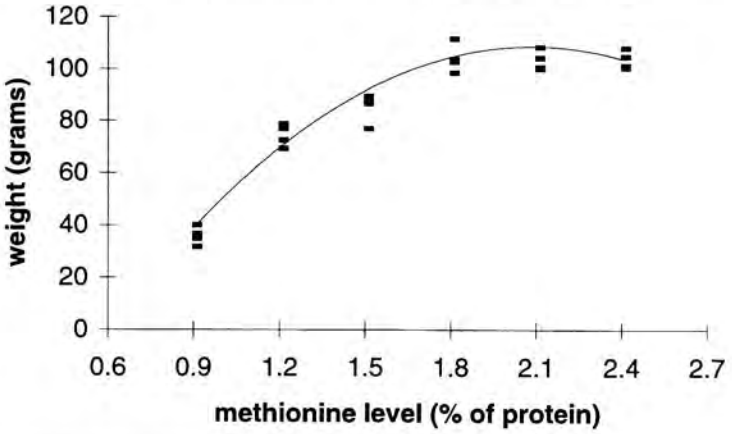

Table 1. Focal length variability and frequency of visually noticeable lens cataracts in Arctic charr fed graded levels of DL-methionine (16 fish examined in each treatment group; variability expressed as the mean ± std. dev.)

Diet	Focal Length Variability	Clear Lenses	Partial Cataracts	Nuclear Cataracts
1. 0.9%	0.58 ± 0.17	0	1	15
2. 1.2%	0.62 ± 0.25	0	1	15
3. 1.5%	0.51 ± 0.13	1	10	5
4. 1.8%	0.36 ± 0.09	5	9	2
5. 2.1%	0.32 ± 0.08	9	4	3
6. 2.4%	0.29 ± 0.09	14	2	0
Control	0.31 ± 0.08	15	1	0

of the requirement value. This model assumes a linear growth response to an incremental increase in the limiting nutrient with the limit of this response marking the requirement. Non-linear models which provide a closer approximation of the growth response of living organisms are regarded as more suitable diagnostic tools with which to estimate nutrient requirements.[5]

The pattern of cataract development observed in this experiment follows that previously described for nutritionally induced cataracts[6] with development originating in the cortical region of the lens and a gradual progression towards the nuclear region. Fifteen of the 16 fish examined in diets 1 and 2 had visually apparent nuclear cataracts. In diet three, while only five fish had nuclear cataracts, 10 had partial or "ring" cataracts. The trend in the remaining diets was towards a decreased prevalence of cataracts with ring cataracts being more common. This suggests that the severity of methionine deficiency combined with the length of exposure to the deficient diet are both important factors in the development of lenticular cataracts.

When focal length variability is the response criteria used to determine the requirement, a value greater than that required for optimal growth is obtained. This finding is similar to a study[2] where the requirement for growth of rainbow trout was estimated to be 0.76% of the diet while a level of 0.96% was necessary to prevent cataracts. The recommended requirement value thus depends on the response criteria deemed most important. Ethics and animal welfare are an increasingly important component of modern animal production systems and although charr are better adapted to feeding in the dark than some species, it would be presumptuous to assume that feeding cataractogenic diets is appropriate. For this reason the methionine requirement of Arctic charr is recommended as 2.3% of dietary protein, or 0.92% of the diet, to optimize the performance and overall well-being of the animal. Further allowances may be necessary for diets in which the ingredients are of lower quality and digestibility.

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Drug resistance of atypical *Aeromonas salmonicida* from Atlantic salmon and rainbow trout in Newfoundland

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Antimicrobial drug resistance patterns of atypical isolates of *Aeromonas salmonicida* from Atlantic salmon or rainbow trout from sites in Newfoundland were determined by disk diffusion assay. There was no resistance against chloramphenicol, kanamycin, nalidixic acid, neomycin or nitrofurantoin, but isolates resistant to erythromycin were found at 3 sites. Oxytetracycline-resistant strains reached 61% in 1995. Resistance to sulphamethoxazole-trimethoprim reached 52%, and a similar trend occurred with ormetoprim-sulfadimethoxine. Simultaneous resistance to the potentiated sulphonamides and oxytetracycline was common at some sites. Enrofloxacin appears to be the most promising drug, since all 72 isolates tested during 1995 were fully susceptible to the drug.

Introduction

Strains of *Aeromonas salmonicida* resistant to oxytetracycline, streptomycin and sulphonamides were detected in Atlantic Canada in 1983[3] and multiresistant classical strains have been found in several countries.[4] The taxonomy of *A. salmonicida* is not fully resolved. Four main subspecies have been described, viz: ssp. *salmonicida*, ssp. *achromogenes*, ssp. *masoucida*, and ssp. *smithia*.[5] The latter 3 subspecies and several unnamed strains are "atypical" members of the species.[6] Some atypical strains are highly pathogenic,[3] but information on drug susceptibility patterns is limited.[7-9] It is important to have information on drug resistance as during outbreaks there is little time for bacteriological and sensitivity testing before commencing treatment. Current data is essential in choosing antimicrobial drugs, especially when multiple resistant strains have been encountered. This study examined the drug resistance of atypical isolates of *A. salmonicida* from Atlantic salmon and rainbow trout with clinical disease in Newfoundland.

Materials and Methods

During 1990-95, 115 isolates of atypical *A. salmonicida* were recovered from cultured fish in Newfoundland (60 from Atlantic salmon, 55 from rainbow trout). Most isolates originated from kidneys and all isolates required culture on blood agar medium for isolation. Isolates were confirmed with an agglutination test using a polyclonal antiserum against a standard strain of *A. salmonicida* (G. Olivier, DFO). Isolates were tested against drugs using standard disk diffusion assay.[10] Tests were done on blood agar plates using Columbia blood agar base (Oxoid/Unipath, Nepean). The plates were read after incubation at 15°C for 3 d, and further checked for up to 5 d if required. We classified only ampicillin-resistant strains as "atypical". Resistance to ampicillin was indicated by an 11-mm zone around a 10 µg disk (Oxoid). The drugs and the disk contents used included: chloramphenicol (30 µg), erythromycin (15 µg), gentamicin (10 µg), kanamycin (30 µg), nalidixic acid (30 µg), neomycin (30 µg), nitrofurantoin (300 µg), novobiocin (30 µg), oxolinic acid (3 µg), oxytetracycline (30 µg), polymyxin B (300 units), streptomycin (10 µg), sulphafurazole (300 µg), sulphonamides (compound) (300 µg), and sulphamethoxazole-trimethoprim (SXT) (25 µg). The SXT disks were used for detecting resistance to sulphadiazine-trimethorim (Tribrissen™). The sensitivity, mild sensitivity or resistance were interpreted according to the zone-size interpretation chart provided by Oxoid.

Isolates recovered in 1995 (total 72) were tested against oxytetracycline, SXT, sulfadimethoxine-ormetoprim (Romet 30™) (25 µg) (DIFCO, Detroit) and enrofloxacin (5 µg) (Oxoid). For Romet 30™, a zone size of 11 mm indicated resistance and 15 mm indicated full susceptibility.

Only isolates falling under the resistant cate-

Table 1. Resistance to 34 atypical *A. salmonicida* isolates from Atlantic salmon during 1990-94 and 24 isolates from 1995 (shown in brackets).

Drug	% Resistant[a]	Site(s) of Origin
Ampicillin	100	all sites
Erythromycin	14.7	9,8,4
Gentamicin	2.9	9[b]
Novobiocin	2.9	9
Oxytetracycline	5.9 (45.8)	4(4 -7[d])
Polymoxin B	2.9	4
Streptomycin	5.9	9
Sulphafurazole	20.6	9,8,4
Sulphonamides (compound)	50	4,9,8
Sulphamethoxazole–Trimethoprim	29.4 (37.5)	9,8,4 (4-7[d])
Sulphadimethoxine-ormetoprim (Romet–30™)	7.1 (45.8)	4 (4-7[d])
Enrofloxacin	(0)	(4-7)

[a] No resistance seen against chloramphemicol, kanamycin, nalidixic acid, neomycin and nitrofurantoin

[b] Strains resistance to erythromycin, gentamicin, novobiocin, streptomycin, sulphafurazole and sulphonamides

[c] Only 14 isoloates were tested against Romet-30™

[d] All 4 isolates from site 7 were simultaneously resistant to OTC, SXT and ROM. From sites 8 and 9 one isolate each was available. The isolate from site 8 showed no resistance and the one from site 9 was resistant to OTC, SXT and ROM.

gory were considered resistant to the drug since it may be possible to treat infections due to mildly sensitive strains. During 1993-94, site 1 (trout) used oxytetracycline (OTC), active ingredient 44%; site 4 (trout and salmon) used OTC and Tribrissen™ (Trimethoprim-sulphadiazine (TRB), active ingredient 40%); site 6 (trout) used OTC and TRB; and site 9 (trout and salmon) used OTC. In 1995, site 1 used TRB, OTC and AquaFlor™ (Florfenicol (AQF), active ingredient 50%); site 2 & 3 (trout) used TRB, OTC and AQF; site 4 used TRB, OTC and AQF; site 6 used TRB and AQF; and site 9 used OTC.

Oxytetracycline was the only antimicrobial used to treat epizootics in the late 1980s and 90s. Following development of some resistant strains of atypical *A. salmonicida* to oxytetracycline, use of Tribrissen™ was started in 1994. Following limited use, resistance to Tribrissen™ developed. Romet-30™ had limited use and resistance developed probably as a result of TRB use (i.e., resistance to the group of compounds).

Results and Discussion

The results obtained from the Atlantic salmon and rainbow trout isolates are summarized in Tables 1 and 2. None of the isolates were resistant to chloramphenicol, kanamycin, nalidixic acid, neomycin, or nitrofurantoin, drugs not in use in Canada. In contrast, 37% of Scottish isolates of typical *A. salmonicida* were resistant to nitrofurantoin in 1990-91.[11]

No strains resistant to chloramphenicol and nitrofurantoin were found in 32 atypical strains collected during 1986-91 in Finland.[9] Isolates resistant to erythromycin, an antibiotic primarily used against Gram-positive bacteria,[6] were found in both trout and salmon in our study. One salmon isolate from site 9 was resistant to 6 drugs, including erythromycin. Resistance to OTC reached 11% in 1990-94 and increased to 61% in 1995. Resistance to OTC was uncommon among isolates in 1991-92.[12,13] In an earlier study[14] of typical *A. salmonicida,* 29% of isolates from Atlantic Canada were resistant to OTC. In comparison, in Finland[9] no resistance was seen against OTC among atypical strains of *A. salmonicida*, though 30% of typical strains were resistant, despite common use of the antibiotic. Resistance to sulphafurazole was 21-33% among our isolates, similar to those obtained[15] from typical *A. salmonicida* strains in Scotland. We found resistance to sulphonamides up to 50% among the 1990-94 isolates, lower than reported for atypical strains in Finland.[9] Resistance to TRB was not found among atypical strains in Finland, whereas 33% of our 1990-94 isolates were resistant to SXT, and the percentage increased in 1995 to 38-52% (Tables 1, 2). This increase is most likely due to increased use of TRB.

The percentage of resistant isolates varied between sites, particularly in the 1995 isolates from rainbow trout. This was possibly related to the difference in number and type of antimicrobial treatments used on site.

All isolates were susceptible to enrofloxacin and the value of this quinolone drug in therapy of furnuculosis is well documented.[16]

A. salmonicida strains from Scotland are inhibited by enrofloxacin at low concentrations.[17] Resistance to enrofloxacin was not detected as of 1994.[4] However, strains resistant to oxolinic acid, an older quinolone are not uncommon in other countries.[4,15]

Table 2. Drug resistance of 9 atypical isolates of *A. salmonicida* from rainbow trout during 1990-94 and 46 isolates in 1995 (shown in brackets).

Drug	% Resistant	Site of Origin
Erythromycin	44.4	4
Oxytetracycline	11.1 (60.9)	1 (1-4, 6,9)
Sulphafurazole	33.3	4
Sulphonamides (compound)	44.4	4
Sulphamethoxazole–Trimethoprim (SXT)	33.3[b] (52.2)	4 (1-4,6,9)
Sulfadimethoxine–ormetoprim (Romet–30™)	(50)	(1-4,6,9)
Enrofloxacin	(0)	(1-4,6,9)

[a] No resistance seen against other 10 drugs (see text)
[b] Simultaneously resistant to erythromycin, sulphafurazole, sulphonamides (compound) and SXT.

Conclusion

Strains resistant to the antimicrobial drugs approved in Canada at the time (oxytetracycline, Tribrissen™, Romet 30™), referred to as triple resistant strains, are common in Newfoundland. Enrofloxacin appears to be useful against resistant strains; however, it should be the drug of last choice to prevent resistance against the fluoroquinolone group of drugs, which have application in both veterinary and human medicine. Florfenicol was approved for use in salmonids in 1997. Prior to this, like erythromycin, it was only available through an emergency drug release under exceptional circumstances. [18] Erythromycin-resistant strains of atypical *A. salmonicida* exist in Newfoundland; therefore sensitivity testing is required before use. Sensitivity to florfenicol needs to be monitored as well, and it is included in our sensitivity panel.

Although treatment was often initiated before sensitivity testing was completed (a necessity due to the delay of 3 to 5 d in obtaining results), the results were used in conjunction with clinical observations to determine the effectiveness of treatment and to make decisions on antimicrobial use. Rarely were isolates with more than one sensitivity pattern isolated from the same "outbreak" or "epizootic".

Factors such as withdrawal time and cost are also important considerations in managing outbreaks with antimicrobials. Close monitoring of resistance patterns is essential, since the inappropriate use of drugs can result in multiple resistant patterns that make the drugs ineffective. Problems with the approval of new antimicrobial agents for treating diseased fish results in a limited number of agents available for future use and therefore isolation of antimicrobial use is not always possible. Apart from the judicious use of antibiotics, better management, better vaccines and the possible application of non-specific immunostimulants and natural antibacterial substances such as chitosan and protamine, may gain importance. In one study,[19] treatment with chitosan provided some protection to brook trout infected with *Aeromonas salmonicida*. The use of non-specific immunostimulants such as glucans in feed may also show promise.[6]

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Saltwater culture of tilapias and possible commercial application in Canada

Thomas T. George [1]

Global aquaculture of tilapias is growing rapidly. Much of the culture activities to date has been in Asia, but there is great interest in other parts of the world, including developed countries where tilapias are becoming increasingly recognized by consumers. Culture activities are not limited to freshwater but have expanded to saltwater because certain species are euryhaline; it is assumed that tilapias evolved from a marine ancestor and that their penetration to freshwater is secondary. This paper highlights the global aquaculture production of tilapias, the euryhaline species and their culture performance in saltwater, and possible commercial application in Canada.

