Bulletin

of the Aquaculture Association of Canada

Proceedings
Aquaculture Canada '95

Edition 96-1
March, 1996

Financial Supporters of Aquaculture Canada 95:

British Columbia Ministry of Agriculture, Fisheries and Food: Aquaculture and Commercial Fisheries Branch

Partners in Progress Program, Demonstration of Applied Technology and Economics (DATE)

British Columbia Ministry of Small Business, Tourism and Culture

Department of Fisheries and Oceans: Pacific Biological Station, Aquaculture and Habitat Science Branch

City of Nanaimo Regional Economic Development Office

National Research Council (TIP)

Moore-Clark Ltd.

Hoffman-La Roche

Syndel Laboratories

Pfizer Animal Health

Bamfield Marine Station

Connors Brothers Ltd.

EWOS Canada

BIOMED

Organizational assistance provided by:

MEL-Mor Science

B.C. Ministry of Agriculture, Fisheries and Food

Department of Fisheries and Oceans

B.C. Salmon Farmers Association

B.C. Shellfish Growers Association

Malaspina University–College

Coregan, Conley and Associates

Donors of seafood for the opening reception:

BC Packers Ltd.

Fanny Bay Oysters Ltd.

Intercan III Aquaculture Corporation

Island Scallops Ltd.

Mac's Oysters Ltd.

Madrona Shellfish Ltd.

Mid-Island Marine Ltd.

Pacific National Group

Stolt Sea Farms Ltd.

Bulletin
of the
Aquaculture Association of Canada

Membership in the Aquaculture Association of Canada (AAC), a nonprofit, charitable organization is available to individuals, institutions, companies and agencies. Receipts for tax purposes are issued for membership dues and donations. For membership information contact: AAC, PO Box 1987, St. Andrews NB EOG 2XO Canada [Telephone 506 529-4766; Fax 506 529-4609; e-mail AAC@WOLVES.STA.DFO.CA].

The **AAC Bulletin** is distributed as a benefit of membership. Annual dues are $40 for students, $50 for individuals and $85 for companies— 25% of dues is designated for *Bulletin* subscription. Subscriptions are available to nonmembers and libraries for $40 per year. One or more of the 4 issues (March, June, September and December) contain the scientific and technical presentations from the annual meeting of the Association. Advertising, news items, information on meetings, jobs available, letters and interesting photographs are welcomed. Although material in the Bulletin is not protected by copyright, AAC would appreciate being cited as the source of material used by other publications. The Bulletin is abstracted and indexed in Aquatic Sciences and Fisheries Abstracts (ASFA). Mailed under Canada Post Publications Mail Product Sales Agreement No. 525375. Change of address notices and undelivered copies should be mailed to AAC. Return postage guaranteed.

ISSN 0840-5417

Printed by Tribune Printing, Sackville, N.B.

Aquaculture Canada '97

14th Annual Meeting

of the

Aquaculture Association of Canada

9 – 15 June 1997, Québec City

Theme:

From Research to Market

Proposed Sessions: (other suggestions welcome)

Harmful Marine Algae and Aquaculture Management

Marine Finfish Aquaculture in Eastern Canada

Aquaculture: What does the Consumer Want?

Information:

Aquaculture Association of Canada
P.O. Box 1987
St-Andrews, N.B. Canada E0G 2X0
Tel: 506-529-4766
Fax: 506-529-4706
e-mail: AAC@sta.dfo.ca

or

Yves Bastien
MAPAQ, 96 Montée Sandy Beach
C.P. 1070, Gaspé, Qc.G0c 1R0
Tel: 418-368-7656
Fax: 418-368-8400
e-mail: ybastien@quebectel.com

Cover: Original Ken Albrecht artwork commissioned by the Aquaculture Association of Canada as a logo for the 1995 annual meeting held in Nanaimo, British Columbia.

Proceedings of the Contributed Papers of Aquaculture Canada '95 — the 12th annual meeting of the Aquaculture Association of Canada

Papers

Aquaculture and the environment

New finfish species

Health and genetics

Nutrition, physiology and energetics

Culture systems and water management

Feeding strategies

Shellfish culture and the environment

Departments

— G.J. Parsons and S.L. Waddy, editors

Meeting Report: Aquaculture Canada '95

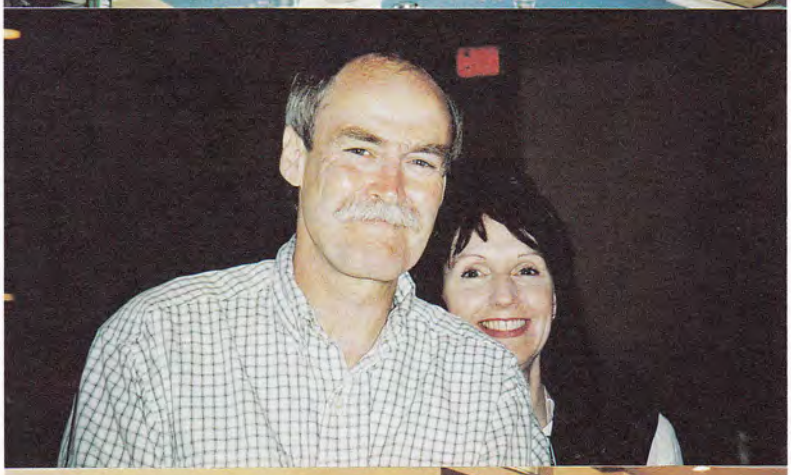
Aquaculture Canada '95, the twelfth annual meeting of the Aquaculture Association of Canada was held in Nanaimo, British Columbia, from June 4-7, 1995. The theme of the meeting was "Aquaculture and the Environment: an essential partnership" and many talks were presented around this theme by local speakers as well as from Norway, Scotland, Germany and Ireland. The balance of the program was an excellent mix of scientific and technical papers on progress in finfish and shellfish aquaculture, farm management, water quality, fish health, nutrition, genetics and physiology and resource planning.

The number of delegates was good with almost 400 participants representing all ten provinces and Yukon, at least eight U.S. states and eight other countries. There was gratifying industry participation, a well-run Trade Show, significant corporate and public support, relevant and well-attended technical and special sessions and the opportunity

to mix, mingle, eat and be entertained. The Association attracted 80 or so new members at the meeting making current membership about 900. Over 30 local volunteers helped deliver the program and as usual, the Association Office (Susan Waddy and Theresia Fawkes) and other Board members played key roles in carrying it all off.

No program would work at all without the individuals who actually make presentations. Our sincere appreciation to all speakers.

The Canadian Aquaculture Industry Alliance (formerly CAP-C), the British Columbia Shellfish Growers Association, the B.C. Salmon Farmers Association and the Salmon Health Consortium all held business meetings during Aquaculture Canada '95. The Department of Fisheries and Oceans met to discuss progress in establishing regional Aquaculture Implementation Committees immediately post-conference. These complementary events helped draw participants and round out the meeting. The AAC appreciates their cooperation and hopes that similar arrangements can be made in future.



The meeting program contained the following message from the Steering Committee as a preface "Aquaculture in Canada is at a critical juncture. Twenty years ago, we talked about the promise of aquaculture in Canada. Today, product sales are in excess of $280 million, primarily in export markets, support services generate $260 million in sales and thousands of new jobs have been created. The Department of Fisheries and Oceans has just released the Federal Aquaculture Strategy which states that aquaculture is a priority of the federal government. Across Canada, a doubling of the value of the in-

dustry by the year 2000 is not an unreasonable expectation. Between six and seven thousand additional jobs would be created in areas of greatest economic need — coastal and rural communities. However, this growth will not be realized unless there is a concerted effort to adapt new technology, gain access to new growing areas and protect those currently in production and, ensure that the goals for aquaculture are in concert with principles of sustainability and are compatible with those of society in general. Aquaculture will only prosper with private sector initiative and capital in an environment of public and government support.

'Aquaculture Canada' is an annual opportunity to exchange information and continue to review the practice of current aquaculture activities, the problems of existing structures and methods and the promise of new technologies and approaches".

The strength of the AAC lies in its ability to provide a well-rounded forum for communication and dissemination of information. The Association is proud to be able to deliver a high quality annual event which serves as an important meeting ground for the various parties interested in aquaculture and its development. The communications function for the 1995 meeting will be completed by production of these Proceedings and in the contents of two further Bulletin issues.

Aquaculture Canada '95 was successful. We look forward to the thirteenth edition of Aquaculture Canada which will take place in June 1996, in Ottawa.

— Al Castledine,
President 1995-96

Evelyn Stillwell (right) receiving the Moore-Clark Award for the best student paper from Dr. R. Whittaker, Chairman of the Department of Biology, University of New Brunswick

CANADIAN AQUACULTURE INDUSTRY ALLIANCE
ALLIANCE DE L'INDUSTRIE CANADIENNE DE L'AQUICULTURE

WHO IS "CAIA"?

The Canadian Aquaculture Industry Alliance (CAIA) is a national organization, a federation of regional and sectoral associations working with and for individuals, businesses and associations whose work is related to the aquaculture industry. This includes those farming finfish and shellfish. Suppliers and supporters of the industry — such as feed manufacturers or veterinarian organizations — are also an important part of our alliance.

"TO ENSURE THE INTERNATIONAL COMPETITIVENESS OF THE CANADIAN AQUACULTURE INDUSTRY."

WHY WAS CAIA FORMED?

The formation of CAIA in 1995 replaced the Canadian Aquaculture Producers Council, with a broader representation of the aquaculture industry. As an umbrella organization for the industry, CAIA provides a framework and direction to optimize the enormous potential and the opportunities aquaculture represents as a globally competitive business.

The position of Canadian aquaculture within a globally competitive industry will be strengthened and maintained by CAIA.

PART OF THE CAIA UMBRELLA

The Sector Council operates as a special committee to CAIA — integrating industry, government, employees and small business — to develop and foster a strong Canadian aquaculture workforce. Tremendous growth is predicted for this rapidly evolving industry, requiring new and existing workers and entrepreneurs with varied specializations, including technical skills, and the ability to work confidently and successfully in a globally competitive climate.

Canadian Aquaculture Industry Alliance
20th Floor, 45 O'Connor Street
Ottawa, Ontario K1P 1A4
Tel.: (613) 788-6851 Fax: (613) 235-7012
Email: CAIAoffice@aol.com

Master of Science in Aquaculture

UNIVERSITY of GUELPH

The master of science in aquaculture is a non-thesis, course-work degree, designed to give students a broad based knowledge of the aquacultural field without emphasis on specialization. Students are encouraged to apply creative problem solving to areas of aquacultural management. Graduates of the program will have the knowledge and skills necessary to enter the field as leaders.

Studies may encompass:

- ***Biotechnology**
- ***Marketing Management**
- ***Fish Nutrition**
- ***Technology in Aquaculture**
- ***Special Research Projects**
- ***Practicum (Hands-on-study)**
- ***Extension**
- ***Marine/Aquatic Sciences**
- ***Food Sciences**
- ***Pathology**
- ***Fisheries Science**

The University of Guelph is one of Canada's top research universities, and our professors consistently earn national and international recognition for the high quality of their research and teaching. Our commitment to provide a learner-centred environment and meet global challenges makes Guelph exceptional. The program is an interdepartmental offering and students have access to state-of-the art research facilities from several departments.

Professor Richard Moccia
Program Coordinator
Department of Animal & Poultry Science
University of Guelph
Guelph, Ontario
Canada
N1G 2W1
(519) 824-4120 ex.6216

Review of impacts of fish farms on prawn fishing — preliminary results

J. A. Boutillier [1]

A review of the logbook and saleslip data from the British Columbia prawn fishery was undertaken to evaluate if fish farming is having a negative impact on prawn stocks. A comparison of sub–areas with and without farms shows that the areas with farms may fluctuate more but in general do not seem to be negatively impacted by fish farming activities. This evaluation was undertaken at a large geographic scale and discussions are presented on possible programs to evaluate impacts on a much more localized scale.

Introduction

This is a report on the preliminary results of a joint federal Department of Fisheries and Oceans (DFO) and British Columbia Ministry of Agriculture, Food and Fish (BCMAFF) initiative to address concerns that fish farming is having a negative impact on prawn stocks in DFO Fisheries Statistical Area 12. Area 12 is an area encompassing the northeastern side of Vancouver Island and the adjacent mainland inlets (Knight and Kingcome Inlets and surrounding waters). These concerns were expressed both on a site specific level (i.e., a favourite fishing location) and on a much broader level, in particular the overall collapse in the fishery in the subareas surrounding Broughton Island. Area 12 has historically been the largest prawn producing area in British Columbia and is also the area that has the highest concentration of fish farms (20 active sites between 1987–1992).

Prawns (*Pandalus platyceros*) are the largest of the six commercial species of shrimp fished in British Columbia waters. All six species of commercial shrimp belong to the family of Caridea shrimp, Pandalidae.[2] Prawns, as with the other pandalid shrimp, undergo a biological process known as protandric hermaphrodism in which they undergo a sex change part way through their lives. The animals mature at age 1^+ and spend one or two years as functional mature males before transforming and functioning as mature females. Mature shrimps breed in the autumn, and the developing eggs are carried on the pleopods of the females from the autumn to the early spring.[3]

The 1994 prawn fishery in British Columbia was valued at ~$10 million. The directed prawn fishery is a trap fishery which is regulated with size limits, escapement modifications, licence limits, trap limits and spawner escapement indices.

Methodology

At this stage, the data that were available allowed for a review of only the large–scale problem. I will discuss the analysis of prawn data collected through fishery-dependant data sources via the commercial logbook and saleslip programs and fish farm data, collected by the provincial aquaculture licensing system. In particular, this review will look for differences in trends in the prawn fishery in sub–areas with farms and without farms. We are assuming that the zone of influence, if any, would be restricted to the sub–area occupied by the farm. It is important to note that this analysis is looking at large–scale effects and not localized effects.

From the licensing records, fish farming tenures in this area started as early as 1987 but most of the new farms were established between 1989 and 1992. Most of these farms remain active to this date. Fish farms are located in 10 of the 48 sub–areas in Area 12, although not all subareas had fish farms in them every year. The total catch and catch per unit of standardized effort (CPUE) for sub–areas with and without fish farms were compared. A comparison was also

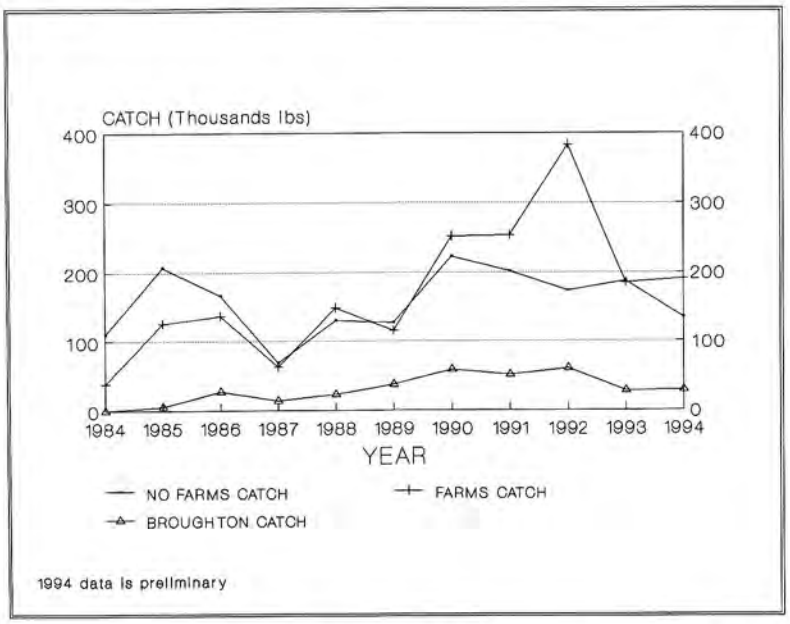


Figure 1. Total catch (000's lbs) trends of prawns from Pacific Region, DFO Statistical Area 12 for the years 1984–1994. Catches were proportioned out into sub–areas with and without fish farms. The catch trends for the Broughton Island area are also shown.

made with a subset of the data which looked at the sub–areas in and around the Broughton Island area.

The fishery–dependant data are a combination of information from the saleslips and the commercial logbook programs. The commercial logbook program was voluntary until 1989 when it became mandatory as a condition of license. Logbook information prior to 1989 is considered as only an index of the fishing pattern, catch, and CPUE in the area. The logbook information is collected in a detailed manner that allows for calculation of catch and standardized catch per unit effort by sub–area.

Results

The Area 12 prawn catch fluctuated between 1984 and 1989 and then increased from 1989 to 1992 (Fig. 1). This increased catch was associated with a relatively good average annual standardized CPUE. There was a drop in production in 1993 and again in 1994, that was accompanied by a decline in the CPUE (Fig. 2). A comparison of the trends in catch for sub–areas with farms and without farms and for the Broughton area showed that the trends in these three subcategories tend to parallel each other. With the exception of the period from 1992 to 1994 when the production from the sub–areas with farms increased substantially, declined substantially in 1993 and increased in 1994. Production in areas without farms during this same period dropped off in 1992, increased slightly in 1993, and dropped off again in 1994. The trends in the Broughton area paralleled the trends in catch in the sub–areas with fish farms; although the increase in 1992 was relatively small, the decline in 1993 was proportionally as great as in other sub–areas with farms.

Since trends in catch may only reflect changes in overall effort in the area, a comparison of the trends in standardized CPUE (Fig. 2) confirms the parallel pattern of fishing success in areas with and without farms and in the Broughton area. Albeit the decline in 1993 is much larger in the sub–areas with farms and in the Broughton area than the decline in the sub–areas without fish farms (declines of 32%, 43% and 7% respectively).

Discussion

From this review, we can corroborate that prawn production in the Broughton area has declined in recent years. However, the decline in production is on a much broader scale than the sub–areas of the Broughton Island area, as is evident by the production and CPUE declines in sub–areas with farms (Fig. 1, 2). The concern for the decline in prawn stock in the Broughton area is real and is further exacerbated by the apparent large decline in CPUE between 1992 and 1993. This substantial difference between 1992 and 1993 where the decline in CPUE is much larger in sub–areas with farms than in

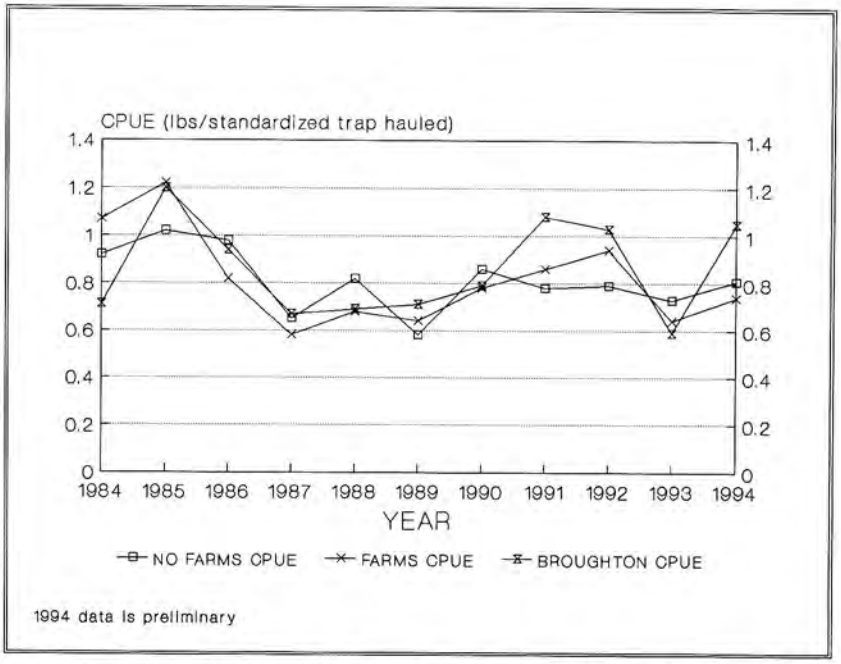


Figure 2. Standardized catch (lbs) per trap hauled for Pacific Region, DFO Statistical Area 12 for the years 1984–1994. CPUE trends for the Broughton Island area are also shown.

sub–areas without farms, may be explained in part by the shift in fishing activity into the sub–areas with farms in 1992, where the catches increased substantially while the catches in areas without farms actually declined. Since the fishery targets two year–classes (age 2 and 3), the intensive 1992 fishery in the areas with farms, including Broughton, would have the effect in 1993 of reducing the availability of age 3 prawns, leading to a reduced fishery and making the drop between the two years more dramatic. There may be a number of other reasons for this larger decline, however the analysis at this time is not detailed enough to confirm the apparent cause. This further analysis will be undertaken this winter for the Pacific Stock Assessment Review, which will not only incorporate the findings from the analysis of the logbook information but will also include biological information from sampling conducted in the area, fishery management actions, and the fleet dynamics in response to the management action. I therefore conclude that the decline in prawn production in recent years in the Broughton area, does not appear to be tied to any negative effects of fish farming activities. It is true that the sub–areas with fish farms have undergone more dramatic changes in relative abundance recently, but these changes have not been all negative. As a matter of fact the sub–areas have actually seemed to increase in production after the fish farms were established.

The concern about the very localized decline in fishing success due to fish farming activities can not be confirmed or denied in this review. To address this problem, our approach will be based on a comparison of historical biological survey data from the mid–1970s with survey information gathered in 1994 and 1995. The surveys in the 1970s were conducted at specific sites in the area (some of which now have active farm sites closely located) 2–3 times a years. The historical survey data measured abundance and biodiversity of organisms in the area and will be compared to data from surveys of the area that are now being planned. The 1995 survey will collect data on the general health of the prawn populations in the area as a whole. We may also have the opportunity to evaluate specific sites and possible zones of influence, if any, of working farm sites.

Many thanks to Claudia Hand for her helpful and insightful comments and corrections on an earlier draft.

Notes and references

1. Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, BC V9R 5K6
2. Butler, TH. 1980. *Can. Bull. Fish. Aquat. Sci.* No. 202.
3. Butler TH, Boutillier JA. 1983. *Selected Shrimps of British Columbia.* Underwater World Fact Sheet. Communications Directorate, Department of Fisheries and Oceans, Ottawa.

Early growth and survival of larval striped wolffish *(Anarhichas lupus)*: a behavioural approach

D. L. Wiseman and J. A. Brown[1]

The rearing of marine fish larvae is constantly being improved as we learn more about these "new" species. The striped wolffish (*Anarhichas lupus*) is considered a "new" candidate species for aquaculture and, as such, research in early rearing techniques is required. Newly hatched wolffish were fed different levels of *Artemia* nauplii in combination with dry pelleted marine diet to develop protocols that produce good growth and survival. Behavioural observations were made in conjunction with the collection of performance data to determine a feeding strategy for newly hatched wolffish. Results showed that larval wolffish fed a higher level of *Artemia* nauplii had better survival, faster growth and weaned onto dry feed earlier than wolffish fed a lower level. Behavioural observations indicated that larval wolffish fed while swimming or while in contact with the bottom. These observations also indicated that larvae took more pelleted food as they grew and "weaned themselves" by the end of the study.