Global Production

Tilapias are the "Miracle Fish" of the past (since biblical times), the present, and the future.[2,3] They have been introduced into 150 countries and are being cultivated by more than 75 nations because of their excellent characteristics for aquaculture.[3] In less than a decade their production, along with other cichlids, expanded by 150% globally.[3] In 1994, world production was about 0.6 billion kilograms with Asia as the leading region; now tilapia culture has expanded to other parts of the world including developed countries.[4] Production in Canada was only about 25,000 kg in 1997,[5] but exceeded 6 million kg in the United States in 1995, a 300% increase in 5 years. Tilapia is the fastest growing aquaculture species in the United States and the third largest imported farmed product behind shrimp and Atlantic salmon.(6,7) In 1996, the United States imported 19 million kilograms of tilapia from 20 countries (frozen whole/fillet, fresh fillet) worth over US$43 million.[8] In 1995, Canada imported 377,000 kg (frozen whole/dressed) of tilapia from 6 countries worth Can$978,000. About 4,400 kg/week of live tilapia are sent to the Toronto market from the United States; the market for fillets is poor.[3] Thus, the growing market appeal of tilapia extends from Japanese sushi bars to seafood-correct Seattle.[9]

Tilapias are cultured primarily in freshwater, but the high degree of salt tolerance of some species has promoted their culture in brackishwater and marine systems.[10] It is assumed that tilapias evolved from a marine ancestor and that their penetration to freshwater is secondary.[11]

Saltwater Species and Culture Performance

The most popular saltwater tilapias are the red hybrids developed by crossing *Oreochromis mosambicus* with either *O. hornorum* (Florida red tilapia) or *O. niloticus* (Taiwanese red tilapia); also popular are the species *O. spilurus* and *O. mosambicus*.[10] Florida red tilapia is capable of reproduction in full strength seawater (36 ppt), but fertilization and hatching success decline markedly at salinities higher than 18 ppt. The Taiwanese red tilapia grows well at salinities of 17 ppt and 37 ppt, but is more susceptible to handling stress at these salinities. *O. spilurus* tolerates direct transfer to seawater of 33 ppt and has a good performance record in high salinity waters. *O. mosambicus* grows well in ponds at salinities between 35 to 40 ppt and reproduces at salinities up to 35 ppt.[10,12]

The growth rates of 2.7 to 3.4 g/day recorded for Florida red tilapia reared from small fingerling to market size in full strength seawater were superior to those reported for *O. spilurus* reared in full seawater and for Taiwanese red tilapia reared in brackishwater and saltwater.[10] Maxi-

mum growth for Florida red tilapia was observed at 18 ppt and 32°C with a high growth rate and feed conversion efficiency.[13] Hybrid red tilapia was also cultured to market size in about 7 months on a pilot/commercial-scale in brackishwater ponds (10 to 25 ppt) with the Pacific white shrimp *Penaeus vannamei*.[14]

A review of the literature[7,9,10,12-17] on tilapia culture revealed the following facts:

- Tilapias are sensitive to accumulating nitrate (100 ppm) and therefore heated indoor intensive recirculating water systems used in temperate climates require anaerobic de-nitrifying systems (biological filters) and complete water reconditioning every 10 to 15 minutes to achieve 100% removal of fish pollutants.
- The use of solar greenhouse structures and insulated buildings which capitalize on inexpensive heat sources is allowing for tilapia culture in increasingly colder locations.
- Eggs spawned in freshwater and hatched in saline waters (less than 15 ppt) resulted in fry with higher salinity tolerance than when eggs were spawned and hatched in freshwater and the fry were acclimated to higher salinity.
- The clutch-removal method is superior to the natural mouth-brooding method of broodstock management for providing Florida red tilapia seed under commercial-scale culture and minimizes seed loss due to cannibalism of eggs and fry by adults and juveniles.
- Pre-acclimation at low salinity and gradual transfer to higher salinities of 5 ppt/day was more effective than a salt-enriched diet (36.5% crude protein + 10% sodium chloride) for adapting tilapias to saltwater.
- Exposure to salinity at early developmental stages and hybridization were found to produce progeny highly tolerant of saline conditions and improve subsequent growth performance.
- Age and/or size at transfer to saline conditions did not have any significant effect on subsequent growth.
- Exposure to a combination of seawater rearing and continuous methyltestosterone (MT) treatment produced a 7-fold increase in growth over that observed in a freshwater tilapia.
- Continuous MT treatment reduces heterogeneity in tilapia growth, leads to a more uniform product that can be harvested all at once and thus saves the aquaculturist time and money.
- Salinity affects temperature tolerance and modifies effects of temperature on growth; temperature also influences salinity tolerance.
- Salinity suppresses territorial aggression in tilapias, resulting in better growth performance in saline waters.
- Stress and physical injury due to handling are the two main causes of disease problems in saline-grown tilapias and can be avoided by minimizing stress.
- Tilapias in freshwater pick up "off-flavor" if certain kinds of algae are present, but when reared in saltwater the flesh has less off-flavor problems and a lower bacteria count.

Application in Canada

The culture potential of freshwater tilapias in Canada was highlighted and emphasized by the author in 1996.[3] The above information on the culture of red tilapia hybrids in saltwater gives insight into the possibility of diversifying the species cultured in Canada, especially in those provinces with access to saltwater resources and inhabited by an ethnic Oriental population, e.g. British Colombia. Saltwater red tilapias could produce a more broadly accepted product for commercial expansion in Canada because they command higher market value and are also preferred in some export markets where they are eaten raw as "sashimi".[9] There is great demand and markets for tilapia in Canada and the United States as indicated from the level of imports and domestic production in both countries. It is important to note that the tilapia market in the United States is growing annually at a rate of 30 to 40% and is predicted to expand into mainline American restaurants and supermarkets across the nation. In 1995, the Palisade, one of Seattle's most up-scale waterfront restaurants, pulled fresh sole off the menu and replaced it with Costa Rican tilapia, indicating consumer acceptance of tilapia.[9] Moreover, tilapias have extremely fast growth rates compared to trout and salmon and do not require high-cost, high-quality, protein-rich feed inputs. Also, their higher growth rates allow maintenance of much higher feeding rates per growing unit, thus reducing production costs attributed to fixed costs (labour, heating etc.).[2] Therefore, provincial aquaculture associations and the industry in Canada are encouraged to promote the culture of freshwater and saltwater tilapias.

Summary and Conclusions

1. The widespread enthusiasm for tilapia culture and the consequent boom in production are due to increased acceptability of tilapia by consumers and the successful culture techniques adopted; tilapias are well on the way to becoming the aquatic version of the chicken.
2. The technology for satisfactorily rearing tilapias in seawater is well developed and the marketability of such saltwater-cultured tilapias is encouraging because they are rated highly in terms of flavour, texture and freshness.
3. Available information suggests the Florida red tilapia is the species best suited for high salinity culture, combining the commercially important attributes of high growth rates in brackish water and seawater and attractive body conformation and colour.
4. The culture of red tilapia hybrids in saltwater could produce a more broadly accepted product for commercial expansion in Canada. Appropriate cost-effective technology is needed to allow producers to compete locally and on the world market.
5. Selection of tilapia species for aquaculture in saline water should consider environmental factors, especially salinity and temperature, that can influence growth performance, reproduction and disease occurrence.
6. Seedstock of saltwater tilapias can only be produced in water of low salinity but better results are obtained when eggs are spawned in freshwater and hatched in saltwater.
7. Use of MT in tilapia production should demonstrate that residuals of this androgen are rapidly eliminated from treated fish to levels that pose no health risk to consumers; likewise, MT treatment of tilapia must be shown to produce no deleterious aquatic environmental effects.
8. In Canada, MT use is banned and therefore production of SuperMale hybrids that have only male-determining sperm would be the best alternative.
9. Tilapias are more sensitive to stress in saltwater and therefore are highly susceptible to secondary infections; this can be avoided by minimizing stress from factors such as crowding, handling, fluctuating temperature, low water quality, chemicals or drugs, and disturbing light or noise.
10. In order to continue the success of tilapia in the marketplace of Canada and the United States, tilapia producers have to increase efforts on their farms and in processing buildings to ensure "on-flavor" and hygienic wholesale tilapia products for the consumer.
11. Aquaculture associations and the tilapia industry in Canada should promote the culture of species with herbivorous and/or omnivorous feeding habits like tilapias which are not dependent upon the use of high-cost, high-quality, protein-rich feed inputs.

In conclusion, tilapias will continue to gain economic prominence as cultured food fishes in North America. In Canada, culture of tilapia started in 1977 in Ontario but will expand quickly because, more than ever before, the federal government has decided to take an active and direct role in aquaculture development to strengthen its domestic farming industry by appointing a commissioner for aquaculture development reporting directly to a cabinet minister.[18]

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Nutritional studies on growth and protein utilization during the juvenile stage of winter flounder *(Pleuronectes americanus)*

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Information on the ability of a species to digest, absorb and metabolize a particular nutrient is necessary in formulating diets that will give the most economic food to product ratio. In a study on juvenile winter flounder, three diets were formulated to contain either 45% protein and 10% lipid (diet 1), 40% protein and 15% lipid (diet 2), or 35% protein and 20% lipid (diet 3). Wet weight and feed consumption were measured at 2-week intervals for 10 weeks. By week six, fish receiving diet 1 were significantly larger (p=0.02) than the fish fed diet 3. The highest specific growth rate (2.11), feed efficiency (0.64) and protein efficiency (1.50) were obtained with diet 1. Oxygen consumption data indicated there was no difference in the metabolic activity of the three groups. These results indicate that a minimum of 40 to 45% dietary protein is necessary to obtain optimum growth rates for juvenile winter flounder.

Introduction

In order for aquaculture to reach its full potential and compete globally, producers must reduce production costs. One of the target areas for reduction is feed costs, which may account for up to 50% of the operating budget. Determination of the optimum nutrient balance is especially important for juveniles, as a large portion of the costs of culturing fish are incurred during the grow-out stage.

The major factor regulating the amount of food consumed by a fish is the energy value of the food relative to the animal's energy requirements. Protein and lipid are the primary energy sources for cold-water marine fish. Protein, as one of the more expensive feed ingredients, should ideally be used for growth rather than energy production. Past studies using trout, *Salmo gairidneri;*[7] salmon, *Salmo salar;*[6] and turbot, *Scopthalmus maximus*[4] have shown that using dietary lipids to meet energy requirements can minimize the use of protein as an energy source.

This study was designed to investigate the effects of varying protein and lipid levels on growth and protein utilization in diets for juvenile winter flounder. Protein utilization can be estimated using data obtained about oxygen consumption and ammonia excretion[5] and allows for an estimation of the protein sparing effect.

Materials and Methods

Three isocaloric diets were formulated with protein to lipid ratios of 45:10, 40:15 or 35:20 (%). Sambro Fisheries Ltd. provided 135 juvenile winter flounder with a mean initial wet weight of 0.812 grams. The fish were housed in nine 25-L glass aquaria on a recirculating water system and held at a stocking density of 15 fish per tank. Wet weight and feed consumption were measured every two weeks for a 10-week period. Fish were fed twice daily and feed consumption recorded. Feed conversion (feed consumed to wet weight gain, ($g \cdot g^{-1}$)) and protein

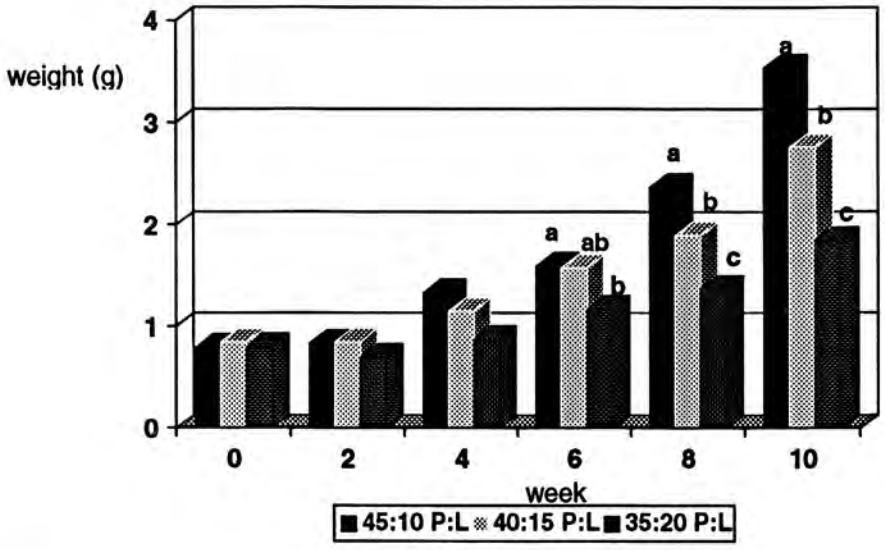

Figure 1. Average weight gain of juvenile flounder on test diets differing in dietary protein and lipid levels (different letters denote statistical significance at the 5% level).

efficiency (protein consumed to wet weight gain ($g \cdot g^{-1}$)) were determined.

An experiment was set up to assess protein utilization based on oxygen consumption and nitrogen excretion. Four 1.5-L containers equipped with oxygen probes and a freshwater supply served as modified respiration chambers. Two fish per diet treatment were placed in each of three containers, while the fourth container served as a blank. The containers were sealed and readings from the probes were obtained at half-hour intervals over 3 hours. Oxygen consumption was determined based on the decrease in oxygen saturation of the water over time. Water samples obtained after 3 hours were analyzed for ammonia levels using the standard method for seawater analysis.[9] Determination of maintenance level consumption was based on tests run after 24 hours starvation.

Statistical analyses were performed using the SAS(8) software package with significance set at the 5% level. The effects of diet on growth, oxygen consumption and ammonia excretion were determined using an ANOVA with growth, oxygen consumption and ammonia excretion as the dependent variables. A Duncan's means separation test was used to assess where specific differences lie.

Results and Discussion

Mortality rates for all three test diets ranged from 5 to 7%. The fish grew from an initial mean weight of 0.812 grams to a final mean weight of 2.715 grams. At 6 weeks (Fig. 1) fish receiving the highest protein diet were significantly larger than those on the lowest protein diet ($p=0.02$). Table 1 shows that specific growth rates were significantly different among all three diets ($p=0.0003$). The best feed efficiency and protein efficiency were also obtained with the 45% protein diet. The indication is that winter flounder grow best with a dietary protein level of 45%. The low protein efficiency of the 35% protein diet suggested that it would not be economically feasible for use in a practical situation. It did not appear beneficial to increase the lipid levels in diets containing less than 45% protein for winter flounder.

Table 1. Information on weight gain, growth rates and feed conversion for juvenile winter flounder on test diets.

Protein %	Lipid %	Weight Gain	Specific Growth Rate	Feed Efficiency	Protein Efficiency
45	10	445	2.12 ± 0.08	0.64 ± 0.03	1.50 ± 0.13
40	15	326	1.68 ± 0.07	0.47 ± 0.01	1.11 ± 0.07
35	20	231	1.16 ± 0.18	0.25 ± 0.02	0.72 ± 0.09

Table 2. Oxygen consumption and ammonia excretion of winter flounder on test diets.

Protein %	Lipid %	Oxygen Consumption (mg/g/h)	O:N Ratio
45	10	0.14 ± 0.07	0.42 ± 0.38
40	15	0.15 ± 0.05	0.42 ± 0.31
35	20	0.14 ± 0.05	0.53 ± 0.27

Oxygen consumption (Table 2) did not differ with dietary treatment (p=0.93). Basal metabolic oxygen consumption for flounder 5 to 7 grams in size at 12°C was 0.10 mg/g/h. Slightly higher ammonia excretion (0.43 µg/g/h) on the 45% protein diet (p=0.06) indicated that some of the protein was being catabolized for energy by the fish. These results indicate that in all cases the protein was being used for growth and the dietary lipid provided no "sparing" effect. Winter flounder appear to require a minimum of 45% dietary protein to obtain optimum growth rates.

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Is there a direct connection between amino acid and lipid metabolism in marine fish embryos and larvae?

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Over the past decade, two major endogenous nutrients, lipids and free amino acids, in developing marine fish embryos and larvae have been studied extensively in order to elucidate the nutritional requirements of these animals. However, the connection between amino acid and lipid metabolism has rarely been investigated. Based on our data for Atlantic halibut (*Hippoglossus hippoglossus*) embryos and larvae, and previous studies on other species, we propose that in the later embryonic stages, towards hatching, a significant amount of amino acid is catabolized to serve as carbon skeletons for lipogenesis. The synthesis of lipid may be related to buoyancy and osmolality regulation during embryogenesis, modification of membrane structure, and may also be an adaptation to hypoxic conditions that may occur at hatch. Although lipogenesis is an energetically inefficient process, its physiological importance may override this disadvantage. These findings suggest that in embryos and larvae there is an interaction between lipid and amino acid metabolism which may relate to physiological and environmental conditions. Thus, from the perspective of broodstock and larval nutrition, lipid and amino acid metabolism should not be considered separately.

Introduction

The mass mortality that can occur at the time when larvae transform from endogenous to exogenous feeding is one of the major difficulties encountered in marine fish aquaculture. It is generally believed that a thorough understanding of biochemical changes that occur during the embryonic and larval stages would help to improve the production of high quality food for first feeding marine fish larvae, and to reduce their mortality.[3] The two most abundant and probably most studied biochemical components of marine fish egg yolk are lipids and amino acids. Both lipids and amino acids are mobilized from yolk during embryonic and prefeeding larval stages to support the development of embryos and larvae. But the direct connection between the metabolism of these two components in marine fish embryos and prefeeding larval stages has rarely been studied. Previously, these two nutrients were considered separately[4-10] or their metabolism was related by means of physiological energetics based on respiratory quotient (RQ) and nitrogen quotient (NQ) calculations.[11-13] Here we investigate the possibility and significance of lipid synthesis from amino acids in marine fish embryos and larvae.

Our interest in a direct connection between amino acid and lipid metabolism arose from our recent finding that there is a significant increase of total lipid in just hatched Atlantic halibut (*Hippoglossus hippoglossus*) larvae compared to the embryos mid-way to hatch.[14] All lipid classes investigated (phosphatidylcholine, phosphoethanolamine, sphingomyelin, triacylglycerol and sterol) increased between 7 and

16%. At the same time, free amino acids (FAA) decreased significantly, without a concomitant increase in total protein. The energy associated with the increase in lipid accounted for about 75% of the total energy decrease associated with the drop in FAA, suggesting the carbon skeleton needed for lipid synthesis may come from the catabolism of FAA. As carbohydrate constitutes less than 1% of total energetic substances in most marine fish eggs studied,[11,15,16] it is not likely to be the major carbon source for lipid synthesis. While it is unusual to find *de-novo* synthesis of lipid during embryogenesis, it is not without precedent. In 1968, Terner[17] demonstrated that trout embryos were capable of lipid synthesis from 1-^{14}C-acetate. Furthermore, total lipid was found to have increased in winter flounder embryos and first day larvae.[15]

Fish usually live on protein-rich carnivorous diets;[18,19] therefore with respect to their primary sources of energy, fish appear to be quite different from mammals. In mammals, carbohydrates are the major substrate for energy production, and excessive carbohydrate is converted to neutral lipid; in fish, dietary amino acids are not only used for energy production, they are also the major precursors for synthesis of lipid and carbohydrate. The utilization of amino acids is similar in marine fish embryos that contain high concentrations of FAA. Since amino acids have easy access to the tricarboxylic acid cycle (TCA) after deamination to α-keto acids,[20] they can be catabolized to produce ATP, and also to provide carbon sources for lipogenesis and gluconeogenesis. Using nitrogen quotients, Finn et al.[11] concluded that FAA which disappeared at embryonic and larval stages are mainly catabolized to produce energy. However ammonia is the only nitrogenous end product they measured, and the existence of an ornithine-urea cycle (which produces urea) in marine fish embryos has not been thoroughly studied. Wright et al.[21] found a functional ornithine-urea cycle during early larval stages in rainbow trout. If a similar pathway functions in marine fish embryos and larvae, NQs obtained in previous studies could be inaccurate. Therefore, amino acid catabolism that has not been taken into consideration may be involved in lipid and glucose synthesis.[22]

During endogenous feeding, the efficiency of metabolic processes becomes very important. Although the synthesis of lipid from FAA is biochemically possible, the question that remains unanswered is: why is this energetically inefficient process necessary? Several physiological and biochemical processes may be associated with the synthesis of lipid.

Osmoregulation

Halibut embryos and newly hatched larvae live in a hyperosmotic environment and do not have a functional mouth or gills. Since FAA contribute about half of the osmotic force in embryos,[23] osmoregulation relies largely on FAA concentration. Because the vitelline membrane is impermeable to FAA and none are excreted to ambient water,[24] osmoregulation is closely associated with the metabolism of FAA. In order to maintain or decrease the osmolality of body fluids,[25] embryos must compensate for the decreasing yolk volume and counteract continuous water loss[25] as development progresses. Therefore excessive amounts of FAA may need to be removed in addition to the amount catabolized for energy production. Lipid synthesis from FAA could provide a means for the removal of additional FAA.

Membrane Modification and Buoyancy Regulation

As proposed by Evans et al.,[22] lipid synthesis from FAA could be related to the restructuring of membranes caused by thermal adaptation when halibut embryos gradually move upward in the water column with the progress of development.[26,27] In a recent study, they found that the PC:PE ratio significantly increased and the percentage of saturated fatty acids increased during development of halibut embryos[22]. These changes in lipid could result in a less fluid membrane structure which would be predicted if embryos/larvae were moving to an environment with higher temperatures and lower pressure.

The position of embryos in the water column depends on changes in buoyancy. The mechanisms of buoyancy regulation include changes of water content,[28] replacement of heavy ions with lighter ions,[25] and changes in specific gravity through varying (protein + FAA) / lipid ratio. The synthesis of lipid from FAA may contribute to the decrease of halibut embryo specific gravity.

Energy Production or Homeostasis Maintenance under Hypoxia

During the hatching process, the mature embryo exhibits vigorous muscular activity to tear open the chorion, and the rate of O_2 uptake increases substantially[4] so that hypoxia may occur. An increase of lipid content has been observed in anoxic perch embryos.[29] van Raaij et al.[30,31] suggested amino acids as acetyl donors for lipid synthesis in gold fish living under anoxic conditions. During anoxia, the operation of the TCA cycle is made possible when reducing equivalents accumulated are being consumed by an unconventional fatty acid chain elongation pathway, as described by van Waarde.[32] A similar mechanism may occur in fish embryos when hatching occurs. One spin of the TCA cycle coupled with fatty acid chain elongation can generate one molecule of ATP even under anoxic conditions. However, van Raaij et al.[32] suggested that this pathway serves mainly to maintain metabolite homeostasis rather than to generate energy.

Summary

We propose that the extensive catabolism of amino acids provides the carbon skeletons needed for the specific synthesis of lipids in some marine fish embryos and larvae. Although it is an energetically inefficient process, the importance of lipid synthesis in physiological events such as osmolality and buoyancy regulation, membrane modification, homeostasis and energy production through the TCA cycle under hypoxia may override this drawback. This is an area with more questions than answers which requires further research.

This study has suggested that, from the perspective of broodstock and larval nutrition, lipid and amino acid metabolism should not be considered separately. Hatchery managers need to be aware that amino acids can be converted to lipids; however, there is still an absolute requirement for essential fatty acids since fish cannot synthesize these acids themselves.

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Growth of juvenile yellowtail flounder (*Pleuronectes ferrugineus*) under three photoperiods

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Yellowtail flounder (*Pleuronectes ferrugineus*) are currently being investigated for their potential as a cold-ocean aquaculture species. Experiments have shown that yellowtail larvae have the highest growth rates and survival when reared under continuous light (24L:0D), but the optimal photoperiod for juveniles is not known. In 1995, yellowtail larvae were produced at Memorial University's Ocean Sciences Centre. In 1996, the fish were divided into replicate groups (n=40) of 0+ juveniles (normally pigmented and hypomelanized) and placed under one of three photoperiods (12L:12D, 18L:6D, 24L:0D). Growth measurements (wet weight, standard length and width) were taken every two weeks over the 10-week study period. No significant differences ($P > 0.05$) were found among the treatments or between the pigmentations for any of the growth measurements. Growth and survival of juvenile yellowtail flounder seem to be unaffected by the occurrence of hypomelanosis or by reducing the length of the photoperiod from 24 to 12 hours per day.

Introduction

Yellowtail flounder (*Pleuronectes ferrugineus*) are a relatively small pleuronectid occurring along the east coast of North America from the Strait of Belle Isle to Chesapeake Bay.[3] Yellowtail, along with several other species, are currently being investigated for their potential as a cold-ocean aquaculture species.

Photoperiod is an environmental factor that influences growth in some species of fish.[4] Previous experiments have determined that higher growth rates and survival of larval yellowtail flounder occur under continuous lighting (24L:0D)[5] Also, a large percentage of yellowtail flounder exhibit abnormal pigmentation under culture conditions. The performance of these fish compared to normally pigmented fish is not known.

The objectives of this study were to determine if continuous lighting is required for optimal growth and survival of juvenile yellowtail flounder, and to determine if yellowtail flounder with hypomelanized pigmentation have growth rates or survival different from normal fish.

Materials and Methods

Three photoperiod regimes (12L:12D, 18L:6D, 24L:0D), with 3 replicates each, were chosen as experimental treatments. Each replicate contained 30 normally pigmented and 10 hypomelanized 0+ juvenile yellowtail flounder. The fish were placed in black buckets immersed in a water bath to control temperature fluctuations. Lighting was provided by a 100-watt incandescent bulb. External light was prevented from entering the experimental tanks. The fish were fed 4% of their body mass every second day.

Sampling was undertaken every two weeks over the 10-week study period. Bulk weights were taken of each replicate by catching the fish in a net, draining the net, and dumping the fish into a pre-weighed basin of water. Seven normally pigmented and 3 hypomelanized fish were arbitrarily chosen from each bucket as

random samples. One of the replicates under continuous light was discontinued during the last week of the experiment because problems with the water supply resulted in a large number of mortalities in that replicate. In order to obtain consistent sample sizes between treatments, 11 normally pigmented and 4 hypomelanized fish were taken from the remaining two replicates under continuous lighting on the final sampling day. For each sample, wet weight (grams), standard length (cm), and width (greatest distance (cm) from base of dorsal fin to base of anal fin) were measured. Data were converted to natural logarithms. Replicate and pigmentation data were analysed independently of treatment with the week of the experiment in a 2-way ANCOVA. The treatment data were then analysed with the week of the experiment in a 1-way ANCOVA.

Results and Discussion

Except for the fish that died because of the water problem, only 4 yellowtail founder died over the study period. As a result, survival data were not further analysed. ANCOVA results showed no significant differences for any of the growth measurements between the normally pigmented and the hypomelanized fish, or among the photoperiod treatments ($P > 0.05$).

The fish grew faster towards the end of the experiment. During this period the average temperature was above 10°C, whereas at the beginning of the experiment it averaged below 10°C. It is possible, however, that the increase in growth rate was due to the fish being acclimated to the experimental conditions for a longer period of time, and not to the increase in temperature.

Conclusions

From this study it seems that continuous light is not required for optimal growth and survival of juvenile yellowtail flounder, as reducing the hours of light from 24 to 12 per day had no apparent effect. It is also evident that normally pigmented and hypomelanized juvenile yellowtail flounder do not have different growth rates or survival.

The authors wish to thank Danny Boyce for his help in conducting this experiment and Dr. David Schneider for his advice on the statistical analysis.

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Do digestive enzymes set a physiological limit upon growth rate and conversion efficiency in Atlantic cod (*Gadus morhua*)?

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The objective of this study was to determine potential sites of maximal growth limitation in Atlantic cod (*Gadus morhua*). The experimental set-up consisted of 5 groups of 8 fish that received different concentrations of bovine growth hormone (0,1,2,4,10 g/g fish/2 weeks) administered by intra-abdominal injections. Fish were fed *ad libitum* during the 4-week experimental period. Individual ingestion rates, conversion efficiencies and growth rates were measured. The following enzyme activities were determined: intestinal glutamyl transferase and alkaline phosphatase, trypsin and chymotrypsin in the pyloric caecum. We found no correlation between treatment with bovine growth hormone and growth rate, conversion efficiency, ingestion rate nor enzyme activities. The relationship between growth rate and trypsin or chymotrypsin activity was stronger than the relationship between ingestion rate and the same enzymes. We suggest that the relationship between growth rate and enzyme activity (trypsin) may partly be explained by the effect of this enzyme on food conversion efficiency.

Introduction

After reaching maximal growth rate, fish tend to increase their ingestion rate with no concomitant increase in growth rate. This suggests that maximal growth rate is not limited by the rate of food ingestion but rather by processes subsequent to food ingestion such as food digestion, nutrient transport, and tissue synthesis.[3] In endotherms, the upper limit to the sustainable energy budget is set by the capacity of the digestive tract to assimilate nutrients from food.[4-8] The same limitation may be present in fish.[3] Torrissen and his team have shown that certain isozymes of trypsin (TRP-2) are linked to higher growth rate in salmon.[9-12] Protein digestion is more efficient in salmon with the izozyme TRP-2.[13] Furthermore, partial or total replacement of fish meal in the diet of rainbow trout (*Salmo gairdneri*) by soybean meal caused a significant decrease in growth rate and in amino acid absorption,[14] which seems related to trypsin inhibition by antinutritional factors in soybean meal. Another study on Arctic charr (*Salvelinus alpinus* L.) has also demonstrated a decrease in food consumption, growth, and proteolytic activity with a diet based on soybean meal.[15] Finally, an unpublished study on Atlantic cod[16] has demonstrated a significant relationship between trypsin activity (expressed in U total g fish^{-1}) in pyloric caecum and maximum growth rate or conversion efficiency.

The objective of the present study was to identify digestive enzymes that could potentially set a limit upon maximal growth rate in juvenile Atlantic cod. In order to obtain fish with maximized growth, we used bovine growth hormone injections.

Methods

Forty-nine cod from the Gulf of St. Lawrence were kept in individual compartments in rearing tanks at the Department of Fisheries and Oceans

Table 1. Linear regression results for all parameters measured and enzyme activities.

	GLT Activity	ALK Activity	TRY Activity	CHY Activity
Digestion Rate	$R^2 = 0.11$ $P = 0.06$	$R^2 = 0.42$ $P < 0.01$	$R^2 = 0.22$ $P < 0.01$	$R^2 = 0.17$ $P = 0.01$
Conversion Efficiency	$R^2 = 0.01$ $P = 0.60$	$R^2 = 0.11$ $P = 0.07$	$R^2 = 0.13$ $P = 0.04$	$R^2 = 0.06$ $P = 0.20$
Specific Growth Rate	$R^2 < 0.01$ $P = 0.94$	$R^2 = 0.44$ $P < 0.01$	$R^2 = 0.37$ $P < 0.01$	$R^2 = 0.29$ $P < 0.01$

in Mont-Joli, QC. Fish were divided into 5 groups of 8 cod and were injected intra-abdominally with different concentrations (0, 1, 2, 4, 10 µg /g fish/2 weeks) of bovine growth hormone (Monsanto, Canada). The control group was sham-injected (saline solution). Fish were fed capelin, *Mallotus villosus*, *ad libitum* 3 times a week during the 4-week experimental period. Fish were weighed and measured at the beginning, after 2 weeks, and at the end of the trial. Individual growth rates, ingestion rates and conversion efficiencies were measured in the last 2 weeks. The different digestive tissues collected were frozen at -80°C.

Intestinal glutamyl transferase (GLT) and alkaline phosphatase (ALP) activities were measured using Sigma© diagnostics kits. Trypsin (TRY) and chymotrypsin (CHY) activities were measured in pyloric caecum following Bergmeyer[17] and Rao and Lombardi[18] respectively. Activities of GLT and ALP were expressed in units by gram of tissue and the other enzyme activities were expressed as total activity per gram (TRY) or kilogram (CHY) of cod wet weight.

Differences among groups were tested using Kruskal-Wallis analyses. The relationships between the different parameters (growth rate, ingestion rate or conversion efficiency) and enzyme activities were tested using simple linear regressions.

Results

We found no correlation between treatment with bovine growth hormone and growth rate, ingestion rate, nor conversion efficiency. However, a relationship between ingestion rate (in the range of 0 to 5% body mass per day) and ALP activity ($R^2 = 0.42$) was noticed (Table 1). The relationship between ingestion rate and TRY ($R^2 = 0.22$) or CHY ($R^2 = 0.17$) activities was significant, albeit weaker. The relationship between conversion efficiency (range of 0 to 50%) and activities were significant for TRY ($R^2 = 0.13$). We found a significant relationship between specific growth rate (range of 0 to 1.5% body mass per day) and activities of the following enzymes : ALP (R2 = 0.44), TRY (R2 = 0.37) and CHY (R2 = 0.29). There was no relationship between ingestion rate, conversion efficiency, nor growth rate (in the same range) and GLT activity.

Discussion

The relationship between growth rate and trypsin or chymotrypsin activity is stronger than the relationship between the same enzymes and ingestion rate. Hence, differences in ingestion rate can not explain all the variation in growth performance among fish. Since trypsin activity is significantly related to conversion efficiency, this could explain why there is a stronger relationship with growth rate than with ingestion rate. However, alkaline phosphatase activity was as much correlated with ingestion rate as with growth rate. Thus it appears that the activity of ALP is adjusted with the quantity of food present in the intestinal tract.

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Stabilisation et conditionnement sexuels en viviers de l'oursin vert, *Strongylocentrotus droebachiensis*, de la région de Pointe-au-Père (Québec, Canada)

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L'oursin vert de l'estuaire du St-Laurent représente un potentiel commercial intéressant, tout particulièrement en regard du marché japonais. Des conditions climatiques contraignantes limitent le développement de cette industrie. Des expériences ont été menées en 1996 à l'INRS-Océanologie dans le but de produire des gonades de taille commerciale en milieu contrôlé. Les résultats de ces travaux ont montré qu'on peut maintenir l'indice gonadique (I.G.) d'oursins matures sur une longue période durant le printemps et l'été. Le conditionnement sexuel d'individus dont l'I.G. est inférieur à 10% est réalisable en automne sur une période d'environ 35 jours. Des gains allant jusqu'à 100% de l'I.G. sont ainsi obtenus. Ces résultats permettent de croire que l'application à l'oursin vert des techniques de stabilisation et de conditionnement sexuels pourrait contribuer de façon significative à la valorisation de la ressource.

Introduction

La forte demande des marchés étrangers, essentiellement le Japon, pour l'oursin a stimulé l'intérêt pour cette ressource. Cependant, les activités commerciales d'exploitation de l'oursin, malgré les efforts investis durant les vingt dernières années[3-5], demeurent marginales. Cette difficulté à développer cette industrie est principalement attribuable aux contraintes climatiques qui empêchent l'accès à la ressource durant l'hiver alors que la qualité de la gonade est optimale et que la demande est la plus forte, et qui imposent un cycle de reproduction déterminant de faibles indices gonadiques durant l'été et l'automne alors que la ressource est accessible.

Les techniques de conditionnement et de stabilisation sexuels, d'abord expérimentées en vue de contrôler la reproduction chez les mollusques[6], ont permis à la conchyliculture de se développer en assurant la disponibilité des gamètes sur une base quasi permanente[7,8]. Plus récemment, la mise au point de diètes semi-naturelles usinées[9,10] a permis de réaliser des progrès importants pour le développement des techniques de conditionnement de l'oursin.

La problématique entourant l'exploitation de l'oursin sous nos conditions climatiques suggère que l'utilisation de techniques aquicoles telles que le conditionnement et la stabilisation sexuels en milieu contrôlé constitueraient des éléments essentiels à la valorisation de la ressource. L'objectif de ce projet était de vérifier le potentiel de ces techniques appliquées à l'oursin vert de l'estuaire du St-Laurent. Les résultats rapportés ici concernent plus particulièrement le suivi de l'indice gonadique réalisé durant les expériences effectuées avec différentes diètes semi-naturelles à la station aquicole de l'INRS-Océanologie en 1996.