Introduction

Since marine fish larvae are typically small and fragile, the mass production of larvae and juveniles tends to be a major bottleneck in marine fish aquaculture. Striped wolffish *(Anarhichas lupus)* have shown great potential as an alternative culture species for many reasons. They produce large robust larvae capable of feeding soon after hatch, they show good growth and survival, and produce a good quality white flesh.

Although several studies have been carried out on newly hatched and juvenile wolffish, early feeding protocols have not been rigorously addressed. Moksness et al.[2] found that survival was higher among larvae fed *Artemia* plus dry diet, but levels of live prey and feeding behaviours were not specifically investigated. In a preliminary study we fed larvae *Artemia* only and found growth was higher among the higher fed fish but survival was low (< 20%) overall.

In this study newly hatched wolffish larvae held in glass aquaria were fed two levels of *Artemia* in combination with a dry pelleted marine diet. Observations were conducted to assist in the interpretation of growth data as well as to help determine a feeding strategy for newly hatched wolffish.

Methods

Striped wolffish used in this study were hatched from a wild collected egg mass incubated at ambient temperature over the winter at the Ocean Sciences Centre in St. John's. Initially 100 larvae were maintained in 30-L glass aquaria at temperatures of 4-7°C. One treatment (replicated) was given 100 *Artemia* nauplii/L plus Lansy N4 dry diet (300-500 µm) while a second treatment was given 900 *Artemia*/L plus dry diet. *Artemia* were enriched with Super Selco and the dry diet was given in excess. All tanks were fed twice a day and mortalities were removed daily. Algae *(Isochrysis)* were also

added to each tank daily. Five larvae were removed every two weeks for standard length and dry weight measurements.

Observations were carried out on three swimming and three resting (touching the bottom of the tank) larvae for two minutes each, twice a week. The first observations were conducted at 10 days post-hatch. At the end of each set of observations the number of larvae swimming or resting was also recorded.

The behavioural Modal Action Patterns or MAPs [3] were Orient, Fixate, Lunge, and Bite. Bite was further differentiated into bites at *Artemia* and bites at dry diet.

Results

Survival was significantly higher ($p < 0.0001$) among larvae fed *Artemia* nauplii at a rate of 900/L in combination with a dry diet. At the end of the study (63 days post-hatch) survival was 93.5% for fish fed 900 *Artemia*/L and 51.1% for those fed 100 *Artemia*/L ($p < 0.0001$).

At the end of the study both standard length ($p < 0.0001$) and dry weight ($p < 0.0001$) were significantly higher for fish fed a higher level of *Artemia*. At 61 days post-hatch low-fed fish were 30.4 ± 0.68 mm and weighed 41.1 ± 3.80 mg, whereas high-fed fish were 36.7 ± 0.53 mm and weighed 76.9 ± 4.84 mg.

The behavioural observations indicated that wolffish will wean themselves onto dry feed over time (i.e., they decrease the number of bites at *Artemia* and increase the bites at dry diet). Feeding behaviour among resting fish was similar for both treatments. Among swimming fish, those fed a higher level of *Artemia* initially performed more bites towards *Artemia* and subsequently weaned onto the dry feed earlier. These fish were biting almost exclusively at dry pellets at about 40 days post-hatch when they measured approximately 30 mm in length. This did not occur among fish fed the lower level of *Artemia* until 56 days post-hatch.

Discussion

Growth and survival was higher among larvae fed a high level of *Artemia* nauplii (900/L) in combination with a dry feed as compared to larvae fed 100/L of *Artemia* plus a dry diet. The results of the behavioural studies indicate that the difference in growth and survival was related to higher consumption of *Artemia* early in development.

Based on previous research that indicated larvae do poorly when fed *Artemia* only, the question arises as to why the level of *Artemia* affects growth and survival when larvae are fed both *Artemia* and dry diet. Several factors may help explain this. One is that *Artemia* alone are a nutritionally inadequate diet but when fed at high levels in combination with a dry diet may provide some nutritional benefit. High levels of moving prey may help to induce feeding behaviour and aid in the transition to dry feed. The behavioural results indicate that larvae fed a high level of *Artemia* swim less and feed more which would allow more energy for growth. *Artemia* may also affect the digestive capacity of larval wolffish. Larvae may utilize digestive enzymes in the live feed [4] to help digest dry feed. It may also be possible for ingested *Artemia* to help induce production of endogenous enzymes [5] for digestion of dry feed.

Conclusions

The results of this study showed that wolffish larvae fed a high level of *Artemia* nauplii (900/L) in combination with a dry pelleted feed grew faster, showed higher survival, and weaned onto dry feed earlier than larvae fed a low level of *Artemia*. With regards to feeding strategy, newly hatched wolffish should be given at least 900 *Artemia* nauplii/L in combination with a dry diet. Levels of *Artemia* should not be reduced until the wolffish larvae are about 40 days post-hatch (or 30.0 mm in length). At this time larvae should be observed feeding primarily on dry pellets.

Notes and references

1. Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, NF A1C 5S7

2. Moksness E, Gjosaeter J, Reinert A, Fjallstein IS. 1989. *Aquaculture* 77:221-228.

3. Barlow GW. 1968. In, *The Central Nervous System and Fish Behaviour.* (D Ingle, ed), p. 217-232. University of Chicago Press, Chicago.

4. Dabrowski K, Glogowski J. 1977. *Hydrobiologia* 54:129-134.

5. Govoni JJ, Bochlert GW, Watanabe Y. 1986. *Env. Biol. Fish* 16(1-3):59-77.

Rearing of lingcod, *Ophiodon elongatus*, to the juvenile stage

W. Craig Clarke, John E. Shelbourn, John Blackburn and John O. T. Jensen[1]

Lingcod larvae readily ingest *Artemia* nauplii within a few days after hatch. Earlier experiments demonstrated that it is possible to produce post-metamorphic juvenile lingcod using *Artemia* supplemented with "Super Selco". Larvae tolerated a wide range of salinities from 30‰ down to 20‰ or less. Reduced light intensity was found to be important for survival of newly hatched larvae in 5-L containers. In 1994, experiments were scaled up with "green water" and presentation of live *Artemia*. Larvae grew well initially, but attempts to wean them to inert diets from 36 days of age were unsuccessful and all were dead by day 78. In 1995, experiments were repeated in large tanks, again with green water and enriched *Artemia*, but with the continual addition of dry feeds. Growth in the latter trial was substantially more rapid than on *Artemia* alone and the juveniles reached a weight of 3.7 g by day 96.

Introduction

The lingcod *Ophiodon elongatus*, a member of the greenling family (Hexagrammidae), occurs from Alaska to California commonly at depths between 10 and 100 m. Its large size, tasty flesh and ease of capture have made it popular with sports and commercial fishermen, resulting in serious declines in lingcod populations in the vicinity of urban areas such as Puget Sound in Washington State and the Strait of Georgia in British Columbia. The decline in catches has stimulated interest in artificial propagation for restoration of depleted stocks[2] and for aquaculture.

Lingcod larvae are fully pigmented and swim actively immediately after hatching from an egg mass attached to the substrate. At hatching, larvae have a mouth gape of 1.5 mm and readily ingest *Artemia* nauplii within a few days. In the first experiments using non-supplemented nauplii, larvae failed to survive more than two weeks. Our preliminary experiments in 5-L containers indicated that survival of lingcod larvae during the critical early stages can be improved by reducing the salinity and by keeping them at low light intensity to reduce their swimming activity.[3] It was also shown that lingcod could be reared through metamorphosis by feeding *Artemia* enriched with essential fatty acids.

The present experiments were undertaken to test the efficacy of green water and the acceptability of inert feeds.

Methods

Larval rearing trials were conducted in 2-m diameter tanks. Three weeks prior to hatch of the larvae, the water supply was shut off and a culture of the green flagellate *Pyramimonas* sp. was added to the rearing tank along with nutrients to allow the algal concentration to build up.

Egg masses collected from spontaneous spawnings of captive lingcod were placed in 200-L tanks supplied with sea water (9.5°C, 30‰) at approximately 1 L/min; a circulating pump generated additional current to assist diffusion of water through the centre of the mass.[4] Just prior to hatch, the egg mass was placed into the rearing tank. This was done so that the larvae could emerge directly into the tank because they are very sensitive to handling. Before adding the eggs, the water was switched on at a rate of 1

L/min to achieve control of temperature and removal of wastes.

Brine shrimp (*Artemia* sp., Platinum Grade Argentemia®, Argent Chemical Laboratories, Redmond, USA) cysts were incubated for 24 h in vigorously aerated filtered 30‰ sea water at 28°C. The nauplii were decanted and placed in 20°C seawater containing Super Selco or high DHA Selco enrichment medium (Artemia Systems N.V., Baasrode, Belgium) at the recommended concentration of 0.6 g/L for a further 24 h. After rinsing with clean seawater, nauplii were added to the rearing tanks.

In the 1994 trials, larvae were fed daily with enriched *Artemia* from day 2 until day 36 after hatching, when Kyowa® fry feed was also presented.

In the 1995 trials, *Artemia* was presented daily for the first 4 days after hatch and then on alternate days. Lansy® dry feed was presented by hand daily from day 5. Kyowa® fry feed was also presented, beginning at day 25; freeze-dried euphausiids were provided from day 45. Feed sizes were adjusted as the fish grew. *Artemia* were no longer fed after day 78.

The water flow was increased at day 26 and the algae were cleared from the tank. Automatic feeders were installed at this point to distribute feed continuously during daylight hours. Water temperature varied between 9.6 and 12.8°C.

Samples of 6-10 juveniles were removed from the tank periodically, given an overdose of anesthetic, blotted dry and measured for total length and wet weight. Specific growth rate was calculated using the formula $\ln(Wt_1/Wt_0) \cdot 100 \cdot d^{-1}$, where Wt_0 and Wt_1 were initial and final mean wet weights.

Results

At the time of hatch, larvae were about 8 mm total length. They swam freely and were distributed through the water column, often obscured from view by the algae. Little "nosing" behaviour was observed along the sides of the tank.

In 1994, attempts to wean them to inert diets beginning on day 36 were unsuccessful and growth tapered off (Figs. 1, 2). The average weight reached a maximum of 112 mg on day 64 and then declined. The experiment was terminated on day 78 at 100% mortality.

Growth in the 1995 trials with *Artemia* plus inert feeds was considerably faster, both in length and weight when compared with 1994. Initially, examination of larval stomachs under a dissecting microscope indicated that *Artemia* nauplii were the principal item in the gut. However, as the larvae grew, ingestion of dry pellets became more apparent. A feeding response to the freeze-dried euphausiids was noticed soon after presentation on day 45. By day 96, the juveniles had an average total length of 89

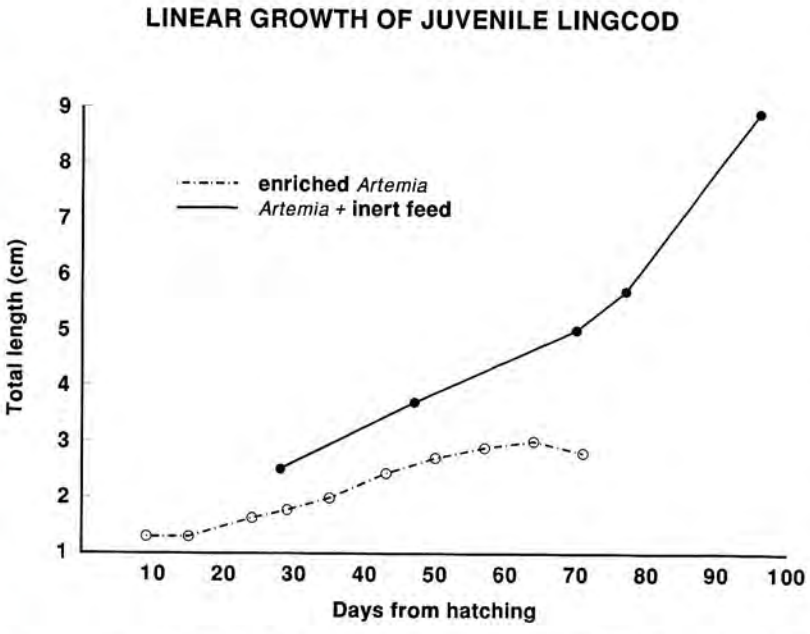


Figure 1. Linear growth of lingcod in two rearing trials, either on enriched *Artemia* alone until day 36 or on enriched *Artemia* plus inert diets from the beginning.

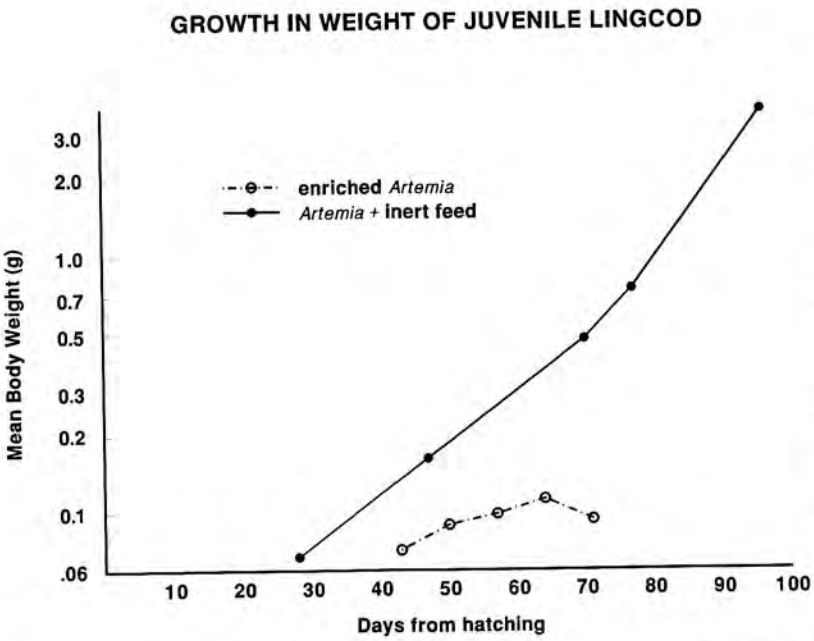


Figure 2. Growth in weight of lingcod in two rearing trials, either on enriched *Artemia* alone until day 36 or on enriched *Artemia* plus inert diets from the beginning.

and weight of 3.7 g (Figs. 1, 2). The average specific growth rate for weight from day 28 to day 96 was 5.9% body weight per day. Growth in length over the same period averaged 1.85% per day.

Mortality in the 1995 trial was high during the first month of rearing but numbers were not recorded. During the second month, daily mortalities averaged 11 fish per day (range from 0-30), with the higher numbers usually associated with the loss of a batch of *Artemia* with the result that no fresh nauplii were available for 4 days. During the third month, mortality averaged only 2 fish per day.

Discussion

The present experiments have shown that juvenile lingcod will accept dry inert diets, if they are presented from the time of first feeding. In contrast, attempts to wean them onto inert diets beginning one month after hatch were unsuccessful.

We conclude that the *Artemia* are required for the first two months of rearing because mortality increased abruptly when it was withdrawn for four days. However, when feeding of *Artemia* nauplii was terminated on day 78, there was no increase in mortality.

Green water was effective in reducing "nosing behaviour" during the critical early stages. This was in contrast to earlier trials in clear water at ambient light intensity where larvae exhibited rapid swimming behaviour at the water surface and along tank surfaces that was associated with reduced survival.[3,5]

The same effect has been obtained with the addition of green algae paste (*Nannochloropsis oculata*) to the rearing containers along with the food.[5]

Now that juvenile lingcod can be weaned onto inert dry diets, further research can be conducted to select suitable diets for maximizing growth.

We are indebted to Dr. J.N.C. Whyte for providing the algal stock culture and to Bill Damon for excellent technical assistance.

Notes and references

1. Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, BC V9R 5K6
2. Wespestad VG, Bargmann GG, Hay DE. 1994. *Aquacult. Fish. Manag.* 25, Suppl. 1:189-197.
3. Appelbaum S, Clarke WC, Shelbourn JE, Jensen JOT, Whyte JNC, Iwama GK. 1995. *Aquaculture* 135:219-227.
4. Giorgi AE, Congleton JL. 1984. *Envir. Biol. Fish* 10:15-27.
5. Marliave J. 1994. *Proceedings Annual Conference of the American Zoo and Aquarium Society*, p. 266-269. American Zoo and Aquarium Society, Atlanta, Georgia.

Culturing Atlantic cod (*Gadus morhua*): importance of diet

Danny Boyce,[1] *Joseph A. Brown* [1]
and A. J. G. Moir[2]

Due to the crises in the fishery throughout Atlantic Canada and particularly in Newfoundland, cod aquaculture is being promoted as a means to diversify the industry. A pilot study using commercial production protocols for larval cod was conducted to evaluate larval performance on two different diets. Eggs were collected from captive broodstock and larvae were reared in intensive and extensive systems. The study consisted of feeding larvae either cultured enriched zooplankton (rotifers and *Artemia*) or a mixture of cultured enriched zooplankton and wild zooplankton. Results indicated that cod larvae fed a diet of cultured and wild zooplankton grew faster (standard length and dry weight) and survived better than the larvae fed only cultured zooplankton. It is suggested that a diet consisting of a mixture of cultured and wild zooplankton will enhance the production of cod larvae. Comparisons between intensive and extensive production systems suggest extensive systems have potential for rearing larval cod.

Introduction

Due to the crisis in the fishery throughout Atlantic Canada and particularly in Newfoundland, cod aquaculture is being promoted as a means to diversify the industry and help replenish wild stocks. Large numbers of cod have been successfully produced using indoor tanks and enriched live feed.[3] Growth of cod (expressed as specific growth rate, SGR) can vary widely with rearing conditions. In Norway, Rosenlund et al.[3] reported growth rates from 7-14% per day up to 70 days post-hatch using live feed (micro-algae, rotifers, and *Artemia*) and growth in extensive and semi-extensive systems (bag enclosures) have been reported to be as high as 21.7% in the first three weeks post-hatch using naturally occurring plankton as a feed source.[4] In the study reported here, we conducted laboratory experiments to 1) replicate the Norwegian experiments using northeastern Grand Bank Atlantic cod, and 2) to determine the impact of diet on growth and survival of larvae reared in intensive and extensive systems.

Materials and methods

Cod larvae were reared at the Ocean Sciences Centre (OSC), Memorial University of Newfoundland, from fertilized eggs collected from northeastern Grand Bank broodstock maintained at OSC throughout the autumn and winter of 1993-1994. The intensive rearing system consisted of 30-liter aquariums that had a water flow of 30 liters/hour. Larval density was 30 larvae/liter and prey levels were kept at 4000/liter. Prey consisted of rotifer, *Artemia*, and/or wild zooplankton. The extensive rearing system consisted of 240-liter circular tanks with a water flow of 120 liters/hour. Larval density was 16 larvae/liter (all that was available) and prey levels were 4000/liter. Prey consisted of rotifers, *Artemia*, and/or wild zooplankton. In both systems, light levels were 600-700 lux with a 24

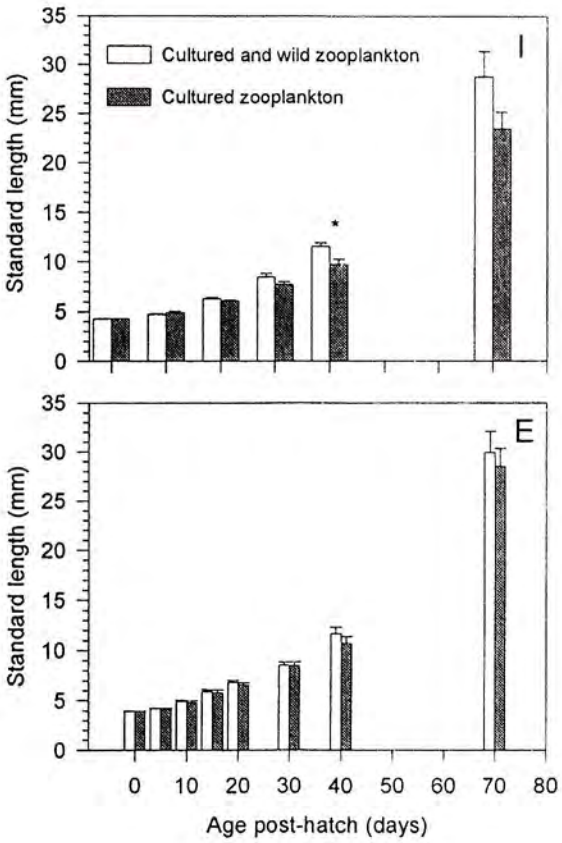


Figure 1. Effect of diet on increase in length of larval cod reared in intensive (I) and extensive (E) systems. * Indicates a significant difference.

Table 1. Survival of cod larvae reared in intensive and extensive systems.

	Intensive				Extensive			
	Tanks 1a and 1b[a] Cultured enriched and wild zooplankton		Tanks 2a and 2b Cultured enriched zooplankton		Tank 1 Cultured enriched and wild zooplankton		Tank 2 Cultured enriched zooplankton	
Day	Number. of Larvae	% Survival	Number. of Larvae	% Survival	Number of Larvae	% Survival	Number of Larvae	% Survival
0	1800	100	1800	100	3840	100	3840	100
40	106	5.88	95	5.27	150	3.90	90	2.34
50	80	4.44	48	2.66	131	3.41	65	1.69
60	57	3.16	26	1.44	62	1.61	29	0.76
70	29	1.61	7	0.38	33	0.86	23	0.59

[a] Intensive rearing system had replicates with combined results

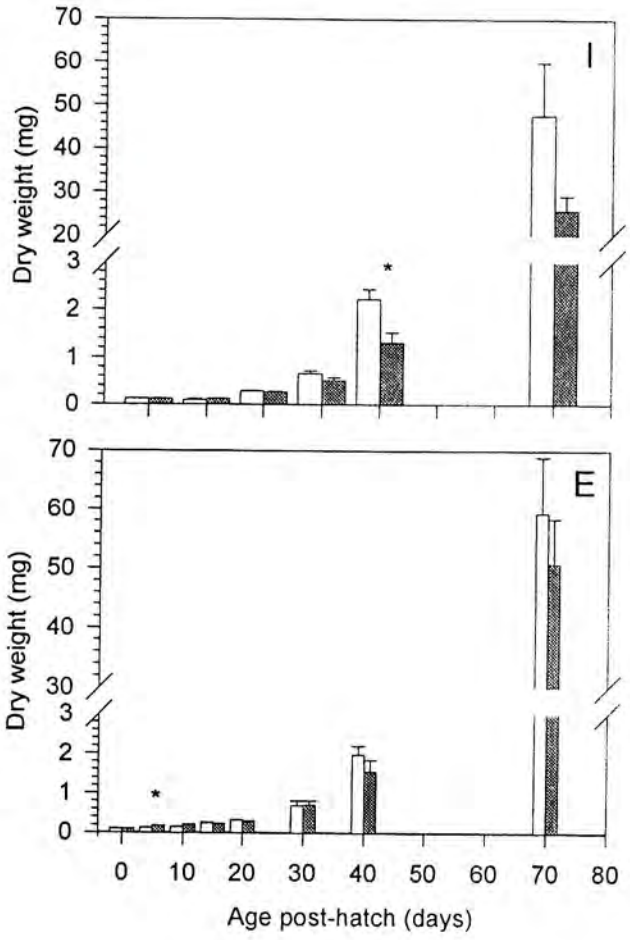


Figure 2. Effect of diet on increase in dry weight of larval cod reared in intensive (I) and extensive (E) systems. * Indicates a significant difference.