Matériel et Méthodes

Les oursins ont été prélevés manuellement en plongée sous-marine dans le secteur ouest du quai de Pointe-au-Père et transférés dans les bassins de la station aquicole. Après quelques jours d'acclimatation en bassins, les oursins ont été sélectionnés pour leur taille (environ 50 mm et 55 g) et répartis dans des paniers de plastique perforés immergés dans des bassins individuels de 50 litres où l'eau de mer filtrée sur gravier circulait au taux de un renouvellement à l'heure. La charge des bassins a été maintenue à 60 kg/m^3. Chaque groupe expérimental était constitué de quatre paniers dont un servait à main-

Tableau 1. Composition des différentes diètes utilisées lors des tests de stabilisation et de conditionnement sexuels de l'oursin vert effectués à la station aquicole de l'INRS–Océanologie en 1996.

Ingrediénts	Diète (% du poids)			
	D1[1]	D2	D3	D4
Algue hydrolysée[2]	41.5	40.0	39.9	33.0
Gluten de blé	21.0	21.0	21.0	21.0
Amidon modifié	29.0	28.0	25.0	24.0
Phosphate de calcium	2.0	2.0	2.0	2.0
Carbonate de calcium	2.0	2.0	2.0	2.0
Sulfate de calcium	2.0	2.0	2.0	2.0
Salmon vit.[3]	1.3	1.3	1.3	1.3
Chlorure de choline	0.7	0.7	0.7	0.7
Acide ascorbique	0.5	0.5	0.5	0.5
Spiruline	—	2.5	2.5	2.5
Huile de foie de morue	—	—	4.0	—
Débris de crevette	—	—	—	10.0

[1] diète D3-92 selon 11
[2] *Laminaria longicuris* préparée selon 12
[3] mélange de vitamines C-1236 de la compagnie Corey Feed Mills Ltd., Frédéricton, N.B.

tenir les oursins destinés à remplacer les individus échantillonnés dans les trois autres paniers. Les échantillonnages (3 oursins par paniers, soit 9 échantillons par traitement) étaient réalisés hebdomadairement ou bi-hebdomadairement selon le cas. Les mesures suivantes étaient alors enregistrées: le poids total de l'animal égoutté pendant une heure, le diamètre du test, le poids des gonades égouttées pendant cinq minutes sur un tampon absorbant. L'I.G. de la population naturelle d'origine des oursins expérimentaux a été suivi parallèlement aux expériences. L'I.G. a été calculé de la façon suivante: I.G.(%) = (poids des gonades/poids de l'individu) X 100.

Les groupes nourris étaient alimentés des diètes décrites au tableau 1. Deux séries d'expériences ont été menées: la première (exp.1) a consisté à stabiliser sexuellement des oursins matures (I.G. de 15%). Deux groupes d'oursins ont été nourris respectivement des diètes D1 et D3 (tableau 1) au taux de 0.5% du poids vif par jour alors qu'un groupe témoin (T1) était maintenu sans nourriture. La température de l'eau de mer a été maintenue entre 4.5°C et 6.5°C. L'expérience a été menée entre le 22 mai et le 17 juillet 1996. La photopériode a été ajustée à 10L:14N. La deuxième expérience (exp. 2) consistait à conditionner quatre groupes d'oursins à partir des diètes D1, D2, D3 et D4 (tableau 1). Deux groupes témoins, l'un nourri d'algues décongelées (*Laminaria longicruris*) (T1) et l'autre ne recevant aucune nourriture (T2) ont été suivis parallèlement. Tous les groupes nourris, y compris le témoin nourri d'algues, recevaient en nourriture l'équivalent de 1% de leur poids vif par jour. La température n'a pas été contrôlée de telle sorte qu'elle a diminué de 9°C à 5°C entre le début (30 septembre 1996) et la fin (7 novembre 1996) de l'expérience. La photopériode a été maintenue à 14L:10N.

Résultats et Discussion

L'ensemble des résultats est montré à la figure 1. Les encadrés B et C montrent les résultats détaillés des deux expériences (B=exp.1; C=exp. 2). L'encadré A montre les résultats des tests *post hoc* aux analyses de variance. Les courbes T et N ont été tracées à partir des moyennes des témoins (T) et des groupes nourris (N) pour chacune des expériences. La courbe (PN) représente le suivi de l'I.G. de la population naturelle de la région de Pointe-au-Père (n=20 pour chaque point). Un effet très net de la nutrition sur la croissance des gonades est observé dans les deux expériences. Les groupes nourris se démarquent de façon hautement significative des groupes témoins à la fin des expériences tel que le confirment les analyses de variance effectuées sur ces données (exp.1: $F(2, 51)=22.86$, $P<0.001$); exp.2 : $F(5, 113) =20.19$, $P<0.001$). Les tests *post hoc* de Tukey n'indiquent cependant aucun effet de la composition de la diète dans les deux expériences (fig. 1A), cela même si le groupe D3 affiche dans chaque cas un avantage sur les autres diètes (fig. 1B et 1C). Des gains de l'I.G. dépassant 30% (I.G. passant de 15% à 20%) ont été observés avec la diète D3 durant l'expérience 1 alors que cette même diète donnait des gains de 100% de l'I.G. (I.G. passant de 8.5% à 17%) durant l'expérience 2. Ces résultats ont été obtenus après une période de nutrition de 4 à 5 semaines. Le conditionnement automnal (exp. 2) a été obtenu à la température ambiante. Ce résultat est intéressant puisqu'il

montre qu'on peut conditionner les oursins dans la gamme de températures de 5°C à 10°C sans avoir à contrôler la température, opération qui s'avère généralement coûteuse. La diète composée uniquement d'algue (T1 de l'exp. 2) n'a pas donné de résultats positifs. Ce résultat a déjà été observé ail-leurs[11]. Il se pourrait que la quantité d'algue administrée (1% du poids vif par jour) ait été insuffisante pour supporter la croissance des gonades. On a d'ailleurs observé que les algues étaient très rapidement consommées dans les heures qui suivaient la distribution de nourriture. La spiruline et les débris de crevette ne semblent pas conférer d'avantages pour la croissance des gonades. Ces produits, riches en pigments caroténoïdes, pourraient contribuer à l'amélioration de la couleur des gonades. L'analyse préliminaire des données portant sur cet aspect de l'expérience ne permet cependant pas d'identifier une tendance en ce sens.

Les résultats de ces travaux montrent qu'il est possible d'appliquer avec succès les techniques de stabilisation et de conditionnement sexuels à l'oursin vert de l'estuaire du St-Laurent. Les avantages qui en résultent pour la croissance des gonades sont suffisamment intéressants pour justifier l'expérimentation de cette technologie à une plus grande échelle. L'amélioration et la standardisation de la couleur et du goût des gonades ainsi que la mise au point d'un système d'élevage à forte densité devraient permettre, dans une prochaine étape, de décrire une technique applicable industriellement.

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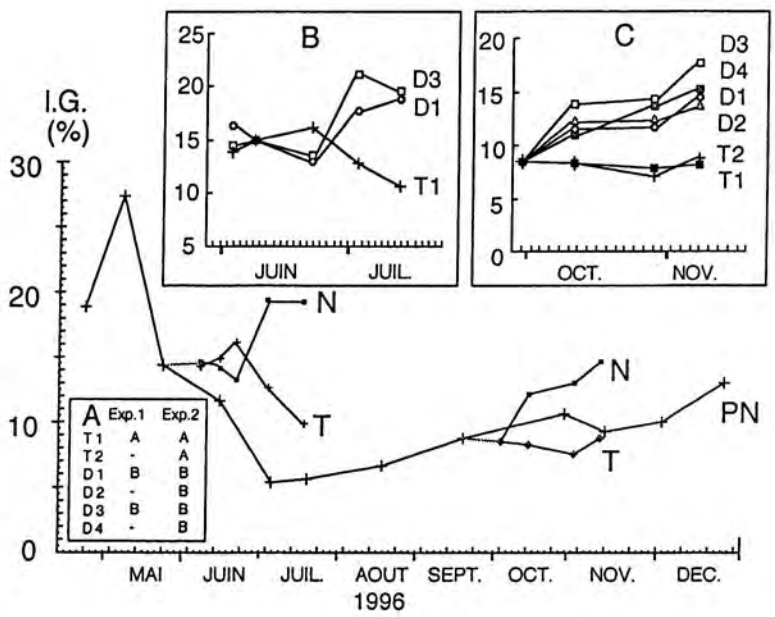

Figure 1. Suivi de l'indice gonadique mesuré dans le milieu naturel et dans l'ensemble des lots expérimentaux durant les tests de stabilisation et conditionnement sexuels réalisés à la station aquicole de l'INRS-Océanologie en 1966.

Détermination de la zone de tolérance à la salinité du turbellarié *Urastoma cyprinae* à l'état libre et en association avec l'huître américaine *Crassostrea virginica*

E. Bataller and A.D. Boghen[1]

Urastoma cyprinae est un turbellarié retrouvé dans plusieurs espèces de mollusques bivalves, dont l'huître américaine *Crassostrea virginica*. Des recherches récentes nous laissent croire que le parasite peut nuire à son hôte. Une série d'expériences sur la résistance à la salinité de ce turbellarié ont été effectuées en soumettant des parasites prélevés des huîtres, à des salinités variant entre 0 et 60 ‰, pour chacune des températures suivantes: 5, 15 et 22°C. Les résultats démontrent que les seuils de tolérance inférieur et supérieur de ce ver sont respectivement de 12 et 40 ‰. Ces données s'appliquant aux parasites isolés de leur hôte, il fallait déterminer si des *U. cyprinae* laissés dans les huîtres démontreraient le même niveau de sensibilité. Des huîtres parasitées ont donc été soumises à des salinités de 11 et 55 ‰ (dont on sait qu'elles sont létales à la suite de l'expérience précédente) ainsi qu'à 28 ‰ (salinité témoin), à chacune des températures suivantes: 5, 15 et 20°C pour une période de 6 jours. Les résultats préliminaires semblent indiquer que les parasites présents dans les huîtres maintenues à une température de 15 ou 20°C, à une salinité de 11‰ meurent au bout de 1 à 4 jours. Notre objectif ultime est de proposer un protocole visant à éliminer *Urastoma cyprinae* des huîtres infectées.

Introduction

L'industrie ostréicole du Nouveau-Brunswick a connu divers problèmes durant les dernières décennies, soit reliés à des facteurs environnementaux spécifiques à certaines régions, soit reliés au fait que le Nouveau-Brunswick constitue la limite nord de la zone de répartition de l'huître américaine *Crassostrea virginica*.

La côte est du Nouveau-Brunswick a été jusqu'ici épargnée par la plupart des maladies importantes telles que Dermo (*Perkinsus marinus*) ou SSO et MSX (*Haplosporidium sp.*), qui ont nui à l'industrie ostréicole américaine. Cependant, depuis quelques années, un Turbellarié, *Urastoma cyprinae*, est considéré comme une espèce nuisible à l'huître. Ce ver à d'abord été décrit par Drinnan et Burt.[2] Au Canada Atlantique, on a rapporté une incidence élevée d'*Urastoma cyprinae* dans des huîtres affaiblies de la baie des Chaleurs.[3] Plus récemment, Robledo *et al.*[4] ont observé des dommages significatifs aux branchies de la moule bleue (*Mytilus edulis*) parasitées par *U. cyprinae*. Ils ont aussi insisté sur le fait que ce parasite pourrait représenter un danger sérieux pour l'industrie mytilicole du nord de l'Espagne. A cause de l'importance croissante de l'industrie ostréicole dans le Canada Atlantique, il est important de déterminer jusqu'à quel point le parasite représente une menace pour l'industrie.

Une série d'expériences utilisant des parasites isolés à partir d'huîtres infestées ainsi que des huîtres parasitées ont été élaborées pour déterminer le niveau de tolérance de *U. cyprinae* à diverses combinaisons de température-salinité.

Matériel et Méthodes

Turbellariés isolés

Les huîtres infestées ont été récoltées dans la baie de Caraquet et transportées sur glace à l'Université de Moncton. Elles ont ensuite été ouvertes, et les parasites prélevés à l'aide d'une pipette Pasteur ont été entreposés dans des béchers d'eau salée (22 ‰, 5°C).

Trois groupes de 3 pétris contenant 10 parasites ont été placés dans un réfrigérateur (5°C), un incubateur (15°C) et à 22°C, température de la pièce. Les températures choisies représentent respectivement l'environnement naturel de l'huître américaine en hiver, au printemps/automne et en été au Canada Atlantique. A chaque température, la salinité de l'eau des trois groupes était respectivement de 15, 23 et 30 ‰, correspondant à peu près aux conditions d'une rivière, d'un estuaire et de la pleine mer dans la région d'où proviennent les huîtres. Des salinités additionnelles de 0, 4, 8, 10, 11, 12, 13, 45, 55 ‰ et saturation ont aussi été testées.

Les parasites morts ont été enlevés chaque jour pour 65 jours et les L_{T50} déterminés.

Turbellariés infestant les huîtres

18 huîtres infestées par *U. cyprinae* ont été gardées à chacune des températures étudiées pour les parasites isolés, soit 5, 15 et 22°C. A chacune des températures, 6 huîtres étaient maintenues dans de l'eau de salinité de 11 et 55 ‰, salinités létales aux parasites isolés (tel que déterminé dans l'expérience précédente) et à une salinité optimale de 28 ‰.

Chaque jour, 1 huître provenant de chacune des 9 combinaisons de température-salinité était ouverte afin de déterminer si les parasites étaient toujours vivants.

Résultats et Discussion

Turbellariés isolés

Le taux de survie de *Urastoma* est principalement influencé par la température, le L_{T50} est de 4 jours à 22°C, de 8 à 20 jours à 15°C et de 64 jours à 4 mois à 5°C. D'autre part, les salinités inférieures à 12 ‰ et supérieures à 40 ‰ sont létales pour *U cyprinae* (Fig. 1).

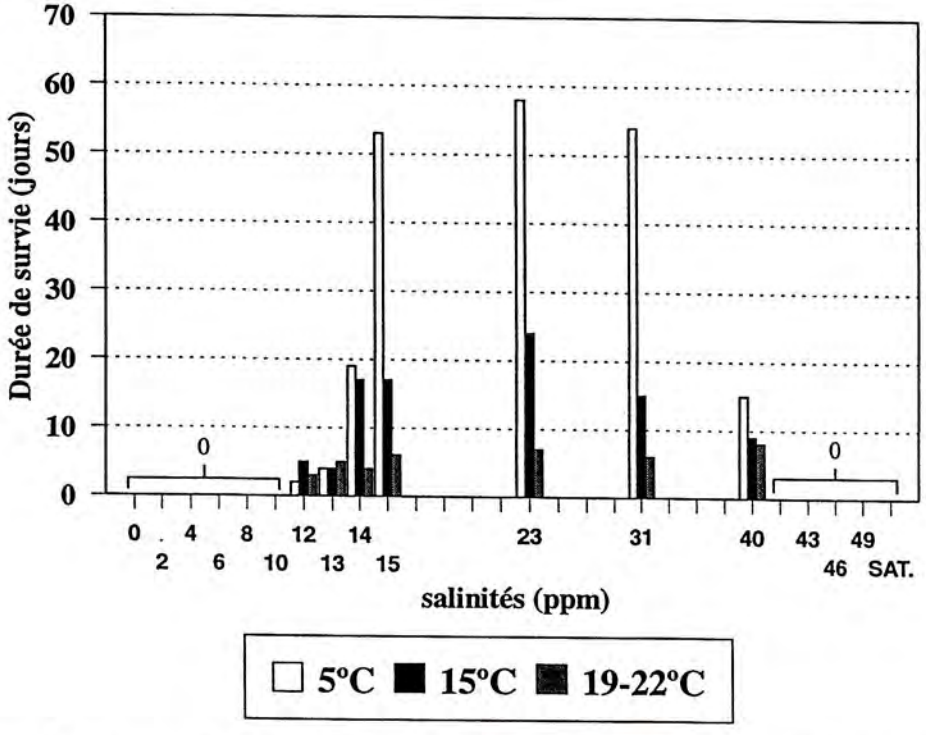

Figure 1. Figure montrant le taux de survie d' *Urastoma cyprinae* maintenu à des températures de 5, 15 et 19-22°C à diverses salinités.

Turbellariés infestant les huîtres

Tous les parasites présents dans les huîtres maintenues à 28 ‰ ont survécu jusqu'à la fin de l'expérience. Cependant, dans le cas des huîtres maintenues à 11 ‰ et à 55 ‰, les parasites présents dans les huîtres gardées à 20°C sont morts en moins de 24 heures. Ceux présents dans les huîtres maintenues à 15°C ont survécu 2 à 4 jours. Les vers sont morts après 5 jours dans les huîtres gardées à 5°C (Fig. 2).

L'objectif final de notre recherche est d'élaborer une technique permettant d'éliminer le parasite des huîtres infestées sans causer de dommage à l'hôte. L'approche que nous prévoyons utiliser est celle appliquée pour la dépuration, où les huîtres parasitées sont immergées dans un milieu de salinité et de température optimales pour une certaine période de temps.