LD24:0). Temperatures were maintained at 9 ± 1°C.

Evaluation of growth and survival was done using two diets: 1) cultured, enriched zooplankton (rotifers and *Artemia*) and wild zooplankton, and 2) cultured, enriched zooplankton (rotifers and *Artemia*) only. Larvae were measured for standard length (mm) and dry weight (mg). Survival estimates were also recorded.

Results

Overall, there was no significant difference ($P > 0.05$) in standard length (mm) or dry weight (mg) between larvae fed the two diets in either the intensive or extensive rearing systems (Fig. 1 and 2). However, survival was greater in both the intensive and extensive systems when the larvae received a mixed diet of cultured enriched zooplankton (rotifer and *Artemia*) and wild zooplankton (Table 1) than when fed just cultured enriched zooplankton.

Conclusion

Diets were important in determining growth and survival of cod larvae. Results indicated larvae fed a diet consisting of cultured enriched and wild zooplankton showed greater growth and survival than those larvae fed only cultured enriched zooplankton.

Wild zooplankton are an important part of wild larval diets and, if available, should be used in feeding cultured larvae. Lipid levels in wild zooplankton decreased over the season; therefore, if these experiments had started in early spring, when lipid levels of wild zooplankton were high, greater growth and survival may have occurred. Cultured, enriched zooplankton (rotifers and brine shrimp) should be used as a supplement to wild zooplankton rather than as an exclusive feed for larval fish.

Notes and references

1. Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, NF A1C 5S7
2. Sea Forest Plantation Co. Ltd. St. John's, NF A1C 5K4

3. Rosenlund G, Meslo I, Rodsjo R, Torp H. 1993. In, *Proceedings of the first international conference on fish farming technology.* (H Reinersten et al., eds), p. 141-148. Trondheim, Norway.
4. van der Meeren T, Naess T. 1993. *Mar. Biol.* 116: 637-647.

Non-specific factors important to innate disease resistance in coho salmon from British Columbia

Shannon K. Balfry and George K. Iwama[1]

Communally reared coho salmon (*Oncorhynchus kisutch*) from Robertson Creek, Quinsam River, Kitimat River, Capilano River, and Chehalis River were compared for differences in innate disease resistance. At 60 g average weight, the fish were experimentally infected with *Vibrio anguillarum* (Vang) by two methods, immersion and injection. Strain differences in percent cumulative mortality and mean time to death occurred for the immersion challenged group, whereas no differences were observed among the strains challenged by injection. To determine the role of external barriers on the observed strain differences, the immersion challenge was repeated on the fish from the most resistant (Robertson), least resistant (Quinsam), and moderately resistant (Kitimat) strains. The number of Vang cells within the gill tissue, immediately post-challenge, was found to be lowest in the Robertson strain. Furthermore, a heat-sensitive bactericidal factor was found in the mucus. The Robertson strain, which was the more disease-resistant, also had the greatest mucus bactericidal activity.

Introduction

Selection for disease resistance in cultured salmonids may be an effective solution to the increasing problems of maintaining fish health in intensive fish production. Strain differences in disease resistance have been well documented,[2] however the underlying mechanisms are poorly understood. Natural selection for enhanced innate, non-specific immunity has been implicated in the evolution of strain differences in disease resistance.[3] The non-specific immune system of fish includes external barriers, soluble and cellular factors. External barriers such as mucus, skin, and scales inhibit the attachment of pathogens to the surface of the fish. Bactericidal substances such as complement, antibody, agglutinins and lysozyme have been found in mucus, and are able to prevent the entry of pathogen.[4,5] In addition, phagocytic cells in the epidermis[6] can also protect the fish from infection.

The objective was to compare strains of coho salmon *(Oncorhynchus kisutch)* for differences in disease resistance. Aspects of the innate immune system of the fish were examined in an attempt to identify possible mechanisms for the strain differences in disease resistance.

Material and methods

Gametes from 10 pairs of returning adult coho salmon were randomly collected from five different rivers in British Columbia (Robertson Creek, Kitimat River, Chehalis River, Quinsam River, and Capilano River). Each strain was comprised of equal numbers of individuals, representing each of 10 (unmarked) full-sib families. Fish were fin-clipped at 3 g for identification and combined into communal freshwater tanks.

Disease challenge experiments were performed when the fish reached 60 g (mean ±1 SD). Immersion and intraperitoneal injection challenges were carried out simultaneously, where 10 fish per strain were placed into four replicate tanks receiving freshwater at 10°C. Four tanks containing unchallenged control fish were also included. Primary isolates of the marine fish pathogen, *Vibrio anguillarum* (Vang) were used for the disease challenges. Immersion challenges were performed by immersing each tank of fish in 2.23×10^{10} Vang/mL peptone-saline (P-S; 0.1-0.85% peptone-NaCl, respectively) for 20 min. The injection challenge group were anaesthetized (MS222) and injected intraperitoneally (ip) with 2.28×10^{6} Vang/fish

suspended in phosphate buffered saline (PBS; pH 7.4). Mortalities were monitored for three weeks post-challenge, and the cumulative percent mortality per strain for each challenge was calculated. Kidney, spleen and liver smears were taken from each dead fish and cultured on tryptic soy agar (TSA) supplemented with 1.5% NaCl. Colonies were Gram-stained and the presence of gram-negative, curved rods used to confirm the cause of death as vibriosis.

Ten fish (60 g) from each of the Robertson, Quinsam, and Kitimat strains were immersion challenged as described above ($1.26x10^9$ cfu Vang/mL P-S). Immediately following the challenge, each fish was killed, bled and 7 gill arches were removed into sterile, iced saline. The gills were washed to remove any attached Vang from the gill surface, then the gill arches and associated cartilage were removed. The remaining gill filaments were homogenized in 10 mL saline, and centrifuged. The supernatant was discarded and a 50% homogenate (w/v) prepared from the pellet and tryptic soy broth (TSB). The homogenates were drop-plated onto TSA, incubated overnight and Vang colonies were counted. The number of Vang cells per gram of gill tissue for each fish was calculated.

Ten fish were sampled from each of 3 strains: Robertson, Quinsam, and Kitimat. The fish were anaesthetized (MS222) and the mucus was scraped off the body with a rubber policeman into a sterile test tube. Mucus was diluted 1:4 v/v with sterile, iced PBS, and stored at -70°C.

Protein concentrations of the mucus samples were measured in a microplate reader using the bicinchoninic acid procedure.[7] The bactericidal activity of mucus was determined for untreated mucus, heat treated,[8] and sterile TSB. Equal volumes (100 µL) of the mucus preparations, and a saline control (0.9% NaCl) were combined with a suspension of Vang (final concentration $4.3x10^5$ Vang cfu/mL). The preparations were immediately drop plated onto TSA, and incubated on a rotator at 18°C. Samples from the preparations were drop plated onto TSA, after 1.5 and 7 h incubation. Inoculated plates were incubated overnight and the Vang colonies counted. The number of Vang cells per µg mucus protein was determined, and the survival of Vang at the two sample times was calculated as the percent change from the starting number of Vang present in the preparation.

Strain differences in mortality were determined from chi square tests. Strain differences in Vang/g gill and mucus bactericidal activity were examined from analyses of variance tests. Comparisons between the strains were performed using Student-Newman-Keul multiple comparison test. Strain differences were noted where $p<0.05$. Sigmastat software (Jandel Scientific, San Rafael, CA) was used for all analyses.

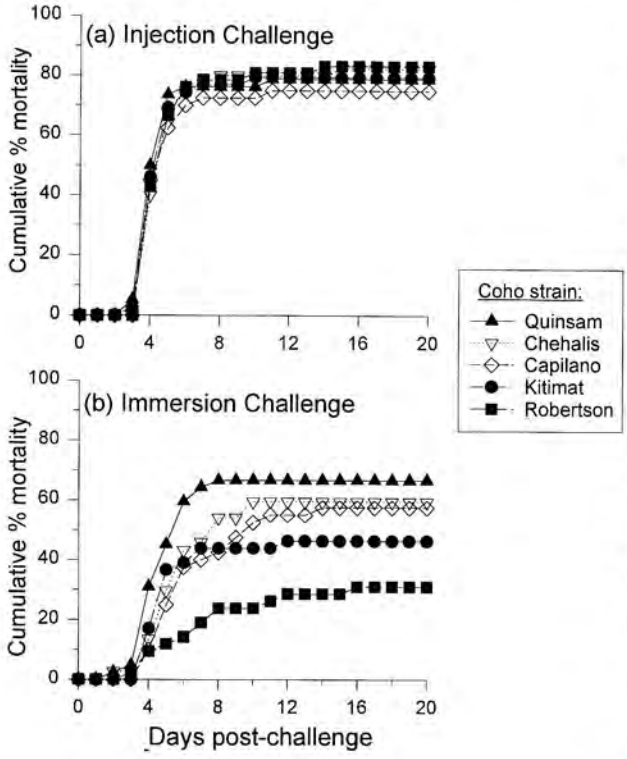


Figure 1. Post-challenge cumulative percent mortality for 5 strains of coho salmon challenged with *Vibrio anguillarum* by immersion and intraperitoneal injection. Ten fish per strain were combined in a single tank, with 4 tanks per challenge method.

Results and discussion

Significant strain differences in total percent cumulative mortality and mean time to death were detected when the fish were challenged by immersion, while no strain differences were detected when injection challenged (Fig. 1). The Quinsam coho

were found to be the most susceptible to vibriosis, followed by the Chehalis, Capilano, Kitimat, and Robertson coho.

The external barriers of the fish were bypassed when the fish were challenged with the injection challenge. In such a direct challenge to the immune system, all the strains responded equally poorly. In contrast, in the more natural immersion challenge, external barriers to infection probably played a more significant role in disease resistance. It would therefore appear that Robertson coho, with lowest mortality, possessed the more effective external defense system.

Gills are a major site of pathogen entry.[9] The experiment examining the uptake of Vang at the gills supports the mortality results. Greater numbers of Vang were isolated from the gills of the Quinsam coho, followed by the Kitimat and Robertson (Fig. 2a), indicating that the observed low mortality rate may be associated with a reduced uptake of Vang following the immersion challenge. The Robertson coho may have had fewer bacteria entering via the gills, so that a systemic infection was more readily resisted.

Further investigations into the mechanisms of the observed strain differences in disease resistance revealed the presence of a heat-sensitive bactericidal factor in the mucus. The Robertson coho mucus possessed significantly greater bactericidal activity against Vang (Fig. 2b). However, when the mucus was heat-treated the bactericidal activity was destroyed and the survival of Vang was not significantly different from the control. Such a heat treatment applied to the mucus is known to inactivate complement in coho salmon.[8] It is possible that complement was the factor responsible for the observed bactericidal activity in the mucus; ongoing studies in progress could verify this.

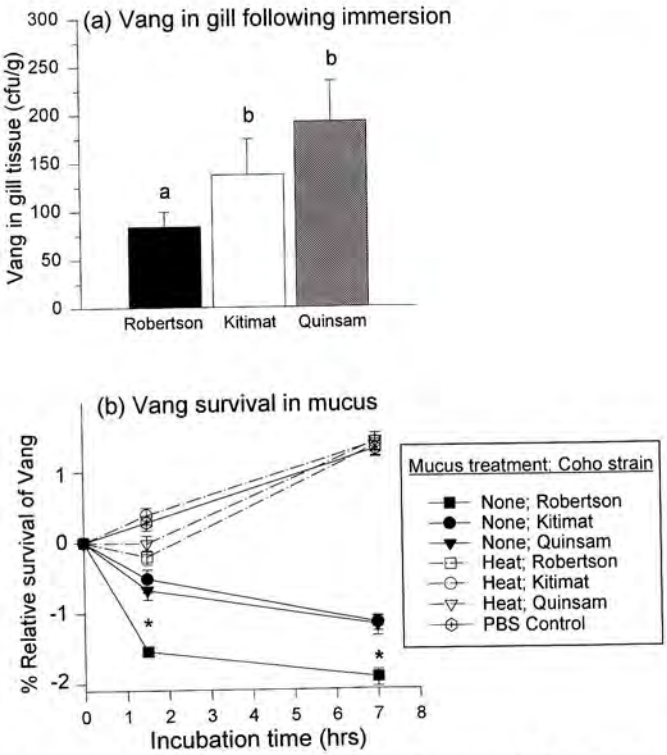


Figure 2. Comparison of the role of external barriers in resistance to experimental infections of *Vibrio anguillarum* (Vang) among 3 strains of coho salmon *(Oncorhynchus kisutch)*. (a) Number of Vang isolated from gill tissue homogenates immediately following an immersion challenge (n=10). Vertical bars represent SEM. Different letters represent significant differences (p<0.05). (b) Percent relative survival of Vang in heat-treated and untreated mucus preparations (n=10). Significant differences between Robertson untreated mucus and other strains (*p<0.05).

Notes and references

1. Department of Animal Science, Canadian Bacterial Diseases Network, University of British Columbia, Suite 208-2357 Main Mall, Vancouver, BC V6T 1Z4

2. Chevassus B, Dorson M. 1990. *Aquaculture* 85:83-107.
3. Beachem TD, Evelyn TPT. 1992. *Trans. Am. Fish. Soc.* 121:456-485.
4. Harrell LW, Etlinger HM, Hodgins HO. 1976. *Aquaculture* 7:363-370.
5. Ingram GA. 1980. *J. Fish Biol.* 16:23-60.
6. Peleteiro MC, Richards RH. 1990. *J. Fish Dis.* 13: 225-232.
7. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC. 1985. *Anal. Biochem.* 150:76-85.
8. Sakai DK. 1981. *Bull. Jap. Soc. Sci. Fish.* 47:565-571.
9. Bowers A, Alexander JB. 1982. *J. Fish Dis.* 5:145-151.

We are grateful to the managers and personnel of the Quinsam River, Kitimat River, Chehalis River, Capilano River, Robertson Creek, and Rosewall hatcheries. Thanks to Dr. TPT Evelyn for his advice and expertise. Technical support was provided by Ellen Teng.

Effects of intestinal helminths on lipid storage in overwintering Arctic charr from northern Quebec

K. J. Kolasa and M. A. Curtis[1]

A lake-resident Arctic charr population heavily infected with intestinal parasites was investigated to assess host-parasite relationships over winter. Fish of age 3^+ to 5^+ infected with the cestode *Eubothrium salvelini* or the acanthocephalan *Echinorhynchus lateralis* were analyzed for total lipid and compared with uninfected fish. In fall and spring both parasites reduced total lipid levels in host muscle and liver tissue compared to control fish. However, charr infected by *E. salvelini* and *E. lateralis* had more visceral lipid in spring than the controls. Infected charr may thus begin foraging and replenishing visceral lipid earlier in spring than uninfected fish to compensate for the energy demands imposed by the parasites.

Introduction

Studies on the effects of intestinal parasites on fish have yielded differing results. In some cases only minor histopathological changes in the fish intestine or negligible effects upon growth were found,[2,3] while in others, the intestinal cestode *Eubothrium crassum* retarded growth in Atlantic salmon[4] and *E. salvelini* had deleterious effects on survival and swimming performance in Sockeye salmon.[5] *E. salvelini* was also reported to reduce the condition index in Arctic charr.[6]

The bioenergetics of host-parasite relationships have been rarely investigated in the context of overwintering stress in fish,[7] and there is no information on this topic for Arctic charr, a species from cold water lakes in the Northern Hemisphere.[8] In such habitats, overwintering fish depend on stored energy reserves and parasitized[9] fish may be subject to additional morbidity or mortality, particularly when heavily infected.[10] In a recent study on seasonal dynamics of intestinal parasites in Arctic charr, we found[11] evidence of increases in parasite biomass over winter for *E. salvelini* and *Echinorhynchus lateralis* and suggested this could cause morbidity in the overwintering fish. The present study attempted to quantify the degree of this morbidity by using lipid storage levels to assess fish condition.

Materials and methods

Lac Kitturiak, in northern Quebec, is a headwater lake (<10 ha, 275 m a.s.l.) inhabited by two fish species: Arctic charr and three-spined stickleback. Charr were collected by gill nets in fall 1992 (before freeze-up) and in spring 1993 (after break-up). In the fall, fish were wrapped individually in polyethylene film and kept on ice for 1-3 days before freezing. In the spring, fish were measured, weighed, wrapped in the polyethylene, frozen on site (-25°C) and stored at -70°C. In the laboratory, the fish defrosted in tap water were necropsied at 4°C. A 1 g sample of muscle tissue from the right dorsal side of the fish was taken as well as the entire liver, gonads, and visceral organs (without digestive system contents). Samples were weighed (0.1 mg) and frozen at -70°C. Water content was assessed by lyophilizing (-50°C, 48 h). Aging and parasite sampling protocols are described in Kolasa and Curtis.[11] Only 3^+ to 5^+ Arctic charr were analyzed (n=254). The fish were categorized as: 1) control group, without parasites;[11] 2) group infected with *E. salvelini*, when the parasite dry weight was ≥1 mg and no *Echinorhynchus lateralis*, *Diphyllobothrium ditremum*, *D. dendriticum*, *Proteocephalus* sp., or *Philonema agubernaculum* were present; 3) group infected with only *E. lateralis* (≥10 mg dry weight). Males and females were selected in equal numbers for the analyses, except for fall when the

Table 1. Summary data on Arctic charr (mean ± standard error) selected for proximate analyses, n=6 in each group.

		fork length (mm)	body weight (g)	digestive system contents (wet weight, g)	number of worms per fish	parasite dry weight (mg)
Fall	Control	165 ± 4	42.2 ± 2.5	0.5976 ± 0.1907	—	—
	Eubothrium salvelini	161 ± 4	39.5 ± 2.9	0.4778 ± 0.1144	15 ± 4	13 ± 4
	Echinorhynchus lateralis	162 ± 7	40.0 ± 5.2	0.5873 ± 0.1455	17 ± 2	29 ± 3
Spring	Control	152 ± 2	31.9 ± 1.7	0.5559 ± 0.0509	—	—
	Eubothrium salvelini	152 ± 9	32.2 ± 4.7	0.7563 ± 0.1107	9 ± 5	15 ± 3
	Echinorhynchus lateralis	165 ± 5	44.5 ± 6.0	1.1854 ± 0.3102	62 ± 15	76 ± 16

group infected with *E. lateralis* was all female. Three groups of 6 fish from each season were analyzed for total lipid and protein content.[12-14] Tissue analyses were performed in triplicate on 30-mg lyophilized subsamples. Tissue contents of lipid, protein, and water were calculated as percentages of wet tissue weights, and lipid and protein were also calculated as dry tissue weight. Influences of season, parasite infection, and sex on the variability of the parameters were tested by ANOVA. Differences between group means were tested with Student's t-test.

Results

The weight-length relationships of Arctic charr did not differ significantly among the parasitized and control groups, between sexes or between seasons (Table 1). However, fish infected with *E. salvelini* and *E. lateralis* tended to have more food items (wet weight) in their digestive systems than did controls, particularly in spring. In spring, those infected with helminths had higher parasite loads per fish than in the fall. Group categorisation accounted for 82% of the variability in total lipid contents of charr muscle tissue in fall ($F_{3.5959}$=16.05, P<0.0002) and 58% in spring ($F_{0.4398}$=3.71, P<0.04). A similar pattern was found for liver lipid (61% in fall, $F_{12.04}$= 4.42, P <0.03; 71% in spring, $F_{0.1494}$=7.59, P<0.005). All fish infected by *E. lateralis* had significantly lower lipid levels in muscle and in liver tissue than controls. Visceral lipid levels seemed to be higher in infected fish than in the control group in spring (Fig. 1). Water content in charr tissue was inversely related to lipid content, except for the control group, where an overwinter decrease in water content of muscle tissue occurred (P<0.01). In fall, charr infected with *E. salvelini* had significantly (P<0.01) more protein in muscle tissue then did control fish (Table 2).

Discussion

Total lipid, water and protein levels in charr tissue from the control group corresponded with findings from other studies.[15-17] Observed differences between fall and spring in the water content of muscle tissue in control charr may be explained by an increase in the protein content in spring. In Arctic charr in northern Norway, total lipid content in the fish muscle was 10.0% (dry weight) ±1.2 (SD) in October,[15] comparable to the muscle lipid level we found in fall

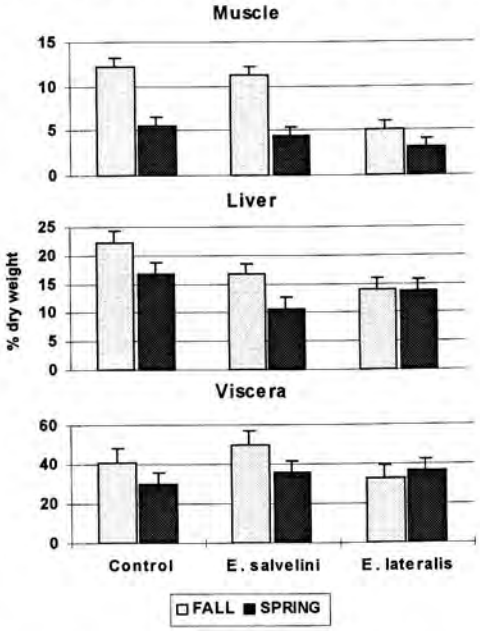


Figure 1. Total lipid content in Arctic charr tissue (%dry weight, means [n=6] ± SE).

Table 2. Proximate composition of Arctic charr tissue (% wet weight, means [n=6] ± SE).