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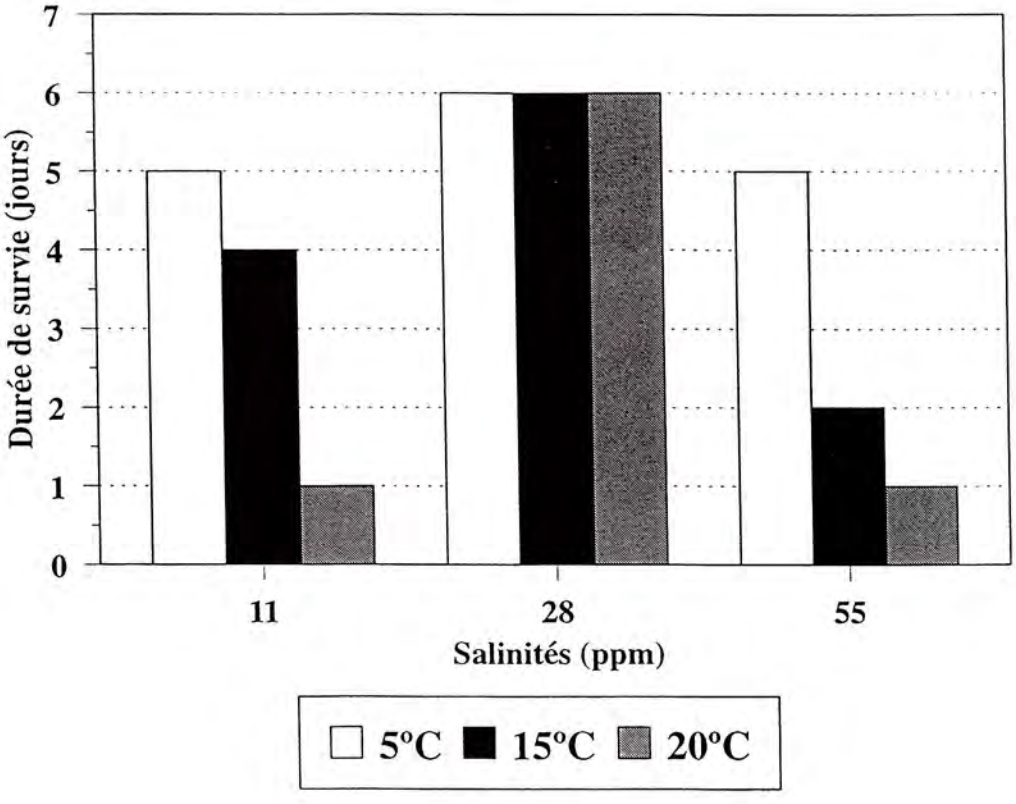

Figure 2: Figure illustrant le taux de survie d'*Urastoma cyprinae* infestant l'huître américaine *Crassostrea virginica* à des températures de 5, 15 et 19-22°C, et des salinités létales (11 et 55 ‰) et optimales (28 ‰) pour le parasite isolé.

The possible role of mucus in the feeding behavior of the turbellarian *Urastoma cyprinae* in the oyster *Crassostrea virginica*

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

The turbellarian *Urastoma cyprinae* occurs on the gills of various bivalve species, including the American oyster, *Crassostrea virginica*. Recent findings suggest that the "gill-worm" might have a negative impact on the health of this molluscan host. Studies were undertaken to establish if *U. cyprinae* is attracted to mucus and/or mucus-bound organs and how this might translate into a specific type of feeding behavior. Turbellarians were removed from infected oysters and exposed to a variety of attractants. Results indicate that *U. cyprinae* displays a significant preference for homogenates containing gill tissue compared to other substrates. Direct observations of feeding behavior using endoscopic techniques reveal that there is extensive contact between the worms and gill tissue, especially in those areas where the flow of mucus is most highly concentrated.

Introduction

To date, research on molluscan diseases has focused primarily on microbial and protozoan pathogens. Less attention has been directed at the potential impact of metazoan parasites. One organism in particular, the turbellarian *Urastoma cyprinae,* is recurrent in oysters from the Gulf of St. Lawrence.[3,4] This "gill-worm" has been reported in various bivalve species throughout the world, including clams[5] and mussels.[6-8] Contrary to earlier interpretations that *U. cyprinae* is an occasional commensal,[9,10] recent investigations have demonstrated that the turbellarian can have a negative impact on the health of its host and may, at least in certain instances, be parasitic.[8] Previous work has demontrated that there is a definite attraction of *U. cyprinae* to oysters.[11] Furthermore, some authors suggest that *U. cyprinae* feeds on mucus that is present in oysters[12] and is known to be concentrated on the gills.[13] This, however, has not been demonstrated scientifically. The objective of the current study is to determine if in fact *U. cyprinae* is more attracted to mucus-coated gills than to other parts of the body and to relate this to the feeding behavior of *U. cyprinae* in oysters.

Material and Methods

Twelve samples of 30 to 35 oysters were collected from the Bay of Shippagan, New Brunswick between July and September 1996. The oysters were usually examined within 48 hours after they were transported to the laboratory. Turbellarians were isolated from the gills of oysters and were subsequently divided into groups of 100. They were maintained in filtered sea water (25 ppt) at 4°C for 1 to 2 days. Turbellarians were acclimated to room temperature (22-23°C) for 12 hours, and experiments were conducted in total darkness. The stimulants tested (6 replicates x 100 worms/replicate) consisted of homogenized oyster tissue, isolated gill tissue and oyster homogenates free of gills.

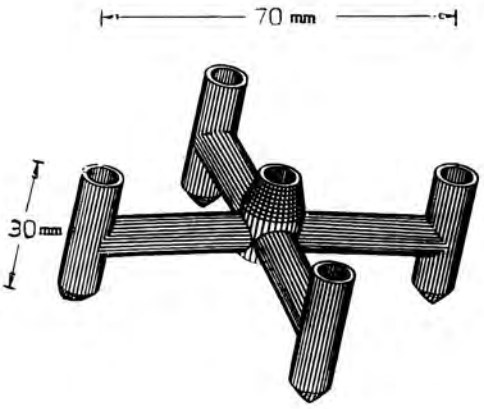

Figure 1. Glass chamber used in experiments conducted with *U. cyprinae*.

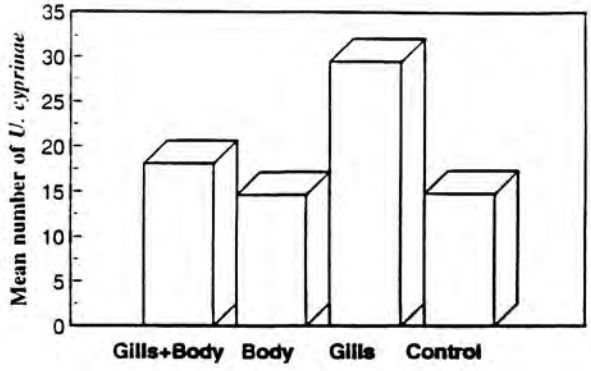

Figure 2. Mean number of *U. cyprinae* counted in the wells containing the stimulants tested. Gills+Body = homogenized oyster tissue; Body = oyster homogenate free of gills; Gills = isolated gill tissue; Control = filtered sea water (25 ppt).

Filtered sea water (25 ppt) served as a control. Concentrates of the stimulants were prepared by centrifuging substrate and sea water in the following proportions: 1:10 (v/v) substrate:filtered sea water (25 ppt). The concentrates were placed in one of four wells of specially designed glass chambers (Fig. 1). The turbellarians were introduced into the central opening of the chamber and after 60 minutes each of the four wells was drained and the worms were counted. The data were analyzed using the Student's *t*- test.

Endoscopic techniques were employed for direct *in vivo* observations of *U. cyprinae* in oysters. Oysters were prepared by trimming a small section (30 mm in length x 5 mm in width) of the inhalent margin of the upper and lower valves. For purposes of observation, the oysters were held in a static seawater system. The endoscope (optical insertion tube, 1.7 mm diameter; Olympus Corp.) was connected to an optical zoom adapter and attached to a video camera (Cohu, Inc.). The optical insertion tube of the endoscope was inserted into the pallial cavity of the oysters. The video camera with attached endoscope was mounted onto a micromanipulator, allowing three-dimensional adjustments. Images were recorded on an 8 mm VCR (Hi8, Sony), and were subsequently analyzed. Results are based on the endoscopic observations of *U. cyprinae* from a total of 12 oysters.

Results and Discussion

U. cyprinae displays a preference for isolated gill tissue compared to the other stimulants (Fig. 2). The results presented in Table 1 demonstrate that while there is indeed a significant difference between the attraction of worms to the isolated gill tissue versus the control (P=0.052) and oyster homogenate free of gills (P=0.064), the difference in the degree of attraction is less evident between isolated gill tissue and homogenized oyster tissue (P=1.000). If we consider that molluscan gill tissue is coated with mucus, it is quite possible that the mucus may be a contributing factor in attracting the worms

Table 1. Results of Student's *t*-test to compare the number of *U. cyprinae* attracted to the tested stimulants. Body+Gills = homogenized oyster tissue; Body = oyster homogenate free of gills; Gills = isolated gill tissue; Control = filtered sea water (25 ppt).

		$\overline{X}_1$	$\overline{X}_2$	SE_1	SE_2	P
Body + Gills	Control	18.2	14.8	6.1	1.7	1.000
Gills	Control	29.5	14.8	3.9	1.7	0.052
Body	Control	14.7	14.8	4.4	1.7	1.000
Body + Gills	Gills	18.2	29.5	6.1	3.9	1.000
Body + Gills	Body	18.2	14.7	6.1	4.4	1.000
Gills	Body	29.5	14.7	3.9	4.4	0.064

to its host. Since the gills of oysters are heavily coated with mucus, our findings support the likelihood that the latter may play a role in attracting *U. cyprinae* to oysters.

Direct observations of *U. cyprinae* on the gills reveal that the worms are distributed along the ventral food groove and the medial regions of the gills. It is noteworthy, however, that they are more frequently observed along the dorsal ciliary tract, where mucus flow is highly concentrated. Furthermore, *U. cyprinae* displays certain behavioral patterns, such as body arching and repositioning on the gill filaments. This behavior maximizes contact between the worm and mucus-coated surfaces, while at the same time reinforcing the potential importance of the platyhelminth's tegument in food tranfer.

Notes and References

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Mussel *(Mytilus edulis)* spawning in the Amherst Basin (Magdalen Islands) between 1991 and 1996 and investigation of the potential trigger mechanisms

Marcel Roussy and Bruno Myrand [1]

Amherst Basin is the only site used for seed collection by the mussel growers of the Magdalen Islands (Gulf of St. Lawrence, Canada). Mussel spawnings were assessed between 1991 and 1996 from larval cohorts. Twelve spawnings were identified over these years and the first spawning of each year occured during the third week of June at the latest. The number of spawnings varied between years. In 1995, it was not clear if the mussels from the bed we were following contributed to the production of larvae. In 1996, two mussel beds were followed and probably only one contributed to the larval production. We were not able to identify clear trigger mechanisms among the following potential prospects: lunar or tidal phases, water temperature, chlorophyll a concentration, salinity, precipitation and wind.

Introduction

Amherst Basin is the only site used for seed collection by the mussel growers of the Magdalen Islands. The spat collected at this site offer a high resistance to summer mortality and have rapid growth. The spat are produced by mussels from natural beds which support some harvesting. Identification of spawnings and spawners would be helpful in managing the site and advising mussel growers on their operations. Furthermore, it should be possible to determine the mechanisms triggering mussel spawnings since Amherst Basin is a small and shallow lagoon with a homogeneous water column.

Material and Methods

Larval samples were taken twice a week at 3 stations between mid May and early September from 1991 to 1996. Approximately 1500 L were pumped from the surface to the bottom with a portable battery-powered pump and larvae were retained on a sieve with 53-µm mesh size. Larvae were counted and measured according to the procedure of Moisan and Myrand[2] using a Leica Q500MC image processing system. The presence of large numbers of 100-120 µm larvae indicated that a spawning had occured 2 days earlier.[2]

About 20 mussels of 55-65 mm shell length were sampled twice a week between mid May and mid September from one natural bed in 1995 and from two beds in 1996.[3] Their total flesh was dried at 65-70°C for 72 hours in pre-weighed aluminum pans and weighed to the nearest 0.0001 g. Sharp declines of tissue dry mass were used as indicators of spawning events since shell length of mussels was kept relatively constant between years and beds.[4, 5]

Circular statistics[6] were used to examine the relationship between spawning and lunar or tidal phases. We calculated a mean vector and axis (corrected for grouping), and we applied a Rayleigh's test for randomness to the circular and axial distributions. Relationships between other environmental parameters and spawnings were examined only for 1995 and 1996 to ease presentation. These two years were representative of the others. Water temperature was recorded hourly at one of the pumping stations. Chlorophyll a concentration was assessed twice

a week at the same station. Salinity was measured at the three pumping stations in 1995 and also above the mussel beds in 1996. Meteorological data were collected by Environment Canada at House Harbor airport.

Results

We identified 12 spawnings between 1991 and 1996 using the abundance of 100-120 μm larvae (Fig. 1). The first spawning of each year always occurred at the latest in the third week of June. The number of spawnings was quite variable between years. In 1995, the tissue dry mass of mussels from Pointe-de-la-Rivière did not decrease sharply, indicating these mussels did not contribute significantly to the larval production (Fig. 2). In 1996, the numerous larvae observed on 8 June were probably produced by mussels from Pointe-à-Marichite since their tissue dry mass decreased sharply between 6 and 10 June. Again, mussels from Pointe-de-la-Rivière did not contribute to the larval production.

Spawnings could not be related to the lunar or tidal phases ($R_c = 0.35$, $\phi = 207^0$, $P = 0.214$; $R_2c = 0.33$, $\phi = 74^0$, $P = 0.299$) even though there was an apparent relationship (Fig. 1). In 1995 and 1996 all the spawnings, except the first one in 1995, occured when the water temperature was over 10°C. Sharp changes in water temperature did not seem to trigger spawnings. Chlorophyll a concentration (max of 3.8 μg/L in 1995 and 2.5 μg/L in 1996) and salinity (25 to 28 ppm) showed no sharp variations before spawnings in 1995 or 1996. In 1995, abundant precipitation (76 mm) on 8 June was followed by a major spawning two days later, but it happened only once. The spawnings of 10 June 1995 and 8 June 1996 were preceded 2 days earlier by strong winds of 71 km/h and 53 km/h respectively. But the majority of the 12 spawnings over the 6 years did not follow this pattern.

Discussion and Conclusion

Larval monitoring is the best way to determine the time of spawning of mussels. The first spawning of each year occured at the latest in the third week of June. The mussel growers should immerse their collectors before the end

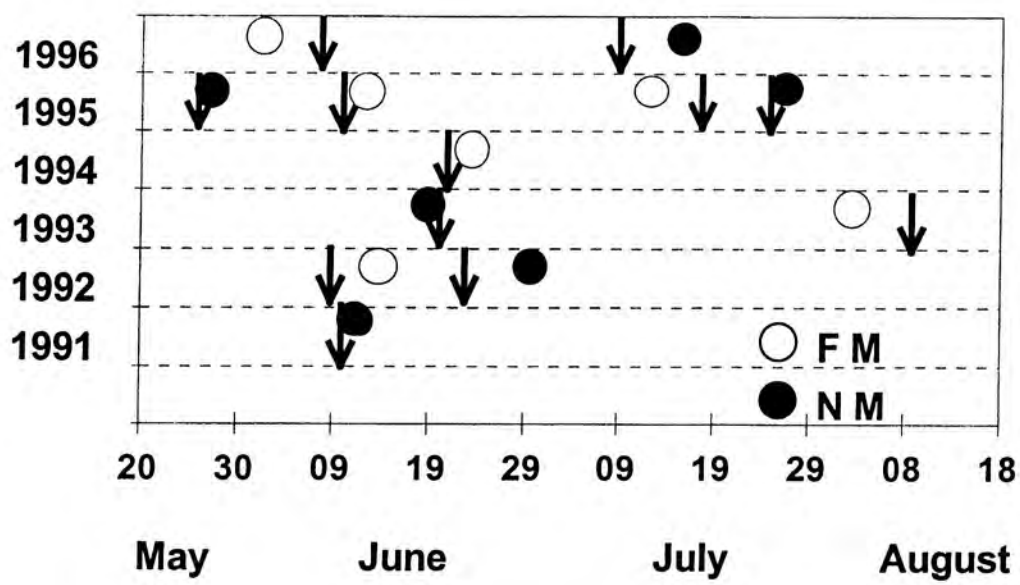

Figure 1. Mussel spawnings in Amherst Basin based on larval density (individuals/L) and length (μm) (arrows indicate spawning dates).

of June since the first spawning usually provides the bulk of the spat. The number of spawnings that occurred each year was variable. The identification of spawners from decreases in dry mass of mussel flesh was difficult because of the jagged patterns. Larval and mussel monitoring should begin as soon as possible in the spring to be sure that the first appearance of larvae and the decreases in tissue mass are detected. There was no statistical relationship between time of spawnings and lunar or tidal phases, but our sample was limited (n=12). The fact that nearly all spawnings occured at a temperature above 10°C suggests the need for a critical temperature to be reached. Spawnings were apparently not related to changes in chlorophyll a concentration or salinity. Yet salinity could change sharply above one mussel bed after strong precipitation. Precipitation and wind did not seem to be associated with spawning either. Although there was no apparent and systematic trigger mechanism, we still cannot exclude the possibility that events related to the lunar phases could induce mussels to spawn. Monitoring of these parameters will be adressed in more detail in 1997.