		Muscle	Liver	Viscera	Gonads
			Water		
Fall	Control	81.0 ± 0.3	74.3 ± 2.1	72.8 ± 2.1	80.1 ± 1.4
	E. salvelini	80.4 ± 0.3	77.9 ± 2.1	68.5 ± 2.2	76.7 ± 1.6
	E. lateralis	81.3 ± 0.8	78.2 ± 0.9	70.1 ± 4.2	77.8 ± 1.4
Spring	Control	79.0 ± 0.6	76.1 ± 0.9	69.9 ± 1.5	80.2 ± 1.0
	E. salvelini	80.9 ± 1.3	78.0 ± 0.5	71.4 ± 2.7	79.6 ± 2.5
	E. lateralis	80.1 ± 0.6	78.9 ± 0.7	72.6 ± 0.4	78.0 ± 2.8
			Protein		
Fall	Control	11.1 ± 0.7	13.4 ± 1.4	11.1 ± 1.4	6.6 ± 0.6
	E. salvelini	13.5 ± 0.4	10.6 ± 1.1	11.4 ± 0.9	7.5 ± 1.1
	E. lateralis	11.4 ± 0.9	10.0 ± 1.5	12.6 ± 1.4	9.0 ± 0.8
Spring	Control	12.1 ± 0.4	13.0 ± 1.1	13.4 ± 1.7	7.1 ± 1.3
	E. salvelini	11.1 ± 0.8	10.4 ± 1.8	10.4 ± 2.1	7.3 ± 0.7
	E. lateralis	12.5 ± 0.8	11.3 ± 0.9	12.7 ± 0.6	9.4 ± 0.5
			Lipid		
Fall	Control	2.3 ± 0.2	5.7 ± 0.6	11.4 ± 1.4	2.7 ± 0.5
	E. salvelini	2.2 ± 0.2	3.9 ± 0.8	15.9 ± 1.8	3.9 ± 1.3
	E. lateralis	0.9 ± 0.1	2.9 ± 0.5	10.7 ± 3.5	4.4 ± 0.7
Spring	Control	1.1 ± 0.2	3.9 ± 0.4	9.0 ± 1.2	2.9 ± 0.7
	E. salvelini	0.8 ± 0.1	2.3 ± 0.3	10.7 ± 3.0	2.7 ± 1.3
	E. lateralis	0.6 ± 0.0	2.9 ± 0.6	9.9 ± 1.2	4.0 ± 1.3

sampling of the controls (12.2% ± 2.5) and in fish infected with *E. salvelini* (11.2% ± 2.8). Muscle lipid content of fish infected with *E. lateralis* was on average half that of the uninfected controls (5.0% ± 1.3). The differences in liver lipid observed among groups followed the same pattern as that for muscle tissue but were less pronounced.

It seems that heavily infected charr were affected by their parasite infections by the end of open water season when they already had decreased lipid stores compared to uninfected fish. In spring, this pattern was still observed in terms of muscle and liver lipid, while the visceral stores showed an opposite trend; infected fish had more food items in their digestive systems. Thus in spring, infected fish may begin foraging and replenishing lipid stores before the fish that are free of parasites. These findings suggest that parasites, such as *E. salvelini* and *E. lateralis*, can affect the levels of their hosts' energy stores, and support the hypothesis that lipid depots are controlling food intake in Arctic charr.[18]

Notes and references

1. Dept. Natural Resource Sciences, McGill University, Macdonald Campus, 21,111 Lakeshore, Ste-Anne-de-Bellevue, QC H9X 1C0

2. Ingham L, Arme C. 1973. *J. Fish Biol.* 5:309-313.
3. Hine PM, Kennedy CR. 1974. *J. Fish Biol.* 6:521-535.
4. Bristow GA, Berland B. 1991. *Aquaculture* 98:325-330.
5. Boyce NP. 1979. *Can. J. Zool.* 57:597-602.
6. Hoffman R, Kennedy CR, Meder J. 1986. *J. Fish Dis.* 9:153-157.
7. Lemly AD, Esch GW. 1984. J. Parasit. 70(4):475-492.
8. Johnson L. 1980. In, *Charrs, salmonid fishes of the genus Salvelinus.* (EK Balon, ed), p. 15-98. W. Junk, The Hague.
9. Curtis MA. 1979. *Natur. Canadien* 106:337-338.
10. Bérubé M, Curtis MA. 1986. *Can. J. Fish. Aquat. Sci.* 43:1626-1634.
11. Kolasa K, Curtis M. 1995. *Nordic J. Freshw. Res.* (in press).
12. Folch J, Lees M, Stanley GHS. 1957. *J. Biol. Chem.* 226:497-509.
13. Hopkins CCE, Seiring JV, Nyholmen O. 1984. *Oceanogr. Mar. Biol. Ann. Rev.* 22:211-261.
14. Gornall AG, Bardawill CJ, David MM. 1949. *J. Biol. Chem.* 177:751-766.
15. Ringo E, Burkow IC. 1990. *Fisk. Dir. Skr., Ser. Ernaering* III:19-25.
16. Miglavs I, Jobling M. 1989. *J. Fish Biol.* 35:1-11.
17. Boivin TG, Power G. 1990. *Can. J. Zool.* 68:2284-2289.
18. Jobling M, Miglavs I. 1993. *J. Fish Biol.* 43:487-489.

Elemental iodine as a fungicide for Pacific salmon

J. O. T. Jensen,[1] W. E. McLean,[2] W. Damon,[1] T. Sweeten[3] and D. O'Dowd[4]

Elemental iodine was tested for fungicidal efficacy on coho salmon eggs and on chinook salmon and steelhead trout prespawning adults. Intermittent exposure to 0.2 mg/L iodine for 1-12 hours/day significantly reduced egg mortality. However, daily exposures of 8 to 24 h to iodine levels of 0.2 mg/L caused hardening of the egg capsule and alevin and fry mortality. In chinook and steelhead adults, continuous exposure to iodine concentrations of < 0.1 mg/L reduced mortalities due to fungus in prespawning fish.

Introduction

The Salmonid Enhancement Program, Department of Fisheries and Oceans, has been attempting to find a fungicide that leaves no deleterious residues in salmon and has minimal environmental impact. Aqueous iodine has had federal approval as a treatment for domestic drinking water since 1987 and thus is being tested on fish. Iodine persists longer than other halogens as it is the least soluble, has the lowest oxidation potential, and has the least reaction with organic compounds.[5] Solubility varies from 150 mg/L at 0°C to 250 mg/L at 20°C. Hydrolysis of iodine produces I_2, HIO, and OI-; I_2 and HIO have germicidal properties.[5,6]

Methods

Iodine Application. Aqueous iodine was generated by passing water through two cartridges filled with elemental iodine (Iomech Ltd.) and arranged in series so that at design flow rates the iodine concentration was near saturation. In some experiments, two dilution steps were used to reach target levels. This value, the *nominal iodine concentration*, was calculated from the final dilution rates. The actual iodine concentration at the eggs or fish was lower and depends on many factors including the water/iodine contact time and the organic load. Because the interaction of iodine and water may be important to the effectiveness of the treatment, contact time was determined for each experiment.

Iodine demand and measurements. Iodine measurements were taken for each water source (Big Qualicum River (BQ), Puntledge River, Robertson Creek, Pacific Biological Station (PBS), plus distilled water for comparison). Iodine was dissolved in distilled water to produce a solution of 200 ppm. Stock solution was diluted to a concentration of 1.5 ppm iodine in each type of water. Iodine concentration was measured spectrophotometrically over 60 min using the DPD method(7) which measures both I_2 and HIO.

Adult treatment. At Puntledge Hatchery, 30 male chinook salmon were divided into three 10-ft (3m) circular ponds on 6 October 1994. Fish were maturing and would die after spawning. One group were held as untreated controls. The other 2 groups were held in Puntledge river water with iodine added continuously at a nominal concentration of 0.1-0.15 mg/L for 24 d. Iodine concentrate was mixed with the main flow and then directed immediately to the ponds. Water flow rate was approximately 100 L/min (mean residence time 67 min).

Adult female and male steelhead brood stock were treated with iodine at Robertson Creek Hatchery. Fish were held in 10-ft (3 m) circular ponds and also in rectangular condominiums. Iodine was delivered to the ponds as at Puntledge. The condominiums (water volume 184 L) were supplied with iodine in 2 stages. First, the concentrate was diluted to 5 mg/L and held for about 4 min in a mixing chamber. The final dilution to 0.1 mg/L occurred in the condominiums (mean residence time 13 min). Iodine treatments were carried out on several groups of fish between 15 December 1994 and 11 April 1995.

Egg treatment. A preliminary test with coho eggs was conducted at Big Qualicum River in

1993-94. Coho eggs were fertilized and placed in incubation trays (650 eggs/tray; 3 trays/treatment) and exposed to iodine (nominal treatments of 0, 0.18, 0.26, 0.34, and 0.42 mg/L) until the "eyed" stage (37 d; 241°C-d; mean temperature 6.6°C). Another test was conducted in 1994-95. Loaded trays of fertilized coho eggs were treated with iodine (nominal concentrations of 0.49 mg/L for 1, 4, 8, and 24 h/d) until the "eyed" stage (55 d; 291°C-days; mean temperature 5.4°C). The iodine delivery system failed on 5 days, so eggs received the target dose on only 50 d.

A multifactorial test (3 x 3 x 3, plus 3 untreated controls; total of 30 treatments) was conducted at PBS using a subsample of the coho eggs tested above (BQ 94/95). The experimental factors were: nominal iodine 0.2, 0.5, 1.0 mg/L; temperature 6, 9, 12°C; exposure 1, 12, 24 h/d. Four subgroups (~30 eggs per replicate) of eggs were placed in incubators and 3 "unfertilized", shocked eggs were added per replicate as a source for fungus growth. Iodine treatment was carried out to the "eyed" egg stage (i.e., for 26, 35, and 47 d for each of the temperatures of 6, 9, and 12°C, respectively).

Table 1. Experimental conditions and egg, alevin, and rearing mortality from the coho egg experiments.

Iodine (mg/L)	°C	Days	Hours/ day	Egg Mortality (%)	Alevin Mortality %)	Total Mortality (%)	Rearing (%)
				BQ 93/94			
0	6.6	0	0	22.53	0.70	23.24	
0.077	6.6	37	24	27.09			
0.111	6.6	37	24	07.70	1.31	9.00	
0.145	6.6	37	24	09.03	0.72	9.75	
0.179	6.6	37	24	06.16	1.57	7.72	
				BQ 94/95			
0	5.4	0	0	39.76	2.26	42.02	1.57
0.205	5.4	50	1	15.79	1.38	17.17	
0.194	5.4	50	4	14.88	0.83	15.71	2.43
0.196	5.4	50	8	13.74	1.18	14.92	13.73
0.196	5.4	50	24	05.45	8.62	14.08	
				PBS 94/95			
0	6.1	0	0	30.7	02.6	33.3	
0	8.8	0	0	15.8	02.4	18.2	
0	10.8	0	0	19.4	02.6	22.0	
0.33	5.3	47	1	11.9	02.5	14.4	
0.26	8.3	35	1	22.4	02.6	25.0	
0.23	10.0	26	1	22.3	06.0	28.3	
0.27	5.4	47	12	13.6	02.0	15.6	
0.24	8.3	35	12	04.3	02.2	06.5	
0.36	10.1	26	12	13.2	12.4	25.6	
0.21	5.5	47	24	13.0	03.3	16.3	
0.25	8.4	35	24	10.5	02.3	12.8	
0.24	9.8	26	24	06.3	12.6	18.9	
0.45	5.4	47	1	10.2	03.2	13.4	
0.48	8.4	35	1	18.7	06.0	24.7	
0.44	9.9	26	1	10.6	08.7	19.3	
0.55	5.8	47	12	09.8	06.8	16.6	
0.30	8.4	35	12	06.0	04.0	10.0	
0.69	10.0	26	12	07.6	23.2	30.8	
0.53	5.7	47	24	20.7	18.5	39.2	
0.51	8.6	35	24	14.1	17.0	31.1	
0.43	9.9	26	24	12.0	47.9	59.9	
0.96	5.6	47	1	10.5	01.5	12.0	
0.95	8.5	35	1	06.5	06.2	12.7	
0.83	10.3	26	1	11.6	05.6	17.2	
0.87	5.6	47	12	13.9	33.1	47.0	
0.90	8.5	35	12	07.2	13.1	20.3	
0.87	10.0	26	12	05.4	44.3	49.7	
0.98	6.2	47	24	81.2	11.5	92.7	
0.83	8.8	35	24	33.5	03.4	36.9	
0.81	10.0	26	24	17.1	44.8	61.9	

Results

The timed series of iodine measurements with different water supplies illustrated differences in iodine demand, with as much as 30% of iodine loss occurring after 5 min and 60% after 1 h (Fig. 1).

Iodine concentrations at Puntledge were measured twice and averaged 0.06 mg/L at the inflow and 0.03 mg/L at the outflow of ponds. Treatment of adult chinook males slowed the normal rate of mortality by about 50%. At the end of the 24-d test the control group showed 90% mortality, while the treated fish showed 45% mortality. The incidence and severity of fungus lesions was much lower on treated fish. Only 1 of 20 treated fish had fungus while all the control fish had some level of fungus.

Iodine delivery at Robertson Creek was erratic so it was impossible to determine the effectiveness of treatments. Brood stock exposed to these levels (< 0.02 mg/L to 0.05 mg/L) survived the treatment

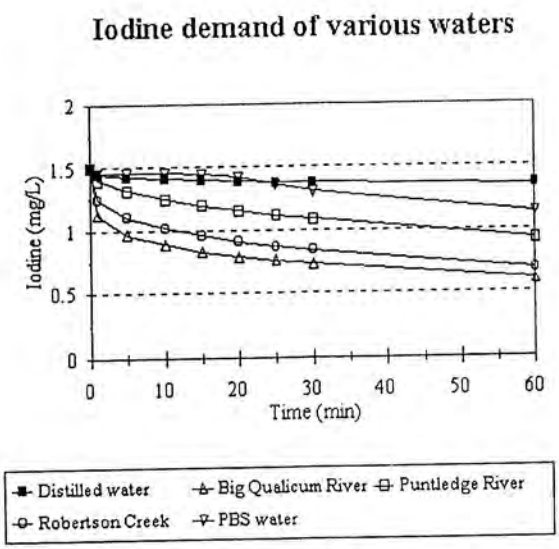


Figure 1. Five curves (fitted using a logistic dose-response model) indicating the differing iodine-demands of various types of water (i.e., distilled water, Big Qualicum River, Puntledge River., Robertson Creek, and Pacific Biological Station (PBS) water) measured for up to 1 h after dilution to 1.5 mg/L.

and produced normal gametes. Several pairs spawned successfully and progeny were monitored to ponding. Experimental conditions and egg, alevin and rearing mortality from the 3 coho egg experiments are summarized in Table 1.

Discussion

Treatment with low concentrations of elemental iodine from fertilization to the eyed stage controlled fungus growth on salmon eggs. Continuously treated groups had losses < 6%, while mortality in untreated groups was 18% to 40%. Lower exposures were less effective but data do not allow prediction of minimum effective treatment.

Iodine treatments at BQ hardened the egg capsule and at higher concentrations the capsule became so hard that it interfered with hatch. Higher alevin mortality in 1994 (Table 1) was due to abrasion of the yolk sac during hatch; damage to the yolk was so severe that some alevins died at hatch. In other cases the abrasion caused coagulation of the yolk resulting in higher mortality. After hatch, the egg shells resisted decomposition and clogged incubator screens. Shells were still intact 4 wk after hatch while untreated shells disappeared in less than a week. This effect can be a problem but in some instances it may be desirable to increase the hardness of the egg capsule (for transportation or when eggs exhibit "soft shell" symptoms.[8])

Hardening of the egg capsule was not observed at PBS at similar and higher concentrations, perhaps due to differences in background water chemistry and the method of application. The importance of background water chemistry was emphasized by the iodine demand curves (Fig. 1). Iodine loss after 15 min of contact with dilution water varied between 50% at BQ to 10% at PBS. These differences were probably due to different organic loads. Background pH and temperature also affect the distribution of different species of iodine.[5,6] Further studies are required to identify treatments that control fungus without hardening the capsule. The range of experimental treatments did allow measure of direct iodine toxicity. An upper lethal level was found with continuous exposure at 12°C to a measured concentration of 0.43 mg/L (Table 1).

Lower concentrations controlled fungus on maturing adults. Continuous exposure to 0.03 to 0.06 mg/L significantly reduced pre-spawning mortality rates of chinook.

Elemental iodine holds promise for control of fungal infections on salmon. However, work is required on the mechanisms involved, including the importance of contact time with the water and the effects of iodine on eggs and fish. Furthermore, the effects of background water chemistry must be determined and practical methods of application are required.

Notes and references

1. Pacific Biological Station, Dept. Fisheries & Oceans, Nanaimo, BC V9R 5K6
2. Quinsam Salmon Hatchery, Box 467, Campbell River, BC V9W 5C1
3. Little Qualicum Project, 4745 Melrose Rd, Qualicum Beach, BC V9K 1V3
4. Iomech Ltd, PO Box 14,054, 2398 Lakeshore Blvd West, Etobicoke, ON M8V 4A2
5. Black AP, Kinman RN, Thomas WC Jr, Freund G, Bird ED. 1965. *J. Amer. Water Works Assoc.* 57:1401-1421.
6. Chang , Morris . 1953. *Ind. Eng. Chem.* 45:1009.
7. Palin, AT. 1967. *Inst. Water Eng.* 21(6):537-547.
8. Cousins K, Jensen JOT. 1994. *J. Can. Zool.* 72:1854-1857.

Morphometric, isozyme and random amplified polymorphic DNA (RAPD) analysis of *Sarotherodon mossambicus* (Pisces, Cichlidae) populations in Java, Indonesia

Yoedono Sovyanhadi
and Ronald Leland Carter[1]

Sarotherodon mossambicus is an important protein source for Indonesians, a strong competitor for some endemic species, and readily hybridizes with other tilapias. A baseline assessment of the present genetic diversity of these populations was conducted to facilitate the management of this species. In this study we compared the effectiveness of three techniques (morphometric, isozyme, and RAPD) in revealing differences within and among nine Javanese populations. The data were also used to estimate the rate of population divergence. Morphometric analysis showed significant size and shape differences among the populations ($F = 25.8$; $p < 0.01$ and $F = 20.80$; $p < 0.01$, respectively). Isozyme and RAPD analysis showed significant population differentiation ($G_{st} = 0.067$; $\chi^2 = 30.15$; $p < 0.01$ and $G_{st} = 0.217$; $\chi^2 = 38.95$; $p < 0.01$ and average D of 0.005 and 0.136, respectively). Morphological differences appear to be primarily environmentally induced, since morphological data were inconsistent with molecular genetic data.

Introduction

Genetic variations found in natural populations represent fundamental resources needed for present and future survival of any species. Therefore, it is essential to conserve the wealth of genetic resources for future generations.[2] Genetic variation in a population can be depleted by population bottle necking, inbreeding, intense selection or by hybridization leading to reticulation.

Sarotherodon mossambicus is an important protein source for human consumption in the developing countries. The genetic resources of this species from natural waters in Java, Indonesia, have never been documented. Sensitive identification protocols are especially needed, since hybridization among tilapia species is common.[3] It is therefore necessary to make fundamental genetic assessments of *S. mossambicus* stocks in Java. Such data will provide information for management of this species. Knowledge of tilapia population genetics may become particularly important for management as this introduced species competes with natural genetic resources in Java. In this study, documentation at the morphological level (morphological data), protein level (isozyme data) and DNA level (RAPD data) was done to compare the effectiveness of each technique in revealing the differences among *S. mossambicus* populations in Java.

Materials and methods

Sample collection

Thirty Javanese tilapia (*S. mossambicus*) were randomly collected from each of nine isolated sites along the island of Java. The selected sites

were on different tributaries and relatively far from each other (about 200 km apart) to reduce the measurement of gene flow. These sites also represented diverse environmental conditions in terms of the size of water body, water salinity and temperature, altitude and tidal fluctuation.

Tissue samples from liver, eye, and muscle for enzyme electrophoresis were separately put in 1.5 mL microfuge tubes to which 0.5 mL of 0.25 M Tris pH 7.0 as the homogenizing buffer was added.[4] Heart tissue was separated for polymerase chain reaction analysis (RAPD, Random Amplified Polymorphic DNA), kept on dry ice during transport, and then transferred to a –86°C freezer upon arrival at the laboratory. DNA was extracted from the heart tissue following the salting-out procedure.[5] The remaining whole body of each individual was preserved for morphometric study.[6]

Data collection

The morphometric measurements were collected according to the truss technique in which the framework of body form is measured.[7,8] The isozyme data were collected from starch gel electrophoresis and staining protocols[4,9,10] employing 16 enzyme systems. RAPD data were collected from the PCR products after the amplification of DNA samples using 17 primers of Operon Technologies, Inc.

Data analysis

All truss lengths from the sampled fish (270 individuals) were entered into the computer and transformed into common logarithms. Principal Component Analysis (PCA) and Discriminant Function Analysis (DFA) were then used to analyze the log-transformed data set.

The gene frequency data from isozyme and RAPD analysis were used to calculate the coefficient of gene differentiation (G_{st}) which measures the degree of divergence among the populations under study.[11,12] The overall structure of population divergence was then analyzed with UPGMA dendrogram and Principal Coordinate Analysis (PCO analysis).

Results

Size factor accounted for 82.93% of total morphometric variance, while shape accounted for less than 3.16% of the total variation. ANOVA on the first Principal Component demonstrated highly significant size differences among the 9 populations ($F = 25.8$; $p < 0.01$). Four size groups could be revealed from the Scheffé post-test. ANOVA on the sheared-PC2 also showed significant shape differences among the 9 populations ($F = 20.80$; $p < 0.01$). Five shape groups could be revealed from the Scheffé post-test. When the recorded environmental factors were qualitatively categorized, correlation between combined environmental factors and shape was significant ($r = 0.673$; $p = 0.047$). The correlation between combined environmental factors and size was not significant. These results suggest that environmental factors contribute to the shape differences among the populations. However, some other factors that were not recorded, such as food availability and the chemistry of the waters that cause local differences, may also have influenced the size variation of the fishes.

Isozyme and RAPD analysis also showed significant population differentiation ($G_{st} = 0.067$; $\chi^2 = 30.15$; $p < 0.01$ and $G_{st} = 0.217$; $\chi^2 = 38.95$; $p < 0.01$ and average genetic distance D of 0.005 and 0.136, respectively). The overall pattern of population divergence based on isozyme was slightly different from that based on RAPD. These molecular patterns of population differentiation were also different from those of morphological patterns.