We would like to thank the technicial staff of the Station Technologique Maricole des Îles-de-la-Madeleine for their valuable work. We also thank the mussel growers who collaborated in this project.

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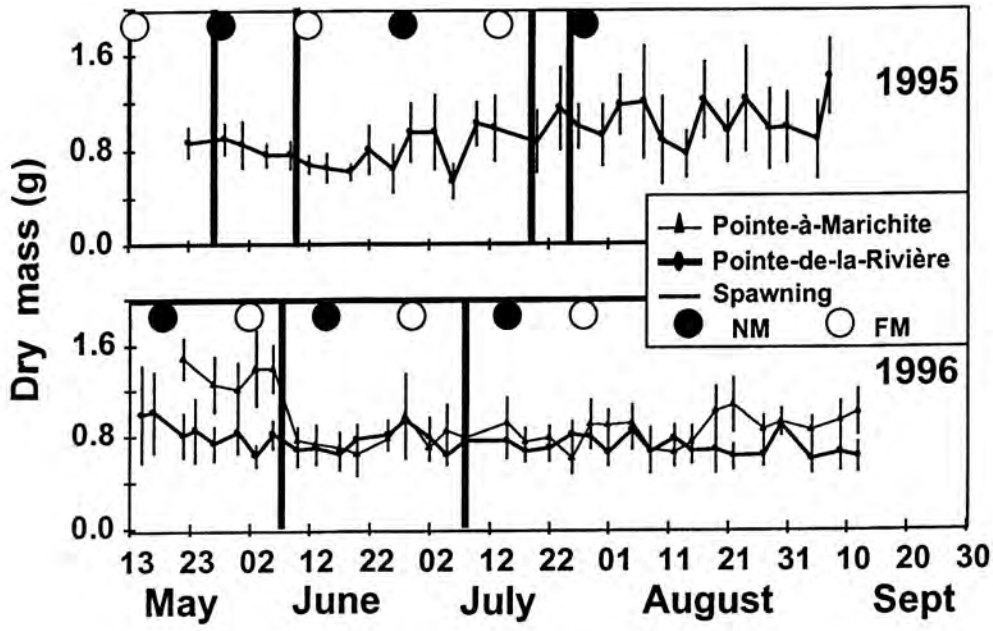

Figure 2 . Mean total flesh dry mass (± S.D.) of mussels of 55 to 65 mm shell length.

Scallop (*Placopecten magellanicus*) seeding trials in Îles-de-la-Madeleine, Québec, 1993 to 1996

M. Nadeau and G. Cliche [1]

Since 1993, 30 000 to 4 600 000 juvenile scallops have been seeded each year off Îles-de-la-Madeleine. To evaluate the success of commercial and experimental seeding, surveys were conducted using scuba divers, video cameras and drags. Results showed that scallop dispersion can reach 95% within one year of seeding. Scallop survival within the studied areas was between 33% and 50%. Most dead scallops were clappers (empty shells joined at the hinge) that were thought to be associated with sea star predation since handling before seeding induced little mortality. Data collected shortly after seeding showed that juvenile scallops from collectors, seeded at a size of 15 to 25 mm, tended to have higher survival than scallops from intermediate grow-out that were seeded at a size of 30 to 40 mm. However, these results may be biased by the fact that larger specimens are easier for divers to see. In contrast, previous laboratory trials showed that smaller scallops were prefered by sea stars. Tagged scallops collected during the surveys had mean growth rates of 2 cm per year.

Introduction

Seeding of giant scallops (*Placopecten magellanicus*) has been conducted on a commercial scale in the Îles-de-la-Madeleine since 1993. This activity is integrated within a research program (REPERE) that is evaluating the feasibility of scallop culture using juveniles seeded on suitable grounds. Different partners are involved in the seeding operation. The scallop fishermen's association of the Îles-de-la-Madeleine (APPIM) supplies and prepares the juvenile scallops for seeding. They also are responsible for the commercial seeding and participate in management of the stock. The Department of Innovation and Technology of the Quebec government (MAPAQ) is mostly involved in the surveys of newly seeded scallops to evaluate predation, dispersion, mortality and growth. The federal Department of Fisheries and Oceans (DFO) is involved in the surveys of natural and seeded populations. This paper presents briefly some data collected on the seeded grounds since 1993.

Sites

Seeding is conducted off southeast Îles-de-la-Madeleine on a natural scallop ground called "Chaîne-de-la-Passe" which is characterized by sand and rocky substrate and a depth of 32 m (Fig. 1). The 1993 to 1995 seedings were done in an area that has been closed to fishing since 1993. The 1996 seeding was performed in an area that is closed from 1996 to 1999. Evaluation of predators using drags, scuba divers and video camera showed a relatively high concentration of sea stars (*A. vulgaris*, *L. polaris* and *C. papposus*) compared to crabs (*C. irroratus* and *Hyas* sp.). Bottom temperature during the period chosen for seeding, November to June, stayed around 6°C. It appears that predatory activity is reduced at this temperature.[2,3] The current speed was generally moderate (20 cm/sec.).

Juvenile scallops

Scallops seeded between 1993 and 1995 measured between 30 and 40 mm and came from intermediate grow-out using pearl nets. However, the 1996 seed came from spat collectors (15 to 25 mm) as well as from intermediate grow-out. Before seeding, collectors and pearl nets were brought to the APPIM plant to be emptied. At this stage, about 30 000 scallops

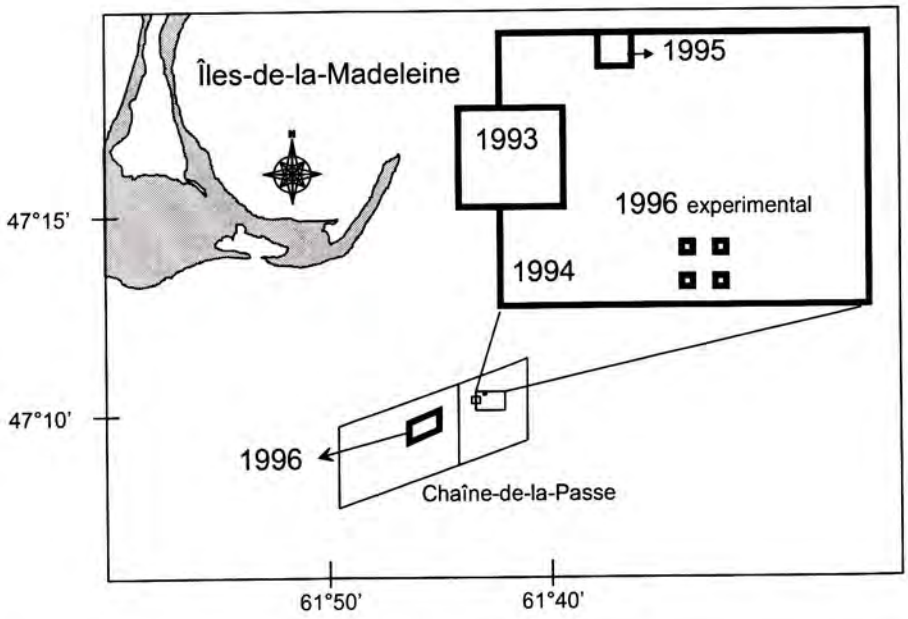

Figure 1. Approximate location of the 1993 to 1996 seeding sites off Iles-de-la-Madeleine.

were marked each year with a plastic colored tag glued on the upper valve. Different colors were used for each seeding year. Scallops were placed in plastic baskets that were easily transportable and taken to the chosen release sites on a fishermen's boat. The scallops were kept moist at all times and transportation time was about an hour and a half. Upon arrival at the site, the baskets containing the scallops were rapidly turned upside down along pre-determined north-south lines to ensure seeding was done at a uniform density.

Seeding

In November 1993, 30 000 juvenile scallops were seeded in an area of 0.2 km^2 (Fig. 1) whereas 1.4 millions scallops were scattered on an area of 1.6 km^2 in November 1994. In August 1995, 300 000 scallops were seeded on a smaller site of 0.05 km^2. The scallop density was thus increased to reach that used in Japanese seeding operations.[4] Finally, 4.6 million scallops were seeded in 1996 on an area of 1.9 km^2. Experimental seeding were also performed in 1996 on small areas of 25 and 50 m^2 in the spring and fall. Scallop survival following two different handling procedures was compared in the spring of 1996. In fall 1996,

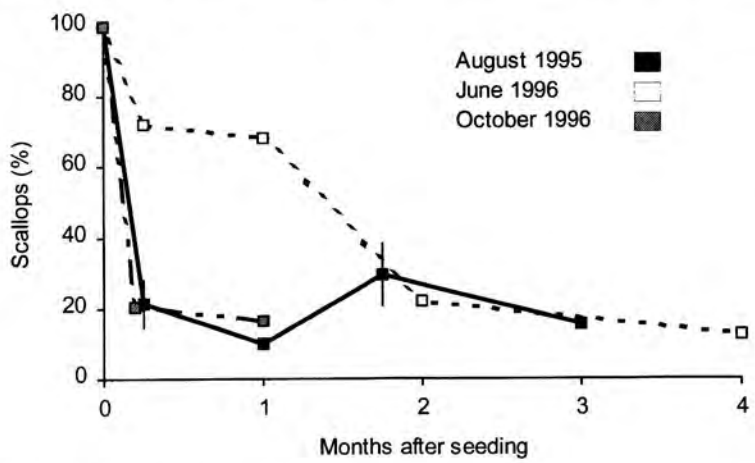

Figure 2. Percentage of juvenile scallops remaining in areas seeded in 1995 and 1996.

survival of smaller scallops from collectors was compared to larger ones from intermediate grow-out. Also, scallop mortality induced by commercial-scale handling was evaluated in November 1996.

Surveys of the commercially and experimentally seeded areas were done with divers and video cameras. The depth of the seeding grounds made it difficult for divers to survey as the time available to collect data on the bottom was very limited and the visibility was often poor. The video camera system provided relatively good results, although some improvements are needed.

Biological data

After only a few months, 85 to 99% of seeded scallops had moved outside the seeded areas. For example, about 80% of the juvenile scallops moved out of the seeded area within one week of the commercial seeding of August 1995 and the experimental seeding of October 1996 (Fig. 2). The scallops used in the experimental seeding of June 1996 were slower to move but after two months 80% had moved out of the area. Rapid dispersion was also noticed during experimental seedings in Nova Scotia[3,5] and in previous studies conducted in Îles-de-la-Madeleine.[6]

Scallop survival in and around the seeded areas was between 33 to 50% after a few months. This estimation was calculated from seeded areas where more than 25% of the seeded scallops have been found. The fate of missing scallops was not determined. They could all be alive or dead, or somewhere between these extremes. In Japan, the survival rate is between 26 and 33% for 30-mm scallops.[4] Survival rate in New-Zealand was 22% for 10-mm scallops.[7]

Most dead scallops sampled during the surveys were clappers with empty valves joined by the hinge (75%) compared to broken shells (25%). These clappers were thought to be associated with sea star predation because only low mortality (<5%) was caused by handling procedures. The experimental seedings revealed that larger scallops (30 to 40 mm) had a greater tendency to be decimated by sea stars. This contrasts with data in the literature[8] and previous laboratory trials that showed higher sea star predation on smaller scallops.

The mean shell length of tagged scallops collected by divers and drags was 52 mm one year after the seeding, 74 mm after two years and more than 87 mm after 3 years.

Future projects

In July 1997, the 1993 to 1995 seeded areas will be fished. More than 1.6 million scallops have been seeded by the APPIM on these sites. About 77 000 of these scallops were tagged. Fishing activity will receive particular attention because the data from catches are essential to evaluate the economic feasability of seeding operations.

Also, surveys of experimental and commercial seeding sites will be pursued. Over the next 5 years, there are plans to seed 5 to 10 million scallops each year. The vitality of juvenile scallops will be evaluated before seeding. The methodology for this evaluation will be fine-tuned this year. In addition, improvements to the video camera system are planned.

The 1997 fall seeding will be performed in the area that will be fished next July. For the next two years, new areas for seeding will have to be identified. Finally, it is important to find means to decrease the density of predators before seeding. It seems that juvenile scallops are particulary vulnerable just after seeding. In Japan, drags are used to clean the bottom before seeding.[4] In oyster culture, lime is currently used.[9] The application of these methods and others will be examined.

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Effect of fouling on current velocities in pearl nets of various mesh sizes

Manjula Devaraj and G. Jay Parsons[1]

The effect of fouling on the internal current velocities in pearl nets of various mesh sizes exposed to external current velocities of 9 to 100 cm/s was studied. Trail marking tape and twine were used to simulate fouling. Fouling significantly reduced current velocities and nets with the highest fouling and smallest mesh size had the greatest reduction in current velocity because the surface area available for the water to pass through was greatly reduced. Also, a higher percent reduction in internal velocity was found in the the fouled nets at the higher external velocities. One implication for scallop aquaculture is in the assessment of suitable sites for grow-out. Bays thought to be unsuitable for scallop growth because of high current velocities may actually be suitable when the mesh size of the pearl nets and the incidence and type of fouling in the area are considered.

Introduction

Scallops are an example of a benthic suspension feeder that depends on water movement to bring food within reach. In aquaculture, where scallops are held in cages or nets in suspension, there is a serious problem with fouling on the nets and cages which can restrict the flow of water.

Eckman and Duggins[2] and Wildish and Saulnier[3] report a dome-shaped response to current velocity in which scallop feeding rates and growth were reduced at the two extremes of current velocities. Wildish and Kristmanson[4] and Wildish and Saulnier[5] found growth limitations at current flows of 10 to 13.5 cm/s. Wildish et al.[6] and Wildish and Saulnier[3] found that velocities greater that 6 cm/s affected the clearance and uptake rate in scallops.

Fouling is greatest near the surface[7] and is a limiting factor in the culture of bivalves.[8] Some fouling organisms themselves are filter feeders and compete for the available food.[9] Claereboudt et al.[9] found a reduction in flow due to fouling on pearl nets containing giant scallops. The fouling organisms caused a decrease in the abundance and size composition of the available seston so that the scallops in the pearl nets had little chance to feed on the seston. Further, Cahalan et al.[10] and Wildish et al.[6] found that the flux of food particles, *per se*, is not a predictor of growth but rather it is a combination of food concentration and flow velocity that is important.

Cole et al.[11] found that the flow through pearl nets decreased as the mesh size of the pearl nets decreased, i.e., the pearl nets reduced the flow of water traveling through the nets.

The objective of this study was to examine the effects of fouling, external current velocity and mesh size on the internal current velocity of the water within pearl nets and the potential impact of these factors on the amount of food reaching scallops grown in suspension culture.

Materials and Methods

Three factors were examined: current velocities of 9.1, 22.3, 48.6, 74.9, and 99.8 cm/s; degree of fouling consisting of macroalgal simulation (orange trail marking tape), filamentous algae or bryozoan simulation (twine), a mixture of the two and a control group with no fouling; and pearl net mesh sizes of 1 x 3, 4.5, 6, 9 and 12 mm were used. The trail tape and twine were attached to the mesh at 5-cm intervals on the top four sides of the net and the tape and twine were cut to a length of 25 cm.

The flume tank at the Marine Institute was used to create current velocities. The pearl nets were placed in the flume tank with a directional

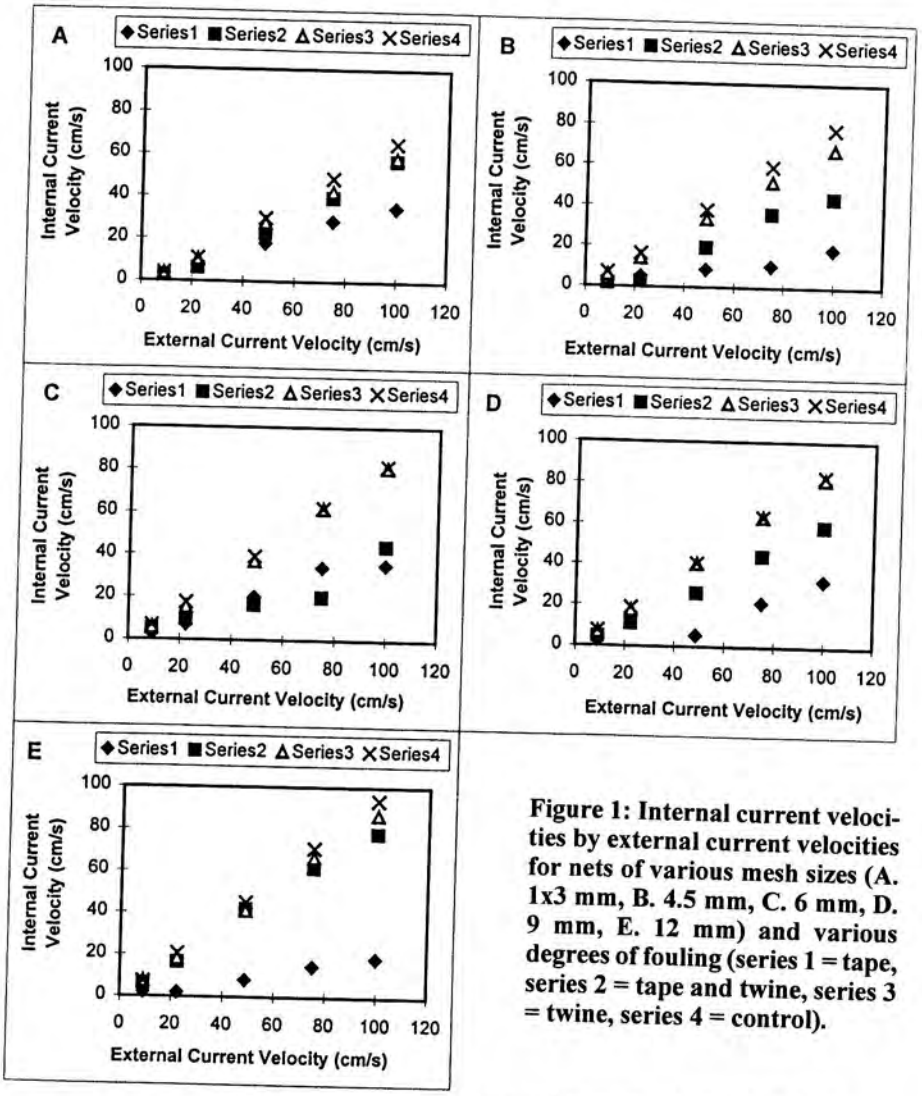

Figure 1: Internal current velocities by external current velocities for nets of various mesh sizes (A. 1x3 mm, B. 4.5 mm, C. 6 mm, D. 9 mm, E. 12 mm) and various degrees of fouling (series 1 = tape, series 2 = tape and twine, series 3 = twine, series 4 = control).

current meter inside the middle of the net. Readings of the internal horizontal unidirectional current speed inside the nets were taken at each of the 5 current velocities using nets of different mesh sizes and degrees of fouling. Three replicates of each of the external current velocity treatments were performed. A 3-way ANOVA was used to determine if there were significant differences in velocities among the three factors (fouling, mesh size, external velocity).

Results

A 3-way ANOVA showed significant differences in internal current velocities among all three factors (mesh, $P<0.001$; fouling, $P<0.001$; external current velocity, $P<0.001$).

There was a linear relationship between internal current velocity and external velocity with internal velocities always being less than the external (Fig. 1). There was a greater reduction in internal velocities in the small mesh nets than in the larger mesh nets and there was a greater reduction in internal velocities caused by the fouling over and above that caused by the mesh size alone (Fig 1). The fouling by tape and tape and twine resulted in a greater reduction than the twine and control, reflecting the percent coverage by the different treatments (Table 1).

The external current velocities resulting in an internal velocity of 6 cm/s are shown in Table 1. The values range from about 7.4 to 49.8 cm/s depending on mesh size and degree of fouling. Generally, the greater the fouling and the smaller

Table 1. External current velocity resulting in an internal velocity of 6 cm/s for different mesh sizes and degrees of fouling. Also shown are the percent coverage's of the different fouling treatments and percent opening of mesh for the different sizes of control nets.

Degree of Fouling	Percent of Pearl Net Covered	Mesh Size (mm)				
		1 x 3	4.5	6	9	12
Tape	68.5	17.2	27.8	19.0	49.8	39.6
Tape and Twine	40.0	21.6	27.7	12.6	13.5	8.8
Twine	3.6	13.9	9.5	9.5	8.1	7.4
Control	0.0	13.0	7.8	8.4	7.5	7.6
Percent Opening (Control)		35.0	70.7	73.1	77.4	77.2

the mesh, the higher the external velocity required to generate a 6 cm/s flow inside the nets.

An interesting observation was recorded in the 9-mm nets with tape at an external velocity of 22 cm/s: the internal horizontal flow of water through the nets was 0 cm/s.

Discussion

The reduction in internal velocity of control (unfouled) pearl nets with decreasing mesh size and increasing external velocity agrees with the findings of Cole et al.[11] In nets with a greater amount of fouling, a greater reduction in internal velocity occurred. The treatments with twine and no fouling had a lower reduction than those wih tape or tape and twine. The reduction in internal velocity was sometimes greater in the nets with tape and twine than nets with tape only. It appeared that the location of the fouling was more important in causing a reduction in internal velocity than the amount of tape. Hence in aquaculture, the distribution of fouling organisms, such as algae, on the surface of nets may be more important that the amount of algae. This needs to be examined further.

The observation that some of the 9-mm nets with tape had an internal horizontal flow of 0 cm/s appears to have been caused by the tape wrapping under the net and causing an undulating or beating action resulting in a vertical exchange of water through the bottom of the net.

Current speed affects the growth of suspension feeders. Wildish and Kristmanson[4] attributed reduced growth to high flow regimes. In 1993, Wildish and Saulnier[3] found that food uptake rate was optimal between 3 and 6 cm/s, beyond which increased velocity corresponds to a reduced uptake of seston and lower current flows resulted in seston depletion. It is clear that, at external current velocities unsuitable for scallops, as the amount of fouling increases (and/or mesh size decreases), the corresponding internal current velocity can be optimal for the animals. The problem that could arise is that some fouling organisms may be in competition with the scallops for food.[6,9] A study of the occurrence of competing fouling organisms in a particular area needs to be determined prior to knowing if the presence of fouling organisms on pearl nets will be a detriment to feeding or a benefit in the reduction of current velocities in high current environments. The selection of an appropriate mesh size is also critical for obtaining the optimal internal velocity at scallop culture sites with high or low water currents.

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Retention and possible transformation of paralytic shellfish toxins in lobster (*Homarus americanus*)

Michel Desbiens [1] *and Allan D. Cembella* [2]

In order to evaluate the potential risk of human intoxication, a study was conducted to describe the occurrence of PSP toxins in a natural population of lobsters in a zone periodically afflicted by *Alexandrium excavatum*. Significant but highly variable concentrations of PSP toxins (as high 1200 µg STXeq·100g^{-1}) were detected in the hepatopancreas, but not in the muscle tissues. Long-term retention in a flow-through seawater system did not lead to the detoxification of lobsters over a 53-day period. Indications from a field population showed that the PSP toxins retention time in lobsters could exceed several months. The persistence of the toxins during the cooking process has been evaluated. HPLC-FD analyses revealed that one third of the samples became more toxic after thermal treatment, likely because of a shift in the toxic derivatives profile in the hepatopancreas.

Introduction

Transfer of PSP toxins in the food web is a major constraint to aquaculture development. Most of the scientific literature on the accumulation of toxins concerns bivalve shellfish, but recent reports indicate the presence of PSP toxins in crustaceans.[3-6] Lobsters have recently been suspected to be a potential vector of phycotoxins, as their habitat is located in coastal zones where PSP-producing phytoplankton species are often observed in high concentrations. The hepatopancreas (tomalley) of lobster is a great delight to numerous seafood consumers, and is the main constituent of lobster paste sold on the market. The objectives of this study were 1) to determine if it is possible to detoxify lobsters by keeping them in seawater for several weeks, and 2) to evaluate the efficiency of standard cooking in hot steam to eliminate the toxins.

Materials and Methods

Three weeks after an intense *Alexandrium excavatum* bloom in the Gaspé Bay (4.5 x 10^5 cells·L^{-1}), 139 commercial-size lobsters were caught and maintained in a flow-through seawater system at 12-14°C without feeding. Periodic samples were taken over a 53-day period. The individual hepatopancreas, hemolymph and muscle tissue subsamples were then submitted to toxin analysis by AOAC mouse bioassays[7] as well as HPLC-FD.[8] To determine if the standard cooking procedure can reduce toxicity, the lobsters were then cooked in hot steam for 20 minutes, to simulate conditions used at home by consumers. Thereafter, the meat and the rest of the hepatopancreas of the same individuals were removed, to obtain paired data. Samples were submitted for HPLC-FD analysis.

Results

Significant concentrations of toxins were erratically found in the hepatopancreas of lobsters immediately after capture, reaching levels as high as 961 µg STXeq·100g^{-1}, estimated by HPLC analysis (Table 1). The variability was very high in spite of the fact that the lobster samples were homogeneous. Detectable toxin concentrations were found in every hepatopancreas examined. No significant toxin concentrations were detected in hemolymph and muscle tissues. Although an apparent toxin decrease in mean toxin concentrations was observed in

Table 1. Variability of PSP toxicity in lobster hepatopancreas during seawater holding, as determined by HPLC.

Holding time (days)	N	Mean toxicity µg STXeq·100 g^{-1}	Standard deviation	Range	% of samples over 80 µg STXeq·100 g^{-1}
0	5	452	530	69 – 961	67
10	14	462	315	62 – 1202	93
23	14	365	246	45 – 972	95
37	21	238	142	67 – 555	95
53	16	241	198	74 – 854	88

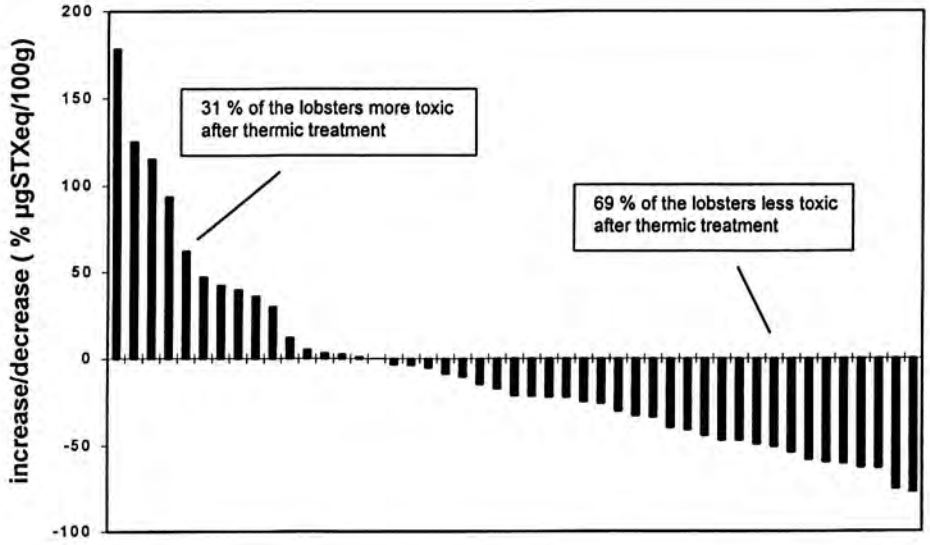

Figure 1. Individual variations of toxicity of hepatopancreas of American lobsters after thermal treatment.

hepatopancreas, holding in the flow-through seawater system did not lead to the detoxification of the lobsters. No significant differences ($P > 0.05$) appeared between the toxin concentrations at time zero and after 10,23,37 and 53 days. We observed an inconsistent response of the toxin concentrations to the thermal treatment. The results showed that the toxicity of the hepatopancreas (in µg STXeq·$100g^{-1}$) increased for 31% of the lobsters, despite a global decrease of the total toxin concentration (in nmol·g^{-1}) in the samples. This apparent gain in toxicity in about one-third of the lobsters seems to be due to a shift in the proportion of the derivatives after thermal treatment. Indeed, we observed a reduction of the concentration of the predominant but less toxic derivatives (C1, C2 and B1) with a simultaneous rise of the most toxic components (STX, neoSTX and dcSTX). The data indicate the partial transformation of some slightly toxic derivatives into more toxic derivatives during cooking. A highly significant correlation was observed between the toxicity obtained by bioassays and HPLC, as calculated from specific conversion factors (µg STXeq·μmol^{-1}) ($r=0.86$); HPLC gave consistently higher results than bioassays.

Discussion

Sampling of lobsters kept in a flow-through seawater system demonstrated the persistence of the toxins over a period of 53 days. Moreover, circumstantial indications from field populations showed that the PSP toxin retention time in the hepatopancreas could exceed several months, even over a summer-winter cycle.[9] Thus, long-term holding in cold seawater could hardly be an efficient process to detoxify lobsters. This suggests a cumulative effect that can make the toxin content increase year after year in the hepatopancreas of lobsters in coastal zones periodically afflicted by PSP-producing organisms. Because of the absence of detectable levels of toxins in hemolymph, it does not appear possible to establish a relationship with the toxin concentrations in hepatopancreas, making it hard to find a non-destructive test to evaluate the toxin content in this organ.

The possibility of conversion of less toxic into more toxic derivatives after thermal treatment is probably related to the initial toxin profile in the raw hepatopancreas. The effect of heating on the PSP toxins in the crustaceans should be the subject of a further study.

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The role of the mussel *Mytilus edulis* in recirculating cysts of the toxic dinoflagellate *Alexandrium fundyense*

F.M. Harper, R.J. Thompson, C.H. McKenzie and E.A. Hatfield

To examine the possible role mussels may play in maintaining populations of *Alexandrium fundyense* within a site, sediment and mussel faecal material were collected from a contaminated aquaculture site and examined using phase and epifluorescence microscopy. Three cell types were predominant: *A. fundyense* cysts, *Scrippsiella trochoidea* cysts and an unknown "A" cyst type similar in appearance to the resting stage described for the toxic dinoflagellate *Alexandrium ostenfeldii*. Adult *Mytilus edulis* from the top and bottom of three separate mussel socks ingested both non-toxic *S. trochoidea* and toxic *A. fundyense* cysts irrespective of their position in the water column (top or bottom of mussel sock), and of the density of these cysts in the underlying sediment. Resuspension effects were evident throughout site, making it impossible to predict the uptake of the cysts based upon cyst densities in the underlying sediment. However, the results demonstrated the potential for the egestion of viable *A. fundyense* cysts from contaminated mussels and their recirculation within an aquaculture site.

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Fatty acid signature compounds of a toxic diatom and a toxic dinoflagellate

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

The determination of fatty acid compositions of aquaculture samples is becoming increasingly common. Fatty acids are of interest for nutritional reasons and also as signature compounds or biomarkers of algae. Certain fatty acids can be used to indicate that specific algae are present in a sample or that an organism has been consuming such algae. Thus, in addition to providing nutritional information, the screening of aquaculture samples for fatty acid content may be particularly useful in providing an early warning of the presence of toxic algae. Fatty acid analyses of cultures of vegetative cells of toxic *Pseudo-nitzschia multiseries* and *Alexandrium fundyense* and of *P. pungens* and *Scrippsiella trochoidea* were carried out in order to determine markers for the toxic organisms. All these species have been found in Canadian waters and the toxic organisms have been responsible for several deaths as well as closures of aquaculture sites. The fatty acid compositions of *P. multiseries* and *P. pungens* were typical of diatoms. However, toxic *P. multiseries* contained larger amounts of 16:4ω1, and the ratio of 16:4ω1/18:2ω6 in the cultures of *P. multiseries* yielded a value that was approximately 13 times larger than that of *P. pungens* grown under the same conditions. This marker ratio was also supported by analyses of field samples. Similarly, the cultures of *A. fundyense* and *S. trochoidea* displayed a fatty acid composition typical of dinoflagellates. The fatty acid data suggested that a marker ratio of 18:4ω3/18:5ω3 would prove useful in differentiating between the toxic and non-toxic species. This marker had a value that was significantly larger in the *A. fundyense* culture than in the *S. trochoidea* culture. Analyses of field samples also supported this marker ratio.

Introduction

In the past decade, eastern Canada has experienced several outbreaks of shellfish poisoning. The best known occurred in Prince Edward Island in 1987. Domoic acid was found in the shellfish and the industry was temporarily closed. A diatom, *Pseudo-nitzschia multiseries*, was implicated as the causative organism.[3] More recently, in northern Newfoundland, several aquaculture sites have been permanently closed due to the presence of a toxic dinoflagellate, *Alexandrium fundyense*.[4] Fatty acid biomarkers may be used to determine the presence of these toxic organisms. There is already much interest in aquaculture in fatty acid compositions for nutritional reasons. The screening of aquaculture samples to determine fatty acid compositions can provide this nutritional information, as well as providing an early warning of the presence of the toxic organisms.

Biomarkers or signature compounds are chemical components of organisms that may be used to qualitatively and quantitatively determine *in situ* biomass. Fatty acid biomarkers may be used to signal the presence of a particular organism. In this study, biomarkers for two species of toxic alga are proposed.

Identifying biomarkers for these toxic algae is particularly challenging as both organisms are similar in appearance and are found in the same environment as non-toxic algae. *P. multiseries* is practically indistinguishable from the non-toxic *P. pungens*. Similarly, *A. fundyense* is

commonly found in the same habitat as the non-toxic *Scrippsiella trochoidea* and the two dinoflagelllates are similar in appearance.

Various methods have been developed to directly measure toxins produced by algae. These include bioassays using mice or rats,[5] as well as several liquid chromatographic techniques.[6,7] However, the gas chromatographic analysis of biomarkers of the toxic organism offers several advantages. The most obvious is the availability of the required gas chromatograph (GC) with flame ionization detection. Also, many aquaculture managers carry out fatty acid analysis to determine the nutritional quality of the shellfish and their food. This GC procedure would also allow determination of the biomarker. Finally, the preparation of samples for fatty acid analyses is relatively simple and rapid.