Discussion

Within this study, the patterns of differentiation among tilapia populations based on size and shape were inconsistent with those based on isozymes or RAPD. This suggests that size and shape differences are not primarily genetic but are most likely environmentally induced.[13] This conclusion needs further confirmation, since such inconsistencies could alternately be obtained if sets of genes that control growth and development were not sufficiently surveyed by isozyme/RAPD analysis. Protein and DNA analysis revealed a significant differentiation among the 9 populations under study. However, these genetic data did not provide unique gene markers or sets of diagnostic band markers for any one of the populations. Such results are consistent with the assumption that the 9 populations are conspecific.[14,15,16]

The results of this study are also consistent with the growing awareness that not all mor-

phometric measures equally reveal relatedness and that morphological features need to be carefully studied in order to evaluate their usefulness in recognizing genetically distinct populations. Whenever possible, morphological documentation should be accompanied by biochemical data. For economical reasons, protein analysis continues to be the most effective tool for population studies. Isozyme data may in itself give a sufficiently broad picture of genetic variation within and among the populations. Isozyme analysis does not satisfactorily reveal genetic variation in some cases and often requires the sacrificing of the subject. If these conditions are limiting, the studies at the DNA level using RAPDs can provide more sensitive, data-rich gels, due to greater polymorphisms that can be detected using this technique.[12,17]

The genetic documentation in this study covers only a part of the data needed for better management of *S. mossambicus* in Java. Further documentation needs to be completed, including samples from fish farms and waters adjacent to the aquafarms to assess possible hybridization and introgression occurring between tilapia in the natural waters with tilapia species being cultured in the farms.

This research was funded by the Government of Indonesia (Overseas Training Office — Bappenas and Hasanuddin University), the US Agency for International Development (USAID), and the Department of Natural Sciences, Loma Linda University, Loma Linda, California.

Notes and references

1. Department of Natural Sciences, Loma Linda University, Loma Linda, California U.S.A. 92350
2. Lannan JE, Gall GAE, Thorpe JE, Nash CE, Ballachey BE. 1989. *Genome* 31:798-804.
3. Chen FY. 1988. In, *Tilapia Genetic Resources for Aquaculture* (RSV Pullin, ed), p. 48. ICLARM, Manila, Philippines.
4. Abdelhamid AA. 1988. M.Sc. Thesis, Auburn University, Auburn, Alabama, U.S.A. 53 pp.
5. Miller SA, Dykes DD, Polesky HF. 1988. *Nucleic Acid Res.* 16(3):1215.
6. Khater AAE. 1985. Ph.D. Dissertation, Auburn University, Auburn, Alabama, U.S.A. 67 pp.
7. Brzeski VJ, Doyle RW. 1988. In, *The Second International Symposium on Tilapia in Aquaculture* (RSV Pullin, T Bhukaswan, K Tonguthai, JL MacLean, eds), p. 439-444. ICLARM Conference Proceedings 15, ICLARM, Manila, Philippines.
8. Strauss RE, Bookstein FL. 1982. *Syst. Zool.* 31:113-135.
9. Morizot DC, Schmidt ME. 1990. In, *Electrophoretic and Isoelectric Focusing Techniques in Fisheries Management* (DM Hillis, C Moritz, eds), p. 23-80. CRC Press, Boca Raton, Florida, U.S.A.
10. Shaw CR, Prasad R. 1970. *Biochem. Genetics* 4:297-320.
11. Nei M. 1983. In *Evolution of Genes and Proteins* (M Nei, RK Koehn, eds), p. 165-190. Sinauer Associates, Sunderland, Massachusetts, U.S.A.
12. Van Coppenolle B, Watanabe I, Van Hove C, Second G, Huang N, McCouch SR. 1993. *Genome* 36:686-693.
13. Yoshiyama RM, Sassaman C. 1983. *Syst. Zool.* 32:52-71.
14. Allibone RM, Wallis GP. 1993. *Biol. J. Linn. Soc.* 50:19-33.
15. Crozier WW, Ferguson A. 1986. *J. Fish Biol.* 28:459-477.
16. Garcia-Marin JL, Jorde PE, Ryman N, Utter F, Pla C. 1991. *Aquaculture* 95:235-249.
17. Meijer G, Megnegneau B, Linders EGA. 1994. *Mycolog. Res. 98:267-276.*

Apparent digestibility and amino acid availability of six feed ingredients by Arctic charr *(Salvelinus alpinus)*

R. M. Gurure, R. D. Moccia and J. L. Atkinson[1]

The apparent digestibility coefficients (ADC) for dry matter (DM), crude protein (CP), fat and gross energy (GE) as well as the apparent amino acid availabilities (AAAA) for herring fish meal (HM), menhaden fish meal (MM), South American fish meal (SAM), corn gluten meal (CGM), wheat middlings (WM) and soybean meal (SBM) were determined for Arctic charr. A reference diet and six test diets containing celite as the diet marker were fed to fish and faeces were collected. Concentrations of celite and the test nutrients were determined in both diets and faeces and the data was used to calculate the ADCs of the nutrients and AAAA for the ingredients. Apparent digestibilities of DM, CP, fats, GE, and AAAA followed different trends among the ingredients. Overall, ADCs for HM, MM, SAM, and CGM were high and were similar to those reported for other salmonids. The DM and GE digestibilities for WM and SBM were generally lower than for the other ingredients tested but higher than those for other salmonids. AAAA of all ingredients were generally high, with values for HM, MM, CGM and WM being similar to one another and higher than those for SAM and SBM.

Introduction

Measurement of feed ingredient digestibility indicates the relative proportions of dietary components such as amino acids (AA), fat, crude protein (CP) , and energy that animals can derive from feeds. This is important, particularly for emerging aquaculture species like Arctic charr, since its nutritional requirements need to be defined precisely in order to produce cost-effective diets. In order to formulate appropriate experimental and production diets it is necessary to establish the availability of vital nutrients derived from common dietary ingredients.

Presently, there is limited digestibility data available for Arctic charr. The objective of this study was to determine apparent digestibility coefficients (ADC) for dry matter (DM), CP, fat and AA, and gross energy (GE) for herring fish meal (HM), menhaden fish meal (MM), South American fish meal (SAM), wheat middlings (WM), corn gluten meal (CGM), and soybean meal (SBM) by Arctic charr.

Materials and Methods

Labrador strain Arctic charr (mean weight ~70 g), from the Alma Aquaculture Research Station, Ontario, were used. Fish were randomly allocated to 7 tanks and were hand-fed a reference diet and 6 test diets to evaluate the nutrient ADCs of HM, MM, SAM, CGM, WM and SBM. The reference diet contained HM, CGM, SBM and WM as the main ingredients, while the 6 test diets consisted of 30% of the test ingredient and 70% of the reference diet. Celite, an indigestible marker, was included at 0.7% of the diet while vitamins and minerals were supplied according to NRC[2] requirements. The experiment was conducted in tanks specially designed for collecting faeces in digestibility studies.[3] Water temperature was maintained at 10±1°C, dissolved oxygen ranged from 8 to 12 mg/L and water flow rate was ~3 L/min. Fish densities ranged between 90 and 100 kg/m^3. The experiment was an Incomplete Latin Square design, replicated in 4 periods. Each period consisted of 3 days for acclimation followed by 3 days of faeces collection. During collection periods,

Table 1. Apparent digestibility coefficients of components of the six ingredients.

Ingredients	Component			
	Dry Matter	Crude Protein	Fat	Gross Energy
HM	89.4 ± 1.8^{a} (85^{1})	96.8 ± 0.8^{b} (92^{1})	97.1 ± 0.8^{a} (97^{1})	95.1 ± 1.9^{a} (91^{1})
MM	87.3 ± 1.9^{ab} (84^{3})	95.0 ± 0.8^{bc} (83^{3})	92.7 ± 3.4^{b} (NA)	92.4 ± 1.7^{ab} (84^{3})
SAM	69.6 ± 3.6^{c} (88^{3})	84.8 ± 1.6^{d} (85^{2})	89.5 ± 1.2^{b} (NA)	77.7 ± 2.8^{d} (91^{2})
CGM	79.7 ± 2.4^{b} (80^{1})	93.3 ± 0.2^{c} (96^{1})	88.7 ± 2.5^{b} (NA)	87.1 ± 1.5^{bc} (87^{2})
WM	52.9 ± 2.7^{d} (35^{1})	103.7 ± 0.6^{a} (92^{1})	89.4 ± 2.6^{b} (NA)	58.3 ± 2.5^{e} (46^{1})
SBM	80.8 ± 2.0^{b} (74^{1})	102.5 ± 0.7^{a} (96^{1})	84.5 ± 3.6^{c} (NA)	82.5 ± 1.9^{cd} (75^{1})

Values in the same column sharing superscripts are not significantly different ($P<0.05$) and those in brackets indicate ADCs reported for [1]rainbow trout (Cho et al.[3]), [2]rainbow trout (Smith et al.[8]) and [3]chinook salmon (Hagen et al.[9]). NA indicates that no values are available.

tanks were cleaned at the end of each day and faeces collected before the next day's feeding. Faecal samples were homogenized, freeze dried, and reground before analysis.

Concentrations of celite and the relevant nutrient fractions were determined in both diets and faeces. Celite was determined as acid-insoluble ash.[4] Concentrations of proximate components were determined according to official analytical methods[5] while AA concentrations were determined by high performance liquid chromatography.[6] The ADCs and AAAAs of the diets were calculated by relating dietary and faecal concentrations of nutrients and marker according to Schneider and Flatt:[7]

$$ADC\ of\ diet = 100 - 100\ x\ \frac{\%\ indicator\ of\ diet\ x\ \%\ nutrient\ of\ faeces}{\%\ indicator\ of\ faeces\ x\ \%\ nutrients\ of\ diet}$$

Subsequently, ingredient ADCs were calculated as follows:

$$ADC\ of\ ingredient = ADC\ of\ test\ diet - \frac{0.7\ ADC\ of\ reference\ diet}{0.3}$$

Arcsin transformed ADC data were subjected to ANOVA and Duncan's multiple range comparisons. Differences among treatments were considered to be significant at $P< 0.05$.

Results and Discussion

ADCs for DM, CP, fat, and GE and the comparisons between test ingredients are shown in Table 1. The values for DM were highest for HM which were similar to MM, while the ADCs for MM, CGM and SBM were similar to one another and higher than for SAM. ADCs for SAM were higher than those for WM. Generally, the present ADCs are similar to those reported for other salmonids,[3,8,9] particularly for the fish meals and CGM. Our results are contrary to findings by Jobling and Wandsvik[10] who reported Arctic charr ADCs to be 5 to 10% lower than those for rainbow trout.[11-13] Differences between the results may be due to strain differences between Norwegian and Labrador strains of Arctic charr. As well, methods of faeces collection can affect digestibility results.[12] Jobling and Wandsvik[10] collected faeces by stripping digesta from the lower gastrointestinal tract; this may result in faeces containing incompletely digested feed or being contaminated with body fluids producing erroneously low ADCs. Similarly, the collection method used here may have affected results because of leaching of faecal components before collection.

Protein ADCs for WM (103.7%) and SBM (102.5%) were higher than for other ingredients and exceeded 100% which is unrealistic. Jobling and Wandsvik[10] also observed higher digestibilities for low protein diets. The higher CP digestibilities for WM and SBM may be due to increased digestion of dietary ingredients that occurs when gut retention time is prolonged by high-fibre carbohydrate ingredients. Improved digestion of the other high protein basal ingredients (HM and CGM) can result in high ADCs being erroneously attributed to the test ingredients (i.e., WM and SBM) because of

Table 2. Apparent amino acid availabilities (AAAA) of the six ingredients tested.

AAS	HM	MM	SAM	CGM	WM	SBM
Ala	102.08 ± 1.71^{a}	103.28 ± 1.42^{a}	94.65 ± 2.64^{ab}	99.70 ± 2.61^{a}	100.65 ± 0.35^{a}	87.13 ± 8.76^{b}
Arg	97.85 ± 1.08^{a}	98.48 ± 0.39^{a}	87.45 ± 2.14^{c}	94.47 ± 0.88^{a}	93.00 ± 0.30^{b}	85.10 ± 1.42^{c}
Asp	98.55 ± 1.82^{a}	96.48 ± 2.58^{a}	98.23 ± 4.80^{a}	97.37 ± 1.39^{a}	81.90 ± 0.90^{b}	89.00 ± 7.40^{ab}
Glu	96.70 ± 3.29^{ab}	100.10 ± 2.97^{a}	66.03 ± 5.80^{c}	90.60 ± 0.80^{a}	84.65 ± 1.35^{b}	66.10 ± 3.30^{c}
His	96.75 ± 1.99^{a}	97.58 ± 1.06^{a}	80.98 ± 3.90^{b}	92.40 ± 1.53^{a}	90.05 ± 0.25^{a}	78.00 ± 2.91^{b}
Ile	99.50 ± 0.21^{a}	99.03 ± 0.26^{a}	94.00 ± 1.13^{b}	99.90 ± 1.80^{a}	94.55 ± 0.65^{b}	95.07 ± 1.66^{b}
Leu	97.28 ± 0.84^{a}	97.93 ± 0.41^{a}	85.38 ± 2.26^{b}	93.75 ± 0.25^{a}	92.70 ± 0.80^{a}	80.13 ± 2.03^{c}
Lys	98.15 ± 0.42^{a}	97.90 ± 0.15^{a}	88.03 ± 1.37^{c}	94.80 ± 0.90^{b}	93.55 ± 0.45^{b}	83.97 ± 1.41^{d}
Met	98.90 ± 0.99^{a}	98.90 ± 0.44^{a}	90.57 ± 2.05^{b}	95.77 ± 1.27^{a}	94.85 ± 0.25^{a}	89.70 ± 2.10^{b}
Phe	94.65 ± 0.62^{a}	93.43 ± 0.35^{a}	73.28 ± 3.69^{c}	88.10 ± 0.78^{a}	82.45 ± 1.05^{b}	67.13 ± 1.71^{c}
Pro	95.95 ± 2.19^{a}	96.55 ± 0.45^{a}	71.58 ± 4.63^{cd}	86.53 ± 4.18^{a}	80.90 ± 0.70^{bc}	67.10 ± 1.93^{d}
Ser	95.93 ± 1.61^{a}	97.83 ± 0.54^{a}	81.25 ± 3.40^{b}	95.67 ± 3.32^{a}	90.55 ± 1.65^{a}	75.87 ± 1.45^{b}
Thr	94.20 ± 4.31^{ab}	95.65 ± 4.87^{a}	76.00 ± 3.59^{cd}	79.67 ± 4.68^{b}	85.10 ± 5.10^{abc}	67.85 ± 1.15^{d}
Tyr	99.00 ± 1.19^{a}	98.85 ± 1.17^{a}	83.90 ± 3.96^{c}	95.77 ± 0.56^{a}	90.75 ± 0.85^{bc}	87.90 ± 1.47^{c}
Val	98.23 ± 3.23^{a}	98.88 ± 2.65^{a}	74.43 ± 3.08^{b}	90.70 ± 0.10^{a}	89.95 ± 0.45^{a}	74.95 ± 3.25^{b}

Values in the same row sharing common superscripts are not significantly different ($P<0.05$).

the method of formulating test diets to determine ingredient ADCs.

In contrast, DM and GE digestibilities for WM and SBM were generally lower than for other ingredients. The lower DM and GE digestibilities of these ingredients is possibly because the test diets contained low concentrations of digestible protein and lipid and high levels of indigestible carbohydrates. These results are similar to those of Jobling and Wandsvik[10] who observed lower energy digestibility for low protein diets. Generally, carnivorous fish do not digest carbohydrates well and consequently derive limited energy from high carbohydrate diets. These ADCs were, however, higher than those for other salmonids. For example, DM digestibility for WM (52.9%) was higher than the 35% reported for rainbow trout.[3] Similarly, GE digestibilities were generally higher than those for rainbow trout,[3] 58.3% vs 46% and 82.5% vs 75% for WM and SBM, respectively. This may be due to species differences. Arctic charr may possess a greater ability to utilize carbohydrates than other salmonids.

Results of AAAAs are shown in Table 2 and these were generally high for all ingredients, ranging between 66.1% to 103.2%. Generally, AAAAs for HM, MM, CGM and WM were similar and tended to be higher than AAAAs for SAM and SBM. Our AAAAs were generally similar to those of other salmonids.

The digestibility data presented here can be used during diet formulation to ensure that sufficient nutrients are supplied in Arctic charr diets.

Notes and references

1. Department of Animal and Poultry Science, University of Guelph, Guelph, ON N1G 2W1
2. NRC. 1993. *Nutrient Requirements of Fishes*. National Academy of Sciences, Washington, DC. 114 pp.
3. Cho CY, Slinger SJ, Bayley HS. 1982. *Comp. Biochem. Physiol.* 73B:25-41.
4. Van Keulen J, Young BA. 1977. *J. Anim. Sci.* 44:282-287.
5. AOAC. 1990. *Official Methods of Analysis*. AOAC, Washington, DC. 1298 pp.
6. House JD, Pencharz PB, Ball RO. 1994. J. Nutr. 124:396-405.
7. Schneider BH, Flatt WP. 1975. *The evaluation of feeds through digestibility experiments*. University of Georgia Press, Athens, GA. 168 pp.
8. Smith RR, Peterson MC, Allred AC. 1980. *Prog. Fish-Cult.* 42:195-198.
9. Hagen WE, Higgs DA, Beames RM, Dosanjh BS. 1993. *Aquaculture* 112:333-348.
10. Jobling M, Wandsvik A. 1983. *J. Fish Biol.* 23:397-404.
11. Austreng E. 1978. *Aquaculture* 13:265-272.
12. Windell EG, Foltz JW, Sarokon JA. 1978. *Prog. Fish-Cult.* 40:51-55.
13. Ince BW, Lone KP, Matty AJ. 1982. *Br. J. Nutr.* 47:615-624.

Energetics and osmoregulation in chum and coho salmon embryos and larvae

E. P. Groot, J. D. Morgan, and G. K. Iwama [1]

Embryos and larvae of chum salmon (*Oncorhynchus keta*) osmoregulate more effectively than coho (*O. kisutch*) and are more salinity tolerant. Embryos and larvae at three developmental stages were acclimated to salinities ranging from 0 to 30‰ and sampled for metabolic rate, growth, and chloride cell distribution and activity. Prior to hatching, embryos acclimated to higher salinities (>12 ‰) had significantly lower metabolic rates and grew less than those in lower salinities (<12 ‰). After hatching, chum larvae responded in the opposite direction with higher metabolic rates in 24 and 30‰ than in lower salinities. Coho larvae responded variably at 6 and 12‰ (none survived acclimation above 12‰). Fluorescent staining of embryos and larvae revealed chloride cells distributed widely over the yolk-sac epithelium, skin, gills, and fin folds of both species. Although cell density was not strongly correlated with acclimation salinity or species, some ontogenic changes were observed. Data indicate that for different species of Pacific salmon, energetic costs of osmoregulation vary with developmental stage and acclimation salinity.

Introduction

Early life stages of chum salmon are more tolerant to hyperosmotic environments than similar stages of coho[2,3] and this physiological difference coincides with differences in life histories. Chum salmon spawn in intertidal areas of rivers and streams and swim-up fry usually migrate directly to the estuary or ocean.[3-5] The physiological basis of these differences have not been examined closely and provide a model to study the energetic implications of osmoregulation during the early life stages. The objectives of this study were to determine and compare the energetic implications of ionic and osmotic regulation in the eggs and larvae of chum and coho salmon by measuring oxygen consumption rates and to examine the ontogeny of chloride cell development and relate this to osmoregulatory capacity and metabolic responses.

Materials and methods

Coho and chum eggs were incubated from fertilization to hatching and reared through to complete yolk sac absorption at 10.0 ± 0.1°C in darkness. Tests were conducted at three predetermined developmental stages (Stage I, eyed embryo; Stage II, 2-3 d pre-hatch; Stage III, larva at 50% yolk sac absorption). Prior to testing, embryos and larvae were acclimated for 7 d to salinities of 0, 5, 10, and 15‰ for Stage I and 0, 6, 12, 18, 24, and 30‰ for Stages II and III. Acclimated Individuals were sampled for oxygen metabolic rate (oxygen consumption), growth (tissue dry weight, TDW), and chloride cell activity (Na^+,K^+-ATPase) and distribution (fluorescent microscopy).

Oxygen consumption measurements were conducted using a custom-built micro-respirometer comprised of four respirometer loops.[6] Tests were run in quadruplicate with 10 eggs (Stage I and II) or three larvae (Stage III) per chamber.

Yolk and tissue dry weights (TDW) were determined by separating the formalin hardened yolk material from the tissue of the embryo or larva and drying at 60°C for 48 h[7] and subsequently weighing to the nearest 0.01mg.

Chloride cell activity levels were estimated by measuring Na^+,K^+-ATPase activity.[8] Chloride cell (CC) distribution and density were estimated using fluorescent microscopy[9,10] on yolk sac epithelium, operculae, skin, and gills.

Data are presented as means standard error (SE). If analysis of variance ($\alpha = 0.05$) indicated significant differences, between treatment comparisons were evaluated using the Bonferroni procedure.

Results

In both species, embryos tolerated higher salinities than larvae and chum larvae tolerated higher salinities better than coho.

Oxygen consumption rates varied with acclimation salinity and, in general, were closely dependent on developmental stage. Salinity had little effect on metabolic rate or growth at the eyed stage. At the pre-hatch stage, both metabolic rate and growth decreased significantly as salinity increased (Figs. 1, 2). A slight peak in TDW was observed at 6‰ for pre-hatch embryos in both species, but was only statistically significant in chum. Obvious interspecific differences in metabolic rate and growth were observed at the larval stage. Coho larvae responded variably at 6 and 12‰ (no coho larvae survived acclimation above 12‰), whereas chum larvae responded oppositely from the pre-hatch embryos; metabolic rate increased with increasing salinity (Fig.1, 2).

An increase in Na^+,K^+-ATPase activity levels occurred with increasing developmental stage. Enzyme activity levels were slightly lower in the embryonic stages (Stage I and II) of chum than coho, whereas in the larval stage, enzyme activity levels in chum were significantly higher than coho. Within a developmental stage, acclimation salinity had little effect on enzyme activity, although a few exceptions were noted. Na^+,K^+-ATPase activity levels in eyed and pre-hatch coho embryos at 5 and 6‰, respectively, were significantly lower than those at 0‰. Also, ATPase activity levels in chum larvae were higher in 6‰ than 0, 12, 18, or 24‰.

Fluorescent staining (DASPMI) of embryonic and larval tissues revealed mitochondria-rich cells (chloride cells) distributed widely over the yolk-sac epithelium, skin, gills, and fin folds with densities as high as 128,000 cells/cm^2. Although cell density was not strongly correlated with acclimation salinity or species, some ontogenic changes were observed. Chloride cell numbers on the operculae of both chum and coho larvae were greatly reduced compared to the embryonic stages.

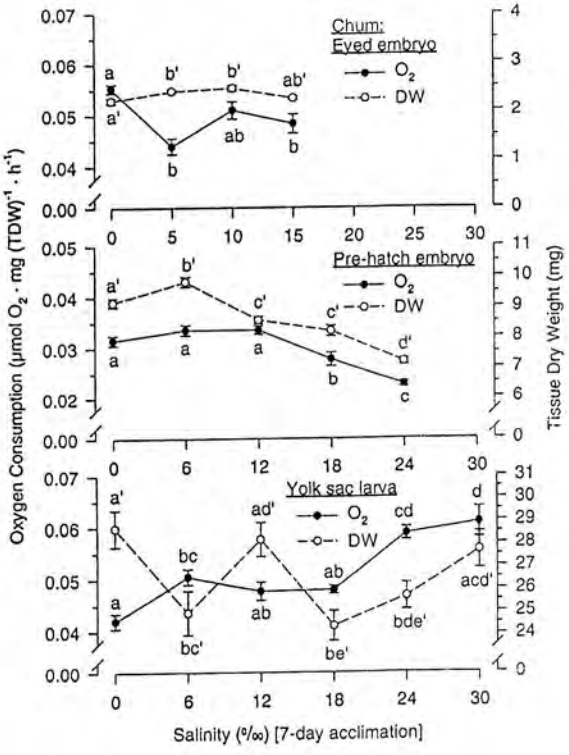


Figure 1. Oxygen consumption rates and dry weights of chum salmon embryos and larvae acclimated to a range of salinities. Means (± SE) that share a common letter are not significantly different (P < 0.06 ANOVA/Bonferroni procedure).

Discussion

Results demonstrated fundamental differences in the way embryos respond to salinity as compared to larvae. The pattern in the embryos was opposite to that in the larvae (chum only). The reduced metabolic rates of pre-hatch chum and coho embryos acclimated in higher salinities (18 and 24‰) were 15 and 30% lower, respectively, than those measured in 0-12‰. This response was similar to that observed in the reduction of tissue growth as a function of salinity. Assuming that after 7 days of acclimation all surviving embryos and larvae are in ionic and osmotic equilibrium, then it is reasonable to state that there was a metabolic cost associated with the higher salinity environment. However, the levels of Na+,K+-ATPase did not reveal a concomitant decrease in activity with increasing salinity. Presumably epithelial chloride cells are the main osmoregulatory mechanism available to these developing embryos and thus the major energy consuming

system for regulation of ions and water.[11,12] In general, ATPase levels were not correlated with metabolic rate and thus our data do not coincide with these statements. Reasons for the lack of correlation remain unclear.

Information on the ontogeny of chloride cell development in the early life stages of salmonids is sparse. Although chloride cells are found in the operculae and skin of coho larvae, they apparently are not functional.[13] The presence of measurable Na^+,K^+-ATPase levels and the widespread distribution of fluorescing chloride cells over the epithelia of all three life stages of chum and coho suggests these cells are functional.

Few studies have investigated the effects of salinity on the metabolic rate of the early life stages of salmonids. Morgan and Iwama[14] reported a similar response of increasing metabolic rate in increasing salinity for chinook salmon and steelhead and rainbow trout fry. Another study of these same species reported no change in metabolic rate in salinities ranging from 0-12‰.[15] Although salinities above 12‰ were not tested, their results coincide with respective values from this study.

Interspecific comparisons of the early life stages of chum and coho based on the physiological variables measured in this study provided only limited support for the life history differences observed for these species in the wild. For the most part, embryos of these species responded similarly, while chum larvae exhibited higher tolerance to salinities above 12‰. Chum larvae ATPase activity levels were also somewhat higher than coho, especially at 6‰ salinity. However, it still seems that the life history differences seen between chum and coho are not easily demonstrated on the basis of differing early life physiology.

Financial support was provided by an NSERC grant to GKI, a BC Science Council GREAT award to EPG, and an NSERC post-graduate fellowship to JDM. Facilities were provided by the Pacific Biological Station, Nanaimo. We thank JOT Jensen for laboratory space and E Teng for technical support.

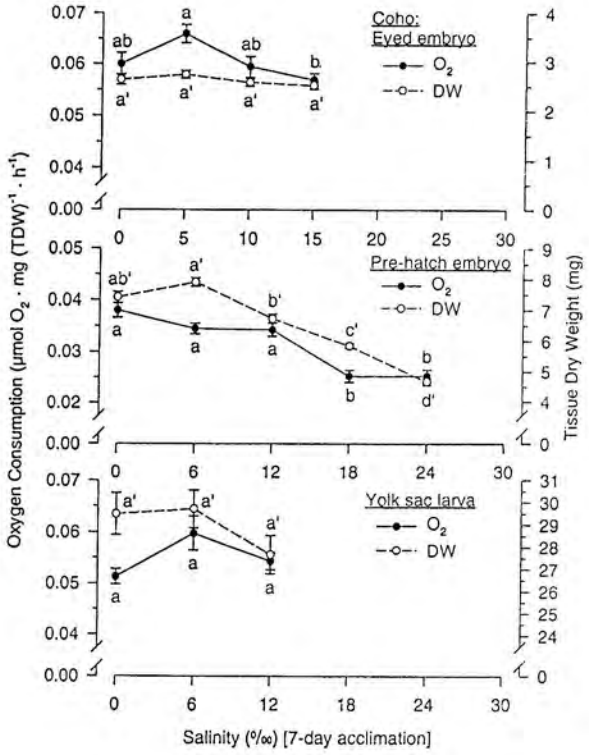


Figure 2. Oxygen consumption rates and dry weights of coho salmon embryos and larvae acclimated to a range of salinities. Means (± SE) that share a common letter are not significantly different (P<0.05, ANOVA/Bonferroni procedure).

Notes and references

1. Dept. Animal Science, University of British Columbia, Vancouver, BC V6T 1Z4
2. Weisbart MW. 1968. *Can. J. Zool.* 46:385-397.
3. Groot EP. 1989. MSc Thesis, UBC, Vancouver, BC.
4. Helle JH, Williamson RS, Bailey JE. 1964. *US Fish Wildl. Serv. Spec. Sci. Rep. Fish.* No. 483.
5. Scott WB, Crossman EJ. 1973. *Fish. Res. Bd. Can. Bull.* 184.
6. Groot EP, Iwama GK. 1994. *Proc. Intl. Fish Physiol. Symp.* p. 263-268. Vancouver, BC, July 16-21, 1994.
7. Rombough PJ. 1988. Can. J. Zool. 66:651-660.
8. McCormick SD. 1993. *Can. J. Fish. Aquat. Sci.* 50:656-658.
9. Bereiter-Hahn J. 1976. *Biochim. Biophys. Acta.* 423:1-14.
10. McCormick SD. 1990. *Cell Tiss. Res.* 260:529-533.
11. Alderdice DF. 1988. *Fish Physiol. 11A:163-251.*
12. Kirschner LB. 1993. *J. Exp. Biol.* 267:19-26.
13. Leatherland JF, Lin L. 1975. *Can. J. Zool.* 53:297-310.
14. Morgan JD, Iwama GK. 1991. *Can. J. Fish.* Aquat. Sci. 48:2083-2094.
15. Morgan JD, Jensen JOT, Iwama GK. 1992. *Can. J. Zool.* 70:1341-1346.

Fatty acid composition and nutritional value of a marine harpacticoid copepod fed various algal diets

Dominic A. Nanton,[1] John D. Castell[2] and Ian A. McLaren[1]

A marine harpacticoid copepod *Tisbe* sp. was isolated from the plankton and raised in the laboratory for over 20 generations. The effects of various algal diets (*Chaetocerus calcitrans*, *Dunaliella tertiolecta*, and *Isochrysis galbana*) on the nutritional value, or essential fatty acid (EFA) composition, of this copepod for use as an alternative live food for cold-water marine finfish larvae was evaluated. Copepods were able to synthesize a significant amount of the EFAs 20:5n-3 and 22:6n-3 from shorter chain n-3 polyunsaturated fatty acids when fed the algae *D. tertiolecta*, which was EFA deficient.

Introduction

The main bottleneck for fry production of many cold-water marine fish is the high mortality rates associated with larval first-feeding. This is partly due to a lack of nutritionally adequate live food organisms. Fish larvae require food with high concentrations of n-3 polyunsaturated or essential fatty acids (EFA) such as 20:5n-3 and 22:6n-3.[3]

Marine copepods, the principal food of many fish larvae, have high amounts of these EFAs. For calanoid copepods, large amounts of long chain n-3 polyunsaturated fatty acids (PUFA) are incorporated directly from their phytoplankton diet. They are unable to elongate and desaturate 18:3n-3 to longer chain PUFA and show reduced growth and egg production when fed *Dunaliella tertiolecta*, an alga deficient in 20:5n-3 and 22:6n-3.[4] Norsker and Støttrup,[5] however, discovered that the harpacticoid *Tisbe holothuriae* has the ability to elongate and desaturate the 18:3n-3 fatty acid (FA) supplied by *D. tertiolecta* to produce significant amounts of the EFAs 20:5n-3 and 22:6n-3.

Brine shrimp (*Artemia salina*) and rotifers (*Brachionus plicatilis*), traditional live foods for the culture of warm-water fish larvae, appear to be nutritionally deficient for cold-water marine fish larvae. Brine shrimp, in particular, cannot synthesize significant amounts of the 22:6n-3 FA, which is crucial for the survival of most cold-water marine fish larvae.[6]

Uhlig[7] claimed that harpacticoids, including *Tisbe* sp., were good live food organisms because they tolerate a wide range of environmental conditions, are able to utilize a variety of food sources, have a short life cycle, have high reproductive capacity, and can be raised at high densities. Another reason, which we evaluated in this study, was their ability to produce relatively large amounts of the EFAs 20:5n-3 and 22:6n-3 from shorter chained PUFA (n-3 series) when longer chain EFA are absent from the diet.

Materials and methods

Copepod culture

Harpacticoid copepods, *Tisbe* sp., were captured from the plankton of the Northwest Arm, Halifax, Nova Scotia, in February 1994. Copepods were isolated and cultured for over 20 generations in 6-L cylindrical plexiglass jars containing seawater (32 ppt) that had been filtered through 10 µm cartridges and UV treated. Air stones at the bottom of each cylinder maintained oxygen saturation and water circulation. Temperature was maintained at 20 ± 1°C. The medium was changed approximately twice a week by screening the copepods through a 40 µm-mesh and transferring them to jars of fresh, filtered seawater. Three replicate cylinders of copepods were fed the 3 algal diets in excess (ca. 1 mg dry weight of diet per liter of seawater) each time the water was replaced.

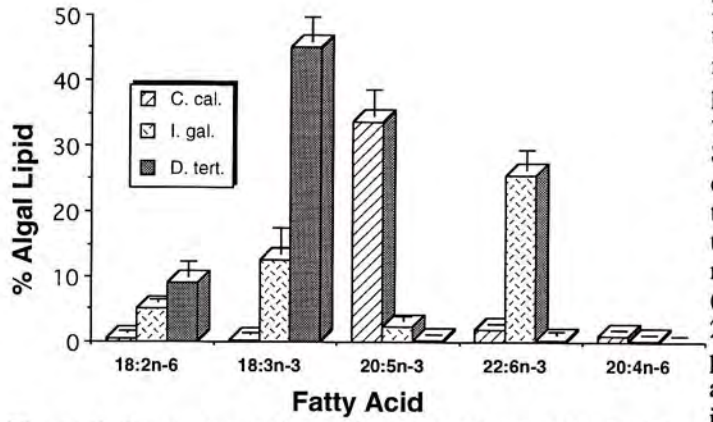


Figure 1. Percentages of some important fatty acids in the lipids of cultured algae used in copepod feeding trials. (I. gal.=*Isochrysis galbana*, C. cal.=*Chaetocerus calcitrans*, D. tert.=*Dunaliella tertiolecta*). Error bars = 1 SD (n=3).

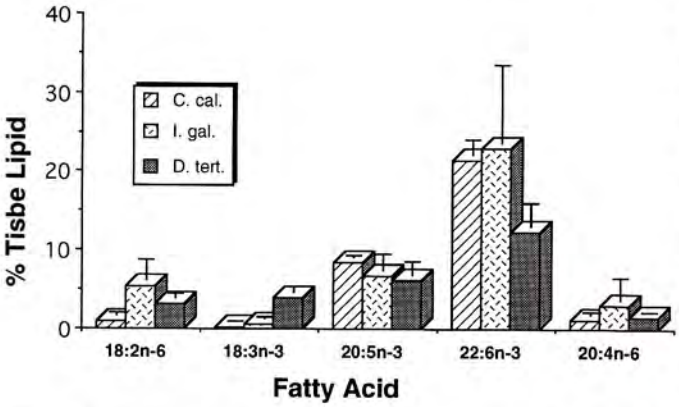


Figure 2. Percentages of some important fatty acids in the lipids of *Tisbe* sp. fed algal diets. (I. gal.=*Isochrysis galbana*, C. cal.=*Chaetocerus calcitrans*, D. tert.=*Dunaliella tertiolecta*). Error bars = 1 SD (n=3).

Algal culture

Isochrysis galbana (UK isolate), *Chaetocerus calcitrans* and *Dunaliella tertiolecta* were grown at 18°C and cultured with f/2 medium.[8] The algae were harvested in the log phase for feeding to the copepods.

Fatty acid analysis

For lipid analysis, algal samples were separated from the seawater medium through centrifugation. Adult copepods were separated from their media with a 200-µm-mesh screen. After transfer to fresh jars they were starved for 24 h to allow for the clearance of the gut and re-screened to remove any accumulated waste products. Over 200 animals were isolated for lipid analysis. Subsequently, copepods were captured on Whatman No. 1 filter paper and rinsed with distilled water. Copepods plus 10 mL of chloroform:methanol (2:1 v/v) were homogenized in a 20 mL glass tube using a polytron. Total lipid for the diets and copepods were extracted using the method of Bligh and Dyer.[9] An exact amount of the internal standard FA (23:0) was incorporated in the sample to estimate lipid weight. After removal of the chloroform, methyl ester derivatives of the FA were prepared by adding 2 mL 7% BF_3 in methanol and heating to 100°C. After cooling, 5 mL saturated sodium sulfate was added and methyl esters were recovered by hexane extraction and purified by thin layer chromatography. Fatty acid methyl esters were then quantified using a Varian 3400 model gas liquid chromatograph (GLC) equipped with a hydrogen flame ionization detector. Individual peaks were tentatively identified by comparing retention times with known standards and references.

Results and discussion

The 3 species of algae used in this study were chosen because of differences in their FA composition (Fig. 1). *Isochrysis galbana* lipid has large amounts of 22:6n-3 and small amounts of 20:5n-3. *Chaetocerus calcitrans* lipid has large amounts of 20:5n-3 and small amounts of 22:6n-3. *Dunaliella tertiolecta* lipid has trace amounts of both 20:5n-3 and 22:6n-3, but large amounts of the 18 carbon fatty acids 18:2n-6 and 18:3n-3. These differences in EFA levels allowed a comparison of the effect of dietary EFA on the fatty acid composition of the copepod.

These algae influenced the fatty acid composition of the copepod lipids (Fig. 2). The amount

of the 18 carbon fatty acid 18:3n-3 incorporated by the copepod corresponded to the amount in the algal diet, although the copepods contained substantially lower percentages of 18:3n-3 than were present in the algae. In the case of the 18:2n-6 fatty acid. *Tisbe* fed *I. galbana* incorporated the greatest amount of 18:2n-6, even though *D. tertiolecta* contained more of this FA.

Amounts of 20:5n-3 and 22:6n-3 were higher in copepods fed *I. galbana* and *C. calcitrans* (which had higher starting concentrations of these fatty acids) than those fed *D. tertiolecta*. The high proportion of 22:6n-3 and lower proportion of 20:5n-3 in the copepods fed *C. calcitrans* compared with the levels of these fatty acids in the lipids of the dietary algae are consistent with the active elongase and Δ-4-desaturase enzyme system in the copepod. There was a large reduction in the proportion of 20:5n-3 in copepod lipid compared with that in the *C. calcitrans* fed to these copepods. There was a corresponding increase in the proportion of 22:6n-3 in the copepods compared with the dietary lipid. The lipid of copepods fed *D. tertiolecta* contained relatively large amounts of 20:5n-3 (6.2%) and 22:6n-3 (12.4%), even though only trace amounts of these EFAs were present in their diet.

Of the algal species, *C. calcitrans* had the highest content of arachidonic acid (20:4n-6) while *D. tertiolecta* had levels below detectable limits (Fig. 1). Castell et al.[10] suggested that 20:4n-6, although required at lower levels than 22:6n-3, is also an essential fatty acid in the diets of marine finfish such as turbot *(Scophthalmus maximus)*. The levels of 20:4n-6 in *Tisbe* sp. lipids were consistently equal to or higher than the lipids of the corresponding dietary algae (Fig. 2). When arachidonic acid was absent from the diet in *D. tertiolecta,* the copepods appeared capable of producing 20:4n-6 from 18:2n-6 by elongation and desaturation.

This study established that the copepod, *Tisbe* sp., consistently incorporates high amounts of the EFAs 20:5n-3 and 22:6n-3 into their lipids, either by direct incorporation from dietary lipids or by elongation and desaturation of 18:3n-3 from the diet. It is hypothesized that *Tisbe* would satisfy the EFA requirements for the survival and growth of cold-water marine fish larvae such as haddock *(Melanogrammus aeglefinus)*, winter flounder *(Pseudopleuronectes americanus)* and halibut *(Hippoglossus hippoglossus)*. In turbot juveniles, changing the dietary ratio of the fatty acids 20:5n-3/22:6n-3 from 13.8 to 2.2 markedly reduced mortalities.[11] In our study the ratio of 20:5n-3/22:6n-3 was less than 1 for *Tisbe* fed all three algal diets, despite the different EFA levels in the algae. Unlike the traditional live food organisms in warm-water fish culture (brine shrimp and rotifers), a *Tisbe* diet would not need enrichment with EFA, thereby making it a desirable live food organism.

The proportion of 18:3n-3 decreased between the algal diet and the composition of the harpacticoid fed that diet for all algal species. Copepods were either selectively catabolizing this FA or, more likely, were elongating and desaturating it to 20:5n-3 and 22:6n-3. There was evidence for this in the *D. tertiolecta*-fed copepods; there were only trace amounts of 20:5n-3 and 22:6n-3 in *D. tertiolecta*, yet there were substantial amounts of these EFAs in the copepods which corresponded to a huge drop in 18:3n-3. These findings were similar to those of Norsker and Støttrup.[5]

The major obstacle in the culture of cold-water marine fish is the provision of a nutritionally suitable live food for early larvae at first-feeding. Brine shrimp and rotifers widely used in aquaculture do not provide a sufficient amount of EFA to larvae. Harpacticoids, however, contain large amounts of 20:5n-3 and 22:6n-3 within their lipids despite varying EFA levels within the diet. Feeding trials must still be conducted to determine if, in fact, this harpacticoid is a satisfactory live food for the culture of commercially important marine larval fish.

Notes and references

1. Biology Dept., Dalhousie University, Halifax, NS B3H 4J1
2. Dept. Fisheries & Oceans, Biol. Sci. Br., PO Box 550, Halifax, NS B3J 2S7
3. Watanabe T. 1982. *Comp. Biochem. Physiol.* 73B:3-15.
4. Støttrup JG, Jensen J. 1990. *J. Exp. Mar. Biol.* 141:87-105.
5. Norsker NH, Støttrup JG. 1994. *Aquaculture* 125:155-166.
6. Watanabe T, Oowa F, Kitajima C, Fujita S. 1978. *Bull. Jap. Soc. Sci. Fish.* 44(10):1115-1121.
7. Uhlig G. 1984. *Spec. Publ. Europ. Maricult. Soc.* 8:261-273.
8. Guillard RL, Ryther JH. 1962. *Can. J. Microbiol.* 8:229-239.
9. Bligh EC, Dyer WJ. 1959. *Can. J. Biochem. Physiol.* 37:911-927.
10. Castell JD, Bell JG, Tocher DR, Sargent JR. 1994. *Aquaculture* 128:315-333.
11. Bell MV, Henderson RJ, Sargent JR. 1985. *Comp. Biochem. Physiol.* 81B:193-198.

Triacylglycerol composition of eggs from captive Atlantic halibut over the spawning season

C. C. Parrish, Z. Yang, J. A. Brown[1]

The triacylglycerol (TAG) composition of batches of eggs from two captive Atlantic halibut (*Hippoglossus hippoglossus*) was studied in detail. Fertilized eggs were obtained during the 1992 spawning season from a first-time spawner and a repeat spawner. The acyl carbon number distributions of intact TAG as well as individual fatty acids in the neutral lipids were measured by gas chromatography. The proportion of C54 TAG, the major TAG in eggs, declined in batches of eggs produced later in the season. These late batches are generally thought to contain eggs of inferior quality. The proportions of two of the highly polyunsaturated long-chain fatty acids, 20:5n-3 and 22:5n-3, also decreased significantly over the spawning season ($r = -0.683$ and -0.836, respectively, $P < 0.01$, $n=16$). Eggs from the repeat spawner had a greater proportion of higher carbon number TAG by comparison with those from the first-time spawner because the TAG contained a higher proportion of long-chain polyunsaturated fatty acids (PUFA). This may relate to differences in fertilization success: the eggs from the repeat spawner had a higher average fertilization success (61%) than those from the first-time spawner (44%).

Introduction

Fatty acids from triacylglycerols (TAG) and phospholipids (PL) are an important form of energy in eggs and larvae of many fish species.[2,3] In our previous studies with eggs from captive halibut we found that eggs with very high proportions of phospholipids may be of inferior quality as determined by fertilization success.[4,5] This could be due to requirements by the eggs for certain proportions of certain TAGs. To investigate this aspect of egg quality in Atlantic halibut, we studied the TAG composition of batches of eggs produced over the spawning season by two captive fish.

Methods

Fertilized eggs were obtained during the 1992 spawning season from a first-time spawner (120 cm, 22.0 kg) and a repeat spawner (146.3 cm, 42.0 kg). Seven batches of eggs were obtained from the first-time spawner and nine from the repeat spawner.

Fertilization success was determined for all batches by examining a sample of 200 eggs for signs of cell division. Eggs were water hardened, rinsed, and incubated at 4.0°C for 24-48 h. A subsample of 50 eggs from each batch was examined to determine the fertilization success. The average fertilization success for the eggs from the first-time spawner was $43.6 \pm 7.2\%$ (mean ± SEM) while for the repeat spawner it was 61.0 ± 3.8 (mean ± SEM).

Egg lipids were extracted and fatty acids and triacylglycerols were determined by gas chromatography as described previously.[4,5]

Results

Over the spawning season, eggs from the repeat spawner had a greater proportion of higher carbon number TAG (Fig. 1) which indicates inclusion of a higher proportion of long-chain

polyunsaturated fatty acids (PUFA). The trends over the spawning season for the major contributors, C_{52} and C_{54} TAG, were quite similar in eggs from the two females (Fig. 2a-b), with batches spawned in the middle of the season having generally higher proportions.

The major fatty acids in the neutral lipids, in order of decreasing proportions, were 22:6n-3, 16:0, 18:n-9, 20:5n-3, 16:1n-7, and 14:0. Together, these fatty acids account for 3/4 of the total. Thus C_{52} TAG could have a fatty acid composition of 16:18:18, 16:16:20, 14:16:22, and 14:18:20, while C_{54} TAG could have one of 16:16:22, 16:18:20, and 14:20:20

The trends for 20:5n-3 and 18:0 fatty acids in the neutral lipids were also quite similar for the two females (Fig. 2c-d): proportions of the PUFA decreased over successive batches with a compensatory increase in the saturated fatty acid, 18:0. Eicosapentaenoic acid, 20:5n-3 and another PUFA, 22:5n-3, decreased significantly over the spawning season ($r = -0.683$ and -0.836, respectively, $P < 0.01$, $n=16$).

Discussion

Eggs from the repeat spawner had a greater proportion of higher carbon number TAG by comparison with those from the first-time spawner because the TAG contained a higher proportion of long-chain polyunsaturated fatty acids (PUFA). Fatty acids in the neutral lipids from the eggs of the repeat spawner had a higher average carbon number, a higher average number of double bonds per fatty acid, and higher proportions of essential n-3 and n-6 fatty acids. This may relate to differences in fertilization success which is thought to be a good indicator of egg quality.[6] The eggs from the repeat spawner had a higher average fertilization success than those from the first-time spawner. In herring, it appears that TAG is particularly important immediately after fertilization when it is catabolized[7] and polyunsaturated fatty acids are preferentially used.[8] Polyunsaturated fatty acids have also been shown to be preferentially used in the early stages of embryogenesis in wild halibut.[9]

The proportion of C_{52} and C_{54} TAG, the major TAG in eggs from both fish, declined in later batches (Fig. 2a-b) that are generally thought to contain eggs of inferior quality. This was consistent with the decline of the essential fatty acid, 20:5n-3

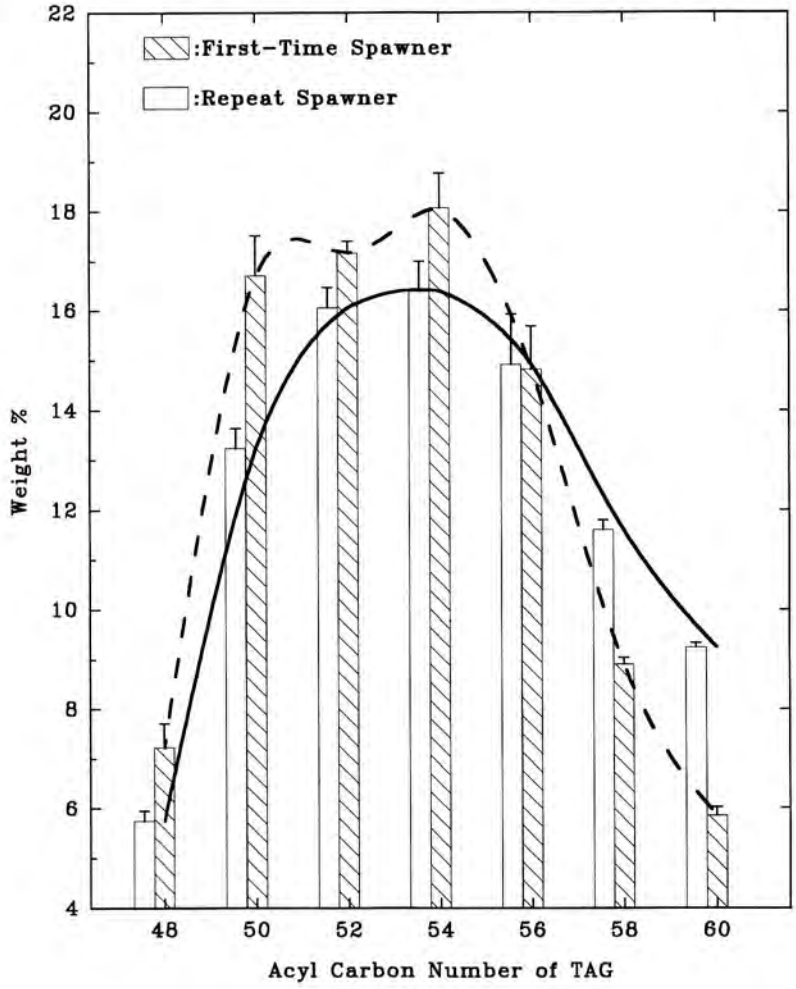


Figure 1: Acyl carbon number distributions in triacylglycerols in Atlantic halibut eggs over the spawning season. Data are mean ± SEM for seven batches from a first-time spawner (dashed line) and for nine batches from a repeat spawner (solid line).

(Fig. 2c) as C_{20} fatty acids were probably a major contributor to C_{52} and C_{54} TAG.

Summary

1. Eggs from the repeat spawner had higher proportions of long-chain polyunsaturated fatty acids (PUFA) in the neutral lipids, which were mainly composed of triacylglycerols, and a higher fertilization success.
2. The proportions of polyunsaturated omega-3 or n-3 fatty acids decreased in eggs from both females over the spawning season.

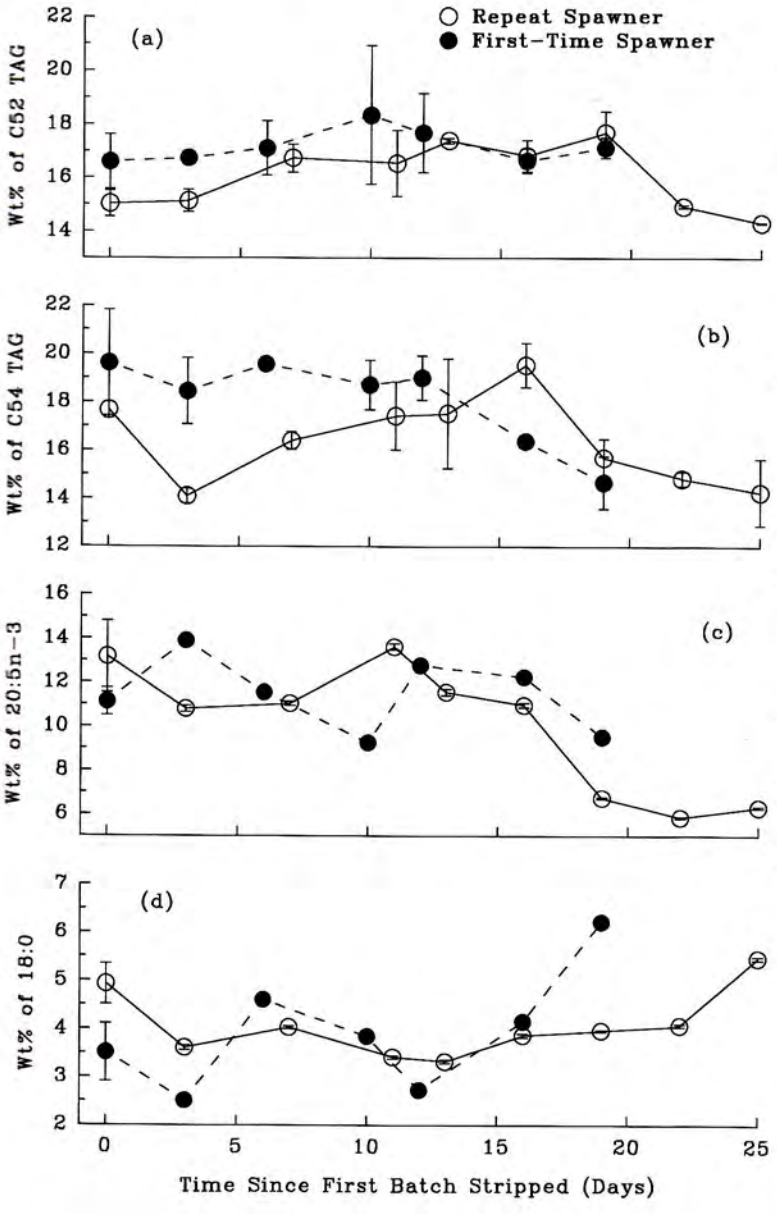


Figure 2: Time courses of (a) C_{52} and (b) C_{54} triacylglycerols and of (c) 20:5n-3 and (d) 18:0 fatty acids in Atlantic halibut eggs over the spawning season. Data are mean ± SD for two samples per batch.

Notes and references

1. Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, NF, A1C 5S7
2. Fraser AJ. 1989. *Can. J. Fish. Aquat. Sci.* 46:1868-1873.
3. Sargent JR. 1995. In, *Broodstock Management and Egg and Larval Quality.* (NR Bromage and RJ Roberts, eds), p. 353-372. Blackwell, Oxford.
4. Parrish CC, Brown JA, Daniel ES, Somerton DC. 1993. *Bull. Aquacul. Assoc. Canada* 93-4:35-37.
5. Evans RP, Parrish CC, Brown JA, Davis PJ. 1995. *Aquaculture,* in press.
6. Bromage NR. 1995. In, *Broodstock Management and Egg and Larval Quality.* (NR Bromage and RJ Roberts, eds), p. 1-24. Blackwell, Oxford.
7. Tocher DR, Fraser AJ, Sargent JR, Gamble JC. 1985. *Lipids* 20:84-89.
8. Tocher DR, Fraser AJ, Sargent JR, Gamble JC. 1985. *Lipids* 20:69-74.
9. Falk-Petersen S, Sargent JR, Fox C, Falk-Petersen IB, Haug T, Kjørsvik E. 1989. *Mar. Biol.* 101:553-556.

We thank D. Somerton and T. Keough for help with egg sampling and determination of fertilization success. This is OSC contribution # 267. Funding for this work was provided by NSERC in the form of a Strategic grant.

Evaluation of particle removal by a microscreen drum filter

T. White [1] *and L. D. Townsend* [2]

A commercially available microscreen drum filter was installed adjacent to the existing pressurized sand filtration units at the Pacific Biological Station, Nanaimo, B.C. It was evaluated with filter panels of 9, 16, 30, and 60 micron pore sizes at a variety of incoming flows and particle densities. Particle removal capacity, hydraulic loading capability, capital and operational cost, and general ease of use of the unit were examined. The microscreen filter met the criteria of removing particles 70 microns and larger, reduced maintenance costs compared to the existing filtration system, and provided a simplified method to process saltwater.

Introduction

The Pacific Biological Station, Nanaimo, British Columbia, is a federal research facility that uses filtered saltwater for research programs on marine fish and shellfish. Traditionally the saltwater has been processed by two pressurized sand filters referred to as rapid sand filters. The existing system is considered to be expensive to operate and maintain. In the autumn of 1994, a project was commissioned by the Department of Public Works and Government Services Canada (PWGSC) to find a filtration method that maintained filtering capacity but was more cost-effective than rapid sand filtration.

The project was divided into three components: a paper study, a laboratory investigation, and a pilot-scale study. The paper study reviewed the types of filtration available and the characteristics of each type. Gravity sand filters, separation filters, hydrocylones, rapid sand filters and microscreen filters were examined. Each filter type was evaluated with criteria appropriate for the facility: hydraulic capacity of 1000 US gallons/minute, removal of particles of 70 µm, ease of use and maintenance, and operational cost. Low head filters showed the greatest potential to reduce operating costs and microscreen filters have the ability to remove particles much smaller than the criterium of 70 µm. Therefore a low head, microscreen drum filter (Rotofilter™, manufactured by P.R.A. Manufacturing, Nanaimo) was chosen for further testing. Operating cost reductions of up to $9,000 per year, in comparison to rapid sand filtration, was expected for this filter. The Rotofilter™ also had the capability to be more effective in particle removal than the existing system.

Microscreen filter description

The microscreen drum filter consists of a rotating drum of five or more filter panels, a spray wash unit and an outer reservoir for the filtered water (Fig. 1). Raw saltwater flows into the centre of the drum and then outward through the filter panels into the outer reservoir. This filter is nominally rated to process 1000 US gal/min when installed with ten filter panels of 60 to 90 µm pore size, (100 US gallons/minute/screen). The filter panels are woven polyester on a polypropylene grid. When the immersed filter panels become loaded and the water level inside the drum rises above a predetermined height, the drum rotates and a fresh water spray cleans the filter panels. The wash water flows to waste at 8 US gallons/minute by a separate outflow.

Laboratory scale study

In the second phase of the project, a laboratory-scale investigation was conducted to determine the particle removal rate and hydraulic capacity of the cloth used for construction of the filter panel. Test cylinders were made from filter cloth with nominal pore sizes of 9, 16, and 30

µm secured onto 15 cm PVC pipe. Phytoplankton naturally present in the laboratory saltwater intake were batch cultured to emulate "bloom" conditions.[3,4] The number and size of phytoplankton particles were evaluated by a Coulter™ Multisizer II.[5] Phytoplankton cell density was 1.3-2.3x10^5 cells/mL and cell diameter ranged from 2.0 to 35.0 µm with an average diameter of 5.6 µm.

The 9 µm filter cloth removed 85% of the particles present, the 16 µm cloth removed 26%, and the 30 µm cloth removed 20%. Based on the data collected, filter panels were constructed of 9, 16, 30, and 60 µm cloth for the pilot phase of this study.

Pilot scale study

In the third phase, a stainless steel version of a standard microscreen drum filter was built and installed adjacent to the existing sand filters at the Pacific Biological Station. The microscreen filter was tested for competence of particle removal, hydraulic capacity, ease of use, as well as being rated for maintenance and operational costs. Filter panels of 9, 16, 30, and 60 µm sizes were evaluated in separate tests while the filter was continuously operated. Water samples were taken hourly from inside the drum (raw saltwater) and from the outer reservoir of filtered water. A minimum of nine filtered and corresponding raw water samples were collected for each of the filter panel sizes. All particles within the range of 1.96 to 350.0 µm diameter were evaluated for number and size using a Multisizer II.[5]

Particle removal results

During these tests, the raw saltwater contained particles of up to 300 µm in diameter. However, the majority of particles were small and average diameter over all the tests was 2.55 µm. Particle size distribution remained relatively constant during the 3 weeks of testing (Table 1). The 9 µm filter panels removed all particles 13.27 µm and larger, the 16, 30, and 60 µm panels removed all particles with diameters larger than 15.0, 24.16, and 32.84 µm, respectively.

Particle removal rates, expressed as a percentage of the particles present in the raw saltwater removed by the filter, are recorded in Table 1. The 9 µm filter removed 21.0% of the total particles present in the unfiltered water; the 16 µm filter removed 11.8%, the 30 µm filter 8.9%, and the 60 µm filter 7.7%. Reported removal

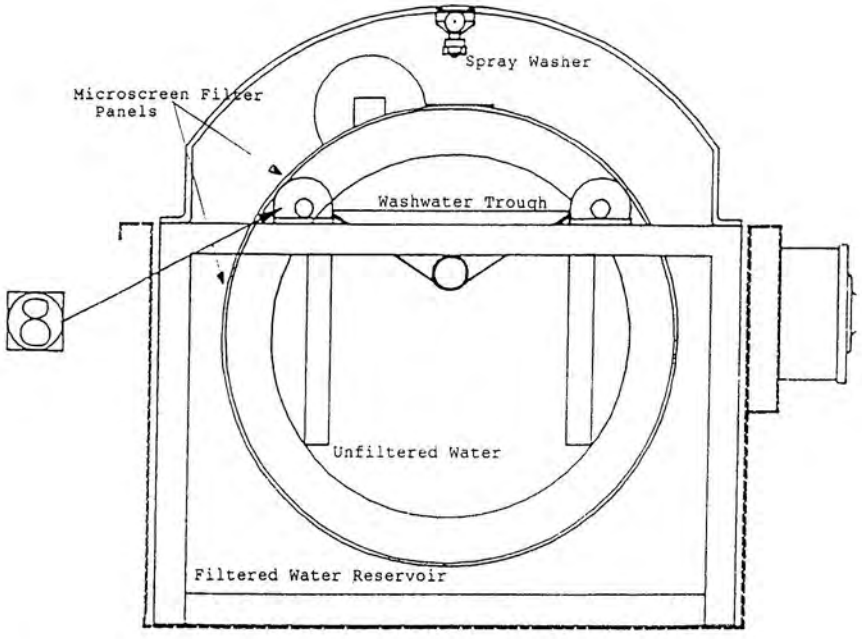


Figure 1. Schematic diagram of the rotating drum filter (Rotofilter™).

rates were influenced by the absence of larger phytoplankton characteristic of spring blooms in Departure Bay[4] and by the presence of a large numbers of particles < 3.0 µm. Further investigations during "bloom" conditions indicated the removal rate improved as the number and size of particles in the raw saltwater increased.

Despite the reduction in the number of particles in the filtered water, the Multisizer II was not sufficiently sensitive to determine differences in the mean diameters of the raw and filtered water, except for the 30 µm panels (Table 1). The 30 µm panels showed a reduction in the mean particle diameter from 2.88 to 2.82 µm.

Hydraulic capacity

The drum filter is nominally rated for the 9, 16, 30, and 60 µm pore filter panels at 318, 1010, 2060, and 2480 L/min with 10 filter panels in place (Table 1). Maximum hydraulic capacity was not determined due to the nature of the filter installation for the pilot study. However, the data collected in these tests supported the nominal rating.

Equipment use and maintenance

The filter panels were changed 5 times during the pilot study. A complete filter panel replacement (10 panels), plus cleaning, was completed in 2 hours. Assistance was necessary during the panel replacement, but the majority of operations, start up, maintenance, water sampling, etc., could be handled by one person. Filter cloth tears were easily repaired by inserting a small rubber plug. The filter was run in automatic wash mode; that is, the drum rotated and the screens were cleaned as demanded by the density of particles in the raw saltwater. This operation ran smoothly without constant maintenance and with continued efficiency over time.

Conclusions

In the laboratory and pilot studies, the 60 µm filter panels removed all of the particles 32.84 µm and larger and the other panels removed all particles at smaller diameters. The nominal flow ratings provided by the manufacturer were supported by the data. The filter was easy to operate and maintain during standard use and during filter panel changes.

This project determined that the rotating drum filter, which has the capability to reduce energy costs at this facility by up to $9,000 per year, was able to meet or exceed the particle removal criteria, hydraulic demands, and the operational and maintenance requirements of the Nanaimo research and culture facility.

Notes and references

1. NovaCulture Inc., 3307 Decourcey Drive, RR#3, Ladysmith, BC VOR 2EO
2. Mel-Mor Science, 3146 King Richard Drive, Nanaimo, BC V9T 3Z9
3. Parsons TR. 1960. *Fish. Res. Bd. Can. Man. Rep.* 81:23 p.
4. Stephens K. 1966. *Fish. Res. Bd. Can. Man. Rep.* 904:32 p.
5. Anonymous. 1989. *Operator's Handbook, Coulter™ Multisizer II,* Coulter Electronics Ltd., Beds, U.K. 38 p.

Table 1. Particle removal data from the pilot study.

Filter pore size	Mean Particle Diameter (µm) Raw water	Filtered water	100% removal [a]	± SE	Particle Density Raw water particles/mL	Particles removed (%)	Open mesh area	Nominal flow rate (L/min)
9	2.47	2.47	13.27	3.4	7.6×10^3	21.0	2.0%	318
16	2.32	2.32	15.00	2.8	1.92×10^4	11.8	8.0%	1010
30	2.88	2.82	24.16	11.6	1.07×10^4	8.9	20.0%	2060
60	2.41	2.41	32.84	14.3	1.12×10^4	7.7	29.0%	2480

[a]The diameter above which 100% of the particles present in the raw water were removed.

Land-based Atlantic salmon commercial production facilities

Walter Butler [1]

Environmental regulations are limiting production both in wild fisheries harvests and aquaculture. Land-based aquaculture systems are better able to accommodate environmental regulations than are ocean facilities and a controlled system can deal with environmental and climatic problems in a more efficient manner. An important consideration is to have the project professionals of the facility, rather than the owners or managers, deal with the regulators. In this way, problems can be solved that could otherwise delay the project. A well designed land-based facility in North America can produce salmon at a cost that is at least comparable, if not lower, than the cost of salmon production in Chile — without the major cost of transportation to the North American marketplace. The future belongs to the producer who provides a quality product at low cost. Those who can do this will be "winners".

It is not the purpose of this paper to compare the merits of a major land-based commercial production facility with those of pen cage systems for the production of Atlantic salmon for the commercial marketplace.

The primary aim of a well designed, land-based system is to create a facility that is environmentally correct and produces fish at the lowest possible cost. Only a few years ago, an Atlantic salmon facility producing 1,000 tonnes annually would require 8,000 to 12,000 US gallons (30,000 to 45,000 liters) of water per minute. That requirement can now be reduced to 500 gpm (1900 liters per minute) and will generally reduce pumping, heating, water treatment and other costs sufficiently to offset increases in costs associated with maintaining water quantity.

To design a cost-effective facility, it is necessary to bring together a team of highly trained professionals to provide the latest technology to meet the increasingly stringent environmental regulations and to establish guidelines to ensure the facility operates at the lowest possible cost. Many failures of major aquaculture facilities have been caused by lack of a strong technical team coordinating efforts to achieve a cost-effective and environmentally sound facility.

In order to build such a facility, it is necessary to acquire a project team encompassing about 15 disciplines including a project manager (usually one skilled in the fish business); a chemical engineer; civil, electrical, mechanical, and structural engineers; an architect; a fish biologist-pathologist; aquaculture specialist; a Certified Public Accountant to develop a financial plan; environmental engineer for securing permits; processing equipment specialists; marketing expert; and construction cost estimators and construction managers.

When the water requirements for production are reduced from 8-12,000 gpm to 500 gpm for 1,000 tonnes of capacity, the land-based facility can be built close to a large market. Of the 260 million people in the United States, 200 million live east of the Mississippi. About 19 million of the 28 million people in Canada live east of Manitoba. Most of the potential consumers are therefore found in these areas. Interestingly, consumption of fish is growing faster in inland areas than on the east and west coasts. A production facility close to a primary market is

highly desirable and 500 gpm from an aquifer is usually readily available.

An essential element of a successful Atlantic salmon production facility is a minimum annual production volume of at least one million pound round weight of salmon with the capacity for daily harvesting and maintenance of water temperatures between 14 and 17°C (58° to 62°F) twelve months of the year. Production should start with the egg and remain in the same facility until the finished fish is processed for the dinner table. Land-based systems eliminate the costly step of adding smolts from another facility or location. Daily harvest helps ensure quality, a stable supply for the customer, and provides a steady source of revenue for the operation.There is no question that Canadian technology for the production of Atlantic salmon is superior to that available in the United States.

Almost daily we read of environmental pressure on the oceans bordering Canada and the United States. Environmental regulations are already limiting production from both the wild harvest and aquaculture. Land-based systems are far better suited to accommodate these regulations than are facilities in the ocean. A totally controlled land-based system can deal with environmental and climatic problems in a far more efficient manner. However, the project technical team must have professional engineers and personnel qualified to deal with government regulators.

It is advantageous to have professional technicians, rather than the owners or managers of the facility, deal directly with the regulators. In this way, problem solving can take place without the insertion of other issues that could considerably delay progress on the project.

As for the future, a well designed land-based facility will produce Atlantic salmon at a cost that will be at least comparable, if not lower, than the cost of producing salmon in Chile — without the major cost of transportation to the North American marketplace.

In summary, the future belongs to the producer who provides a quality product and is a "low cost producer". Those who can do this, will be "winners".

Notes

1. Butler Research and Development Company, 474 Galtier Plaza, Box 86, 175 East 5th Street, St. Paul, MN 55101 USA

Fish farm effluents: cyanobacterial treatment and biomass recycling for fish feeding

J. de la Noüe, A. Dumas, Line McLaughlin and D. Proulx [1]

Using the cyanobacterium *Phormidium bohneri*, a self-flocculating species, in completely mixed 70-L bioreactors with three retention times of 8, 12, and 24 hours, removal of ammonium nitrogen from fish culture tank effluents reached 82% and removal of soluble inorganic phosphorus was 85%. The incorporation of 15% *P. bohneri* biomass into the diet had no adverse effect on the growth of rainbow trout (*Oncorhynchus mykiss*) juveniles over a 15-week period.

Introduction

One promising way of treating effluents loaded with inorganic nitrogen and phosphorus is to use microalgal cultures[2] especially the self-flocculating[3] cyanobacterium *Phormidium bohneri*. One of the aims of the present work was to verify the capacity of *P. bonheri* to remove inorganic nitrogen and phosphorus for fish culture tank effluents and to produce biomass.

Cyanobacteria have potential as a protein source and other nutrients in fish feeds since they contain 41-71% protein (dry mass basis), a high proportion of polyunsaturated lipids, 25-60% of total lipids,[4] and several important vitamins, especially vitamin A.[5]

The second major objective of the present work was therefore to test the hypothesis that substituting part of the feed for rainbow trout (*Oncorhynchus mykiss*) would lead to satisfactory survival, growth, and body composition.

Materials and methods

Effluent treatment experiments

Experiments using *P. bohneri* were conducted with an effluent (12-15°C) produced by adult rainbow trout (n= 19; mean individual mass: 568 ± 308 g) fed (< 1.5% wet mass) at 08:00 hours with commercial floating pellets ("Purina Trout Chow"; crude protein 40%, wet mass basis).

Three 70-L completely mixed triangular photobioreactors[6] were inoculated with 165 mg dry mass of *P. bohneri*. Artificial light (100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the water surface) with a photoperiod of 12 h light (00:00 h to 12:00 h):12 h darkness and aeration (0.1 vvm, i.e. 0.1 L air·L^{-1} bioreactor·min^{-1}) were provided. Three effluent influxes were used: 70 $L \cdot d^{-1}$, 140 $L \cdot d^{-1}$ and 210 $L \cdot d^{-1}$ (retention times of 24, 12, and 8 h, respectively). Water temperature was 20°C.

Water characteristics were continuously measured inside the reactors and included temperature, O_2, pH (continuously); N-NH_3, N-NO_2^-, N-NO_3^-, P-PO_4^{-3} (3·$week^{-1}$); COD (1·$week^{-1}$).

Five diets were given based on a pigment-free commercial trout feed (Corey, closed formula). The unmodified feed (DC) was used as a reference (7.6% H_2O, 51.1% protein, 16.6% lipid, 10.4% ash, 21.8 kJ energy·g^{-1}). The other diets were prepared by grinding this feed and mixing it with 0% (DO; 7.5% H_2O, 50.8% protein, 17.0% lipid, 10.3% ash, 21.6 kJ·g^{-1}), 15% (D15; 7.6% H_2O, 47.2% protein, 14.1% lipid, 13.5% ash, 20.6 kJ·g^{-1}) and 30% (D30; 8.0% H_2O, 45.0% protein, 12.9% lipid, 15.4% ash, 19.2 kJ·g^{-1}) freeze-dried *P. bohneri* biomass (dry mass basis, 5.9% moisture; 35.7% protein, 6.5% lipid, 34.1% ash, 13.2 kJ·g^{-1}d.m.). The fifth diet (D30 + oil; 8.1% H_2O, 42.4% protein, 16.1% lipid, 15.6% ash, 19.6 kJ·g^{-1}) contained 30% *P. bohneri* and 5% cod liver oil. The resulting

Table 1. Treatment efficacy (%) and final biomass concentration achieved with *P. bohneri* on fish culture effluents.

	Hydraulic retention time (day)		
	1.0	0.5	0.33
NH_3 reduction (%)	73/78[a]	78/82	78/82
NO_x reduction (%)	28/48	+14/28[b]	+14/0
PO_4^{3-} reduction (%)	70/85	65/85	63/74
Maximal pH	7.9/8.3	8.2/9.1	7.7/8.7
Maximal biomass (mg $d \cdot m \cdot L^{-1}$)	270/395	320/320	320/375
Biomass growth rate (d^{-1})	0.05/0/06	0.04/0.07	0.06

[a] replicate
[b] (+) increase

mixture was moistened, passed through a meat grinder, dried overnight at 25°C and cut to appropriate size. DO diet (processed like diets incorporating cyanobacterial biomass) was the true control.

Rainbow trout juveniles obtained from Pisciculture du Lac William Inc., St-Ferdinand, QC, were kept (50 fish per tank) in 15 self-cleaning cylindrical tanks (15 L capacity), each supplied with 4 L water per minute (14.0 ± 0.5°C) from a down flow nitrifying filter (hydraulic retention time of 1 d).

Experimental fish were hand-fed for 15 weeks (5.5% down to 2.1% of total fresh mass per day; 8 down to 2 meals·d^{-1}), according to published[7] guidelines. Individual mass and fork length were measured for all fish after anesthesia with tricaine methanesulfonate (MS 222, 40 mg·L^{-1}; Sigma Chemicals, St. Louis, MO).

Moisture, ash, protein, lipid contents, and energy were evaluated by AOAC standard methods[8] on frozen samples for feeds and on freeze-dried samples for fish.

Data were analyzed using one-way Super ANOVA (Abacus Concepts 1989) and tested for significance ($P<0.05$) with Fisher's protected least significant difference test.

Results and discussion

Effluent Treatment

Table 1 reports the results obtained for treatment efficiency and cyanobacterial production. Whatever the hydraulic retention time, the result was the same for $N\text{-}NH_3$ reduction, i.e., around 80%, giving a final effluent content around 0.2 mg $N\text{-}NH_3 \cdot L^{-1}$. This compares well with results obtained with other types of effluents[9,10] and the final effluent was below the 0.5 mg $N \cdot L^{-1}$ level set for drinkable water.

Although $NO^{3-}+NO^{2-}$ showed for two reactors some increase (nitrification), oxidized nitrogen forms remained at relatively low concentration (<1.5 mg $N \cdot L^{-1}$). Removal of inorganic phosphorus reached 75% so that the final effluent concentration reaches 0.02-0.05 mg $P \cdot L^{-1}$. This is much better than the 30% removal obtained with biofiltration[11] and such an effluent would be usable for growing fish.[12] The pH values increased, as expected, to reach the 8.0-9.0 bracket and biomass reached at the end of the experiments a value twice that of the inoculum. This was modest compared to previous results obtained with other effluents[9,10] and the 0.05 d^{-1} growth rate was also low compared to the 0.5 d^{-1} value previously reported.[13]

All fish readily accepted the diets provided. Table 2 summarizes the results obtained for survival and growth of rainbow trout fed the various diets for 15 wk.

Survival was over 95% and did not differ ($P>0.05$) between diets. Growth rates (results not shown) obtained for the reference diet (DC) were close to published values for rainbow trout grown at 14°C,[14] indicating that experimental conditions were good. After 15 wk, body length and mass were the highest ($P<0.05$) for fish fed genuine commercial feed (DC); fish fed control (DO) and 15% cyanobacterial diet (D15) were

Table 2. Survival and final size of juvenile rainbow trout fed the five diets for 15 weeks.

Diet	Survival (%)	Body length (cm)[a]	Body wet mass (g)[a]
DC	95 ± 1	12.8 ± 0.3^{c}	30.1 ± 2.0^{c}
D0	96 ± 4	12.2 ± 0.2^{b}	25.6 ± 1.6^{b}
D15	97 ± 3	12.2 ± 0.3^{b}	26.1 ± 0.9^{b}
D30	96 ± 2	$11.9 \pm 0.1^{a,b}$	23.2 ± 0.6^{a}
D30 + oil	96 ± 2	11.7 ± 0.1^{a}	22.5 ± 1.2^{a}

Values are means ± standard deviation of three replicate tanks. Within each column, means with different letters are significantly different ($p<0.05$).
[a] At the beginning of the experiments there was no significant difference ($p>0.05$) for body length and mass between fish groups.

next and equal and higher ($P<0.05$) for body mass than the fish fed the 30% cyanobacterial biomass (D30, D30 + oil) and for body length (D30 + oil).

The incorporation of *P. bohneri* biomass, which does not produce toxins,[16] into the feed did not produce adverse effects.

Since reference (DC) and control (DO) diets had almost identical compositions and, yet, led to significantly different results for body mass and length, this suggests that some nutritive value was lost during the repelleting process.

The feed conversion ratio (FCR= (final fish mass – initial fish mass)/total feed given) was highest (1.31 ± 0.05, $P<0.05$) for the DC group, followed (1.21 ± 0.03, 1.26 ± 0.01 respectively) for the D0 and D15 groups and lowest (1.14 ± 0.02) for D30 groups.

These FCR values compare favourably to other published values.[14,15] The same situation holds true for the condition factor values obtained (1.35-1.41) that were on the high side of published values (1.22-1.42) for rainbow trout.[15] Final body composition (results not shown) was similar for all fish.

In conclusion, biotreatment of fish culture facilities with the cyanobacterium *P. bohneri* appears feasible and the produced biomass can be incorporated at least to the 15% level into feed for rainbow trout after proper processing.

Financial support from the Conseil des recherches en pêche et agroalimentaire du Québec (CORPAQ) is gratefully acknowledged. We thank G. Turcotte for helpful comments.

Notes and references

1. Groupe de recherche en recyclage biologique et aquiculture (GREREBA), Université Laval, Sainte-Foy, QC G1K 7P4
2. de la Noüe J, De Pauw N. 1988. *Biotech. Adv.* 6:725-770.
3. Talbot P, de la Noüe J. 1993. *Water Res.* 27:153-159.
4. Becker EW. 1988. In, *Micro-algal Biotechnology* (MA Borowitzka, LJ Borowitzka, eds), p. 222-256. Cambridge University Press, Cambridge.
5. Borowitzka MA. 1988. In, *Micro-algal Biotechnology* (MA Borowitzka, LJ Borowitzka, eds), p. 153-196. Cambridge University Press, Cambridge.
6. Talbot P, Lencki RW, de la Noüe J. 1990. *J. Appl. Phycol.* 2:341-350.
7. CPAQ (Conseil des Productions Animales du Québec). 1982. *Guide sur l'aquiculture. Élevage de salmonidés.* Adgex 485, Québec. 102 pp.
8. AOAC (Association of Official Analytical Chemists). 1980. *Official methods of analysis* (W Horowitz ed). AOAC, Washington. 1018 pp.
9. Proulx D, Lessard P, de la Noüe J. 1994. *Environm. Technol.* 15:449-458.
10. Blier R, Laliberté G, de la Noüe J. 1995. *Biores. Technol.* 52:151-155.
11. Metcalf & Eddy Inc. 1991. *Wastewater Engineering, Treatment, Disposal and Reuse.* 3rd ed, McGraw Hill, Boston. 1344 pp.
12. Austrang E, Storebakken T, Åsgård T. 1987. *Aquaculture* 60:157-160.
13. Talbot P, Thébault JM, Dauta A, de la Noüe J. 1991. *Water Res.* 25:465-472.
14. Lee DJ, Roehm JN, Yu TC, Sinnhuber RO. 1987. *J. Nutr.* 92:93-98.
15. Ministère du Loisir, de la Chasse et de la Pêche du Québec. 1983. *Facteur de condition.* Information N° 3-64-1020/83005. Service de l'aquaculture. Gouvernement du Québec, Québec. 5 pp.
16. Codd G. Personal communication.

Feeding end-points associated with different feeding methods in seacage farming of salmonids

K. P. Ang[1] *R. J. Petrell*[2] *and B. E. March*[1]

To design better feeding systems, more information on feeding end-points and feeding and swimming patterns is needed. Underwater video camera and surface observations were recorded of fish feeding at different stocking, feeding, and environmental conditions. Method of feed discharge affected swimming pattern, feeding opportunities and fish distribution. The majority of fish did not commence feeding if fish were evident at the surface but not feeding at the beginning of a feeding event. Aggression occurred when fish were fed small portions throughout the day, and both sub-surface and surface feeding activities were weak at satiation. Surface activities under other test conditions seldom coincided with the actual cessation of feeding as judged from sub-surface feeding activities and pellet loss. Pellet loss as a feeding end-point indicator did not account for feeding rate or loading pattern. Providing a fixed ration resulted in feed wastage or underfeeding. Cessation should be determined using both pellet loss and fish activity at the bottom of the feeding school.

Introduction

Various authors[3,4] have shown that automatic feeding of fish is associated with high levels of feed wastage. Fish have traditionally been fed based on surface feeding activities even though fish may not be feeding uniformly throughout the water column.[5] Newer systems based on pellet loss also fail to ensure that bottom fish are eating, because pellet loss can be caused by many factors including excessively high rate of feed dispensation, poor visibility and variations in appetite. Setting fixed rations for fish based on estimated growth rates results in either feed wastage or underfeeding because appetite and environmental conditions are difficult to predict.

The objective of this research was to compare surface feeding activities with those on the bottom prior to and at feeding end-points. This information will be used in designing feeding systems that are fast, reliable, and effective.

Methods

Observational equipment

Either a Panasonic WV-BD400 or a Cohu 4915-2000/0000 video camera with a Cosmicar (Pentax Canada Inc., Vancouver, B.C.) 4.8 mm lens was used. The camera was sealed in an underwater housing made of an anodized aluminum case with acrylic dome ports and PVC bulkhead (International Hardsuits, North Vancouver, B.C.). The resulting viewing volume was a right rectangular pyramid and the height of the view volume, as measured perpendicular to the base of the pyramid, was considered to be the visibility (in meters) within the water column as defined by the Secchi disc reading. No artificial lights were used. A Panasonic AG-1960 S-VHS recorder was used to record images and a Panasonic TR-930 CB video monitor allowed direct viewing of fish images.

Feeding fish were monitored with cameras placed in the centre of the cages 1-2 m below the feeding zone. This camera position enabled us to check for pellet loss and provided a complete picture of fish distribution in the water column during the entire feeding event. The camera was usually at a depth of 8 m but ranged from 5 to 12 m, depending on fish distribution and water visibility. The camera faced toward the water surface and in that position the feed pellets were viewed as black, round objects.

Feeding trials

Before commencing each feeding trial, the

camera was left in the cage overnight so that fish became accustomed to it. Feeding experiments lasted one to three days (Sites D, E, F, G) or 2 to 3 months (Sites A, B, C) and were conducted at sites rearing either Atlantic, *Salmo salar,* or chinook, *Oncorhynchus tshawytscha,* salmon of various sizes and stocking densities under different environmental conditions.

Feeding Techniques and End-points

A wide range of feeding techniques including semi-automatic broadcast feeders, programmable automatic (rotary) feeders, and hand feeding using a variety of scoop sizes were tested (Table 1).

Surface and sub-surface feeding activities, pellet loss and feeding to fixed ration were used to judge feeding end-points. Surface feeding activities included mouthing, splashing, foraging, and feed capture. Foraging activity was described as fish breaking away from a regular swimming mode to exhibit searching action with the head swerving sharply to the right or left. A regular swimming mode was described as fish swimming in an organised circular manner.[6] When surface feeding activities were used, feed dispensation was discontinued when mouthing, splashing, etc., at the surface could not be detected. When sub-surface feeding activities were used, feed dispensation was discontinued when foraging activity and feed capture were no longer perceivable and pellet loss was observed. When only pellet loss was used (Feedback Feed Control System, Moore-Clark), feed dispensation was discontinued when a reduction in discharge rate did not eliminate pellet loss. Pellet loss not accompanied by sub-surface feeding activities could be stopped by reducing feed discharge rate. Using a fixed ration, the feeding end-point was when the fixed ration was completely discharged.

Results and discussion

The continuous broadcast of feed produced an organised circular swimming and feeding pattern and no obvious foraging behaviour unless visibility was poor (< 3 m). Fish probably did not have to break from a regular swimming pattern because pellets were readily available. Generally, this pattern was not associated with pellet loss except when the rate of feed dispensation was too high or fish were satiated.

Concentrated or point feed loadings and pulsed or handfeeding produced irregular or disorganized swimming patterns and fish congregated in a small area with the point of feed discharge as the epicentre. It was not uncommon for fish subjected to these feed loading patterns to change from an organised to a disorganized swimming pattern, depending on the size of the broadcast area and discharge rate. Concentrated feed loadings appeared to force fish to compete for feed. Pellets were lost, unless the discharge rate was varied to reduce loss, because fish could not feed fast enough. Smith et al.[7] also observed that in a net cage pellets appeared more likely to be missed when two or more fish attacked the same pellet.

Atlantic salmon (site B) at the bottom of the feeding school were often (mean 82 ± 4% and 74 ± 7% for morning and evening feed, respec-

Table 1. Combinations of feeding methods tested.

Pattern of discharge of feed pellets	Mode of feed dispensation		
	Semi-automatic (feed blowers)	Semi-automatic (rotary, programmable)	Hand-feeding
Continuous broadcast	Sites A, B & C		
Intermittent broadcast	Site C	Sites E & F	Sites D_3, E_1, F_2 & G_3
Pulsed broadcast	Sites A, B	Sites E & F	Sites D_3, E_1, F_2 & G_3

Feeding schedule:
Site A: alternate day
Site B,C,D,G: once per day
Site E, F: small portions (0.25-0.5 kg per 3 second burst varied over the entire daylight period)
Scoop sizes: 1 = 0.5 kg; 2 = 1.0 kg; 3 = 2.3 kg
Intermittent: cages fed in rotation (feeding rounds).

tively) the last to finish eating. The fish were not seen eating at the surface but rather engaged in foraging activities at the bottom until pellet loss was evident. The reverse was true for chinook salmon at site C (Table 2).

When visibility was less than 3 m, salmon (1.2 kg average initial weight, site A) could not visually detect pellets as easily. Pellets were wasted despite intense foraging behaviour. When visibility was > 10 m, chinook salmon (2.9 kg average weight, site D) could detect and consume pellets deeper (at the camera level).

When Atlantic salmon (1.8 kg average weight, site E) were hand fed small portions based on the level of surface activity over the daylight period (high visibility, >10 m), there was no perceivable feeding activity and pellet loss at satiation. However during feed discharge, fish were seen to forage intensely. At the surface, fish were aggressive as they competed for pellets and few pellets reached the bottom schooling fish. Fish, especially those at the bottom, probably had to expend significant amounts of energy foraging for food. Kadri et al.[8] also implied that feeding regimes of this nature may be failing to provide enough food when fish are hungry, yet cause substantial wastage at other times. Similarly, feeding a predetermined ration resulted in either pellet loss or underfeeding, because fish were either still eating or had already stopped feeding when the ration was completely discharged.

Whenever fish were at the surface but were not eating or foraging at the beginning of a feeding trial, sub-surface feeding activities were also weak and pellet loss was substantial. Further discharge of feed never initiated feeding.

Conclusion

Sub-surface feeding activities and pellet loss when used together were good indicators of feeding end-points. It was possible to reduce pellet loss and aggressive behaviour if the feeding method generated an organised swimming pattern. Feeding fish to a fixed ration is not recommended as a feeding strategy. Water quality, fish species, stocking condition and feed delivery method affect feeding behaviour throughout the water column and the degree of pellet loss, making the use of underwater visual systems vitally important.

We acknowledge financial support from Agriculture Canada, National Research Council, BC Ministry of Agriculture, Fisheries and Food, BC Packers, PNG Ltd., Pacific Aqua Salmon Farming Partners Ltd., and Paradise Bay Seafarms.

Notes and references

1. Department of Animal Science, University of British Columbia, 2357 Main Mall, Vancouver, BC V6T 1Z4
2. Department of Bio-Resource Engineering, University of British Columbia, Vancouver, BC V6T 1Z4

3. Thorpe JE, Talbot C, Miles MS, Rawlings C, Keay DS. 1990. *Aquaculture* 90:41-47.
4. Seymour EA, Bergheim A. 1991. *Aquacult. Eng.* 10:73-88.
5. Juell JE, Westerberg H. 1993. *Aquacult. Eng.* 12:1-18.
6. Sutterlin AM, Jokola KJ, Holte B. 1979. *J. Fish. Res. Board Can.* 36:948-954.
7. Smith IP, Metcalfe NB, Huntingford FA, Kadri S. 1993. *Aquaculture* 117:165-178.
8. Kadri S, Metcalfe NB, Huntingford FA, Thorpe JE. 1991. *Aquaculture* 92:219-224.

Table 2. Mean incidence (%) of underfeeding (U) and overfeeding (O) based on conventional surface observation of feeding activity, relative to underwater video camera monitoring. (A) indicates agreement in judgement of satiation by the two methods. Triplicate cages were studies at each site.

	Site B Mean % (± S.D.)		Site C Mean % (± S.D.)
	a.m.	p.m.	a.m.
Underfeeding	82 ± 4	74 ± 7	8 ± 6
Overfeeding	12 ± 10	18 ± 8	80 ± 3
Agreement	6 ± 3	8 ± 1	12 ± 6

Notes:
Site B: Atlantic salmon, 3.7 kg average initial body weight; experimental period of 41 days (July-August 1994); net cage 15 x 15 x 12 m deep; Secchi disc reading 3.5-5.0 m.
Site C: Chinook salmon, 1.9 kg average initial body weight; experimental period of 56 days from September to November 1994; net cage 15 x 15 x 16 m deep; Secchi disc reading 4.5-8.0 m.

Effect of meal frequency on winter growth and feed wastage in farmed chinook salmon

Henrik Kreiberg[1]

Chinook salmon *(Oncorhynchus tshawytscha)* in their second sea-winter of cage culture were reared at two sites in British Columbia and fed on a pelleted commercial grower diet at manufacturer's recommended rates. Under similar husbandry conditions, chinook fed once daily achieved better growth and conversion efficiency than chinook fed the same ration delivered in every-other-day feedings. Neither