Results and Discussion

Examination of cultures of *P. multiseries* and *P. pungens* was carried out to determine an appropriate biomarker for the toxic organism. The fatty acid composition of the two organisms was very similar and typical of diatoms. Both diatoms contained an unusual acid, 16:4ω1, which is present in varying amounts in almost all species of diatom and can be used as a general marker for diatoms. This acid, combined in a ratio with a second acid, 18:2ω6, seems to function well as a marker for *P. multiseries*. The ratio, 16:4ω1/18:2ω6, has values of 53 ± 1 (S.D.) and 3.90 ± 0.66 in cultures of *P. multiseries* and *P. pungens*, respectively.

To test this marker, ratios of 16:4ω1/18:2ω6 were determined for field samples from Notre Dame Bay, Newfoundland. Samples containing harmless *P. pungens* consistently had ratio values between 0.62 ± 0.17 and 2.48 ± 0.73, while a sample thought to contain *P. multiseries* had a value of 16.2 ±1.4 (Fig. 1). These field samples offer strong support for the proposed marker ratio, 16:4ω1/18:2ω6, for *P. multiseries.*

Fatty acid analyses of cultures of *A. fundyense* and *S. trochoidea* were also carried out to determine a suitable biomarker for *A. fundyense*. As with the diatoms, the fatty acid compositions of the dinoflagellates were very similar. Both cultures contained an unusual acid, 18:5ω3, in approximately equivalent amounts, while *A.*

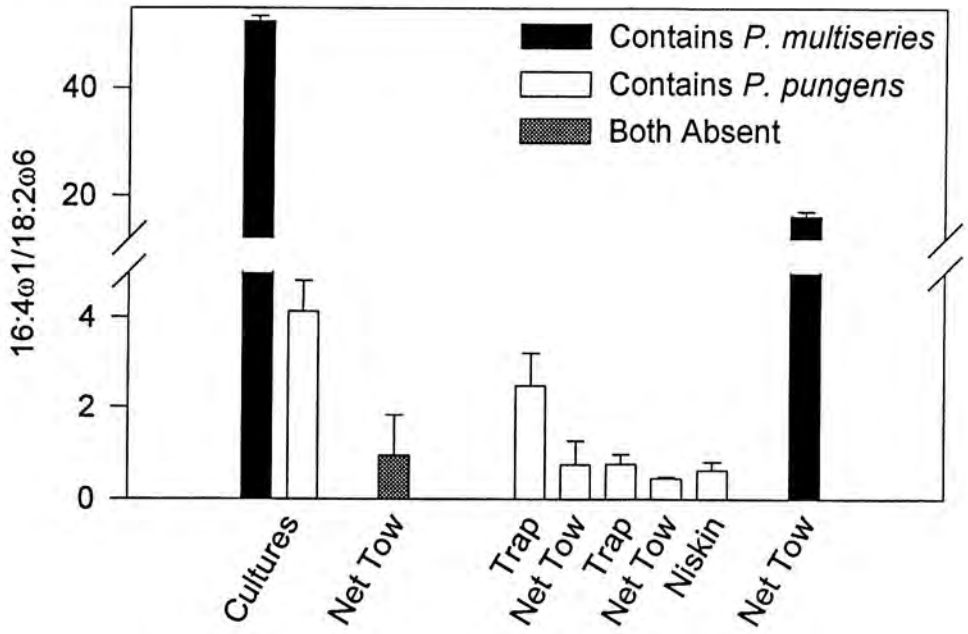

Figure 1. Values of *P. multiseries* marker in field samples (mean ± S.D.)

fundyense contained significantly more 18:4ω3. A combination of these two acids in a ratio, 18:4ω3/18:5ω3, serves as a biomarker. This ratio had a value of 0.99 ± 0.02 in the *A. fundyense* culture and a value of 0.63 ± 0.01 in the *S. trochoidea* culture.

This marker was also tested on field samples from Notre Dame Bay, Newfoundland (Fig. 2). Samples containing both *A. fundyense* and *S. trochoidea* yielded ratios ranging from 1.04 ± 0.16 to 1.9 ± 0.6, while samples containing *S. trochoidea* and not *A. fundyense* consistently had ratios less than 0.84 ± 0.16. A problem did arise, however, when a sample that did not contain *A. fundyense* yielded a ratio of 4.9 ± 0.4. The algal composition of this sample included two other dinoflagellate species, *Ceratium fuses* and *Ceratium longipes*. It appears that these dinoflagellates produce only small amounts of 18:5ω3, causing elevated values of the marker ratio. Fortunately, these effects of *C. fuses* and *C. longipes* on the ratio can be taken into account when interpreting the marker. For example, ratio values less than 0.8 would signal the presence of *S. trochoidea*, while values ranging from 1 to 2 would indicate *A. fundyense*. Very large values, above 4, would indicate the presence of *C. fuses* and *C. longipes*, and the absence of *A. fundyense*.

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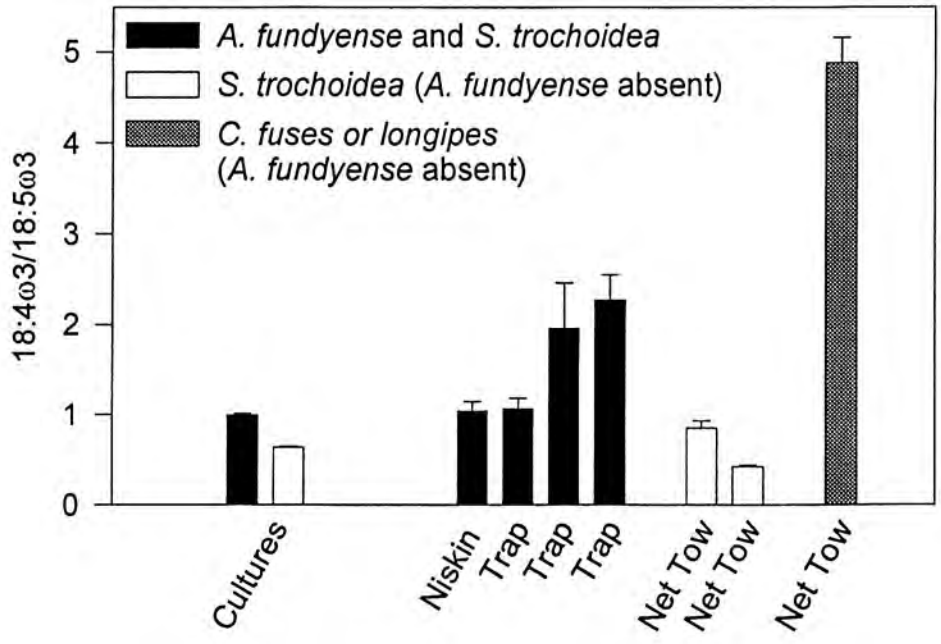

Figure 2. Values of *A. fundyense* marker in field samples (mean ± S.D.)

Potential environmental impact of peat moss harvesting on the Richibucto River in New Brunswick

C. Ouellette,[1] A. D. Boghen,[1] S. C. Courtenay[2] and A. St-Hilaire[1]

Peat moss harvesting is a lucrative industry in New Brunswick. Concerns have been raised that the methods used to excavate peat might be affecting the aquatic ecosystem and shellfish culture. A study was conducted on the impact of peat spilled into a tributary of the Richibucto River to determine the effects of peat on the biodiversity and health of aquatic organisms, as well as on water quality and the bottom substrate. While the project included histopathological and parasitological analyses as well as behavior experiments, the current work focuses on the distribution and densities of vertebrate and invertebrate species and results of sediment core analyses. Stations were established along a 1-km stretch at varying proximities to the peat delta. Beach seining and grab samples for biota and sediment core samples for peat depth were analyzed. Preliminary findings suggest there is an inverse relationship between the density of fish and shellfish species and the depth of peat moss. Detailed quantitative analyses on previously collected and current data are in progress.

Introduction

Peat harvesting is an important activity in eastern New Brunswick and was valued at over 64 million dollars in 1996.[3] The industry is located in close proximity to the ocean and recent expansion in harvesting has raised concerns regarding the effect on the quality of the coastal and estuarine environments.[4] During harvesting, high levels of peat fibers are released and water collects in deep ditches. The water eventually transports the peat fibres to fresh and marine water via a network of streams and rivers. While current regulations require the installation of settling basins to reduce excessive peat run-off, their effectiveness needs to be determined.[5] In addition, the effects of historical damage are still evident in some areas.

Few studies have examined the long-term impacts of peat run-off on the aquatic biota. Evidence suggests, however, that peat particles may influence the chemistry of the water and produce modifications to the bottom substrate.[6] Such changes affect the biodiversity and health of aquatic organisms. Recent information indicates that peat fibers may interfere with the respiratory and feeding activities of bivalves[7] and possibly other invertebrates.

Following the release of excessive peat by a harvester into Mill Creek,[8] the Environmental Sciences Research Centre of the Université de Moncton,[9] in collaboration with the Department of Fisheries and Oceans and the industry, initiated a study on the effects of peat on the biota. The program provides a nucleus around which broader concerns can be addressed.

Materials and Methods

Mill Creek, a tributary of the Richibucto River, is located along the southeastern coast of New Brunswick. Sampling was conducted between 9 July and 18 October 1996 adjacent to a heavily concentrated peat deposit. Ten stations were established along a 1-km stretch at varying proximities to the peat delta (Fig 1). To acquire precise estimates of the extent of peat accumulation on the river bottom, core samples were extracted using 1.25 m and 2.50 m long clear polycarbonate tubes (2.9 cm I.D.). Samples were taken along transects, perpendicular to shore. More detailed sediment analyses were conducted at stations 1,2,3,6,8 and 10.

Beach seining was done on 14 August at low

tide at stations 1,2,3,6,7,8 and 10 (5 m from the low tide mark), using a 14 m long by 1.5 m wide seine (5 mm mesh size). Benthic organisms were sampled on 12 August and 14 September at stations 1,2,3,4,6, 8, and 10, using a metal cylinder grab (17 cm deep, 15 cm diameter) manually driven into the sediment. Five replicates were randomly extracted within a 5-m radius. Sediments were sieved through a 5x5 mm metal mesh and animals were retained. All animals were placed on ice, transported to the laboratory, and frozen. Species identification and specimen counts were conducted. Mean numbers of *Macoma* sp., the most abundant species collected by grab, were compared across stations by ANOVA, followed by Tukey multiple range test.

Results and Discussion

Distribution of peat

The bottom sediment consisted of pale-brown sand and muddy-silt covered by varying amounts of peat. Peat, identified by its black-fibrous texture, was most heavily concentrated between stations 2 and 8 (Fig 1). The entire area of Mill Creek is tidal and the range of salinities and temperature were 11.0 to 22.0 ppm and 13.0 to 27.0°C, respectively. There were no noticeable differences in salinities or temperature between stations. Detailed descriptions of the area are provided by MGI Limited[6] and Berlinsky.[8]

Beach seine

The most abundant species of fish and macroinvertebrates in Mill Creek were: Atlantic silverside *(Menidia menidia)*, banded killifish *(Fundulus diaphanus)*, mummichog *(F. heteroclitus)*, sand shrimp *(Crangon septemspinosa)*, and grass shrimp *(Palaemonetes vulgaris)*. Present in lower numbers were: four-spine stickleback *(Apeltes quadracus)*, crab *(Rhithropanopeus harrisi)*, nine-spine stickleback *(Pungitus pungitus)*, alewife *(Alosa pseudoharengus)*, three-spine stickleback *(Gasterosteus aculeatus)*, striped bass *(Morone saxatilis)*, smooth flounder *(Pleuronectes putnami)*. An inverse relationship between the number of individuals and the concentration of peat was noted (Fig. 2).

Grab

Species identified using the grab method were: macoma clam *(Macoma baltica)*, soft-shell

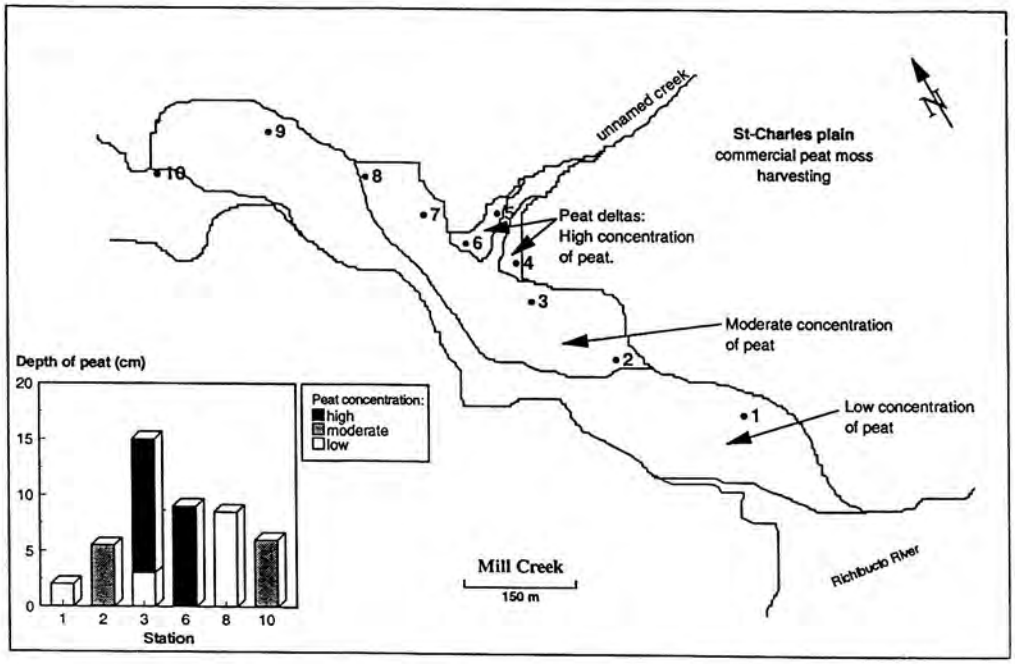

Figure 1. Area of Mill Creek showing peat sill.

clam *(Mya arenaria)*, ribbed mussel *(Modilus dimissus)*, mud welk *(Ilynassa obsoleta)* and marine worm *(Nereis virens)*. *Macoma* sp. was the most abundant (90%) species collected by grab. Similar to the fish and invertebrate species collected using the beach seine method (see above), macoma clams displayed an inverse relationship to peat depth. On both sampling dates there were significantly fewer clams at stations 3 and 6 than at stations 1 and 10 (Tukey test $P < 0.001$). Data suggest there is a modification of habitat for the benthic invertebrates in the immediate area of peat deposition.

Future work will consider the effects of peat on the resident estuarine biota. Emphasis will be placed on defining indices of health of benthic organisms, including bivalves, the decapod, *C. septemspinosa,* and sedentary fish such as the mummichog. It has already been shown in laboratory experiments[10] that there is avoidance of peat substrate when organisms are provided with a choice. It is therefore considered important to quantify the effects of such exposure on both wild and cultured organisms.

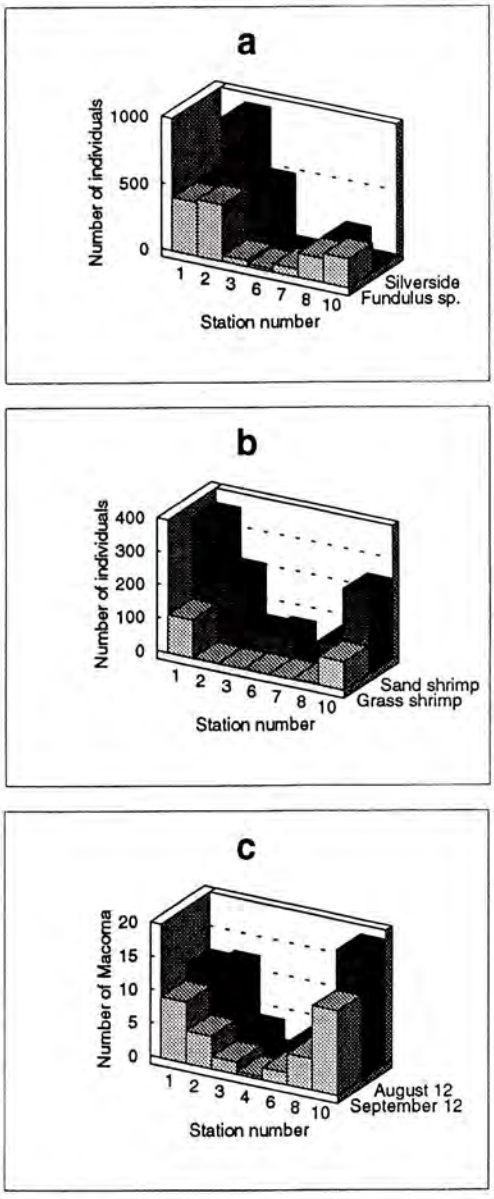

Figure 2. The number of vertebrates and invertebrates by station for Mill Creek: a, most common fish species; b, shrimp; and c, most common bivalves.

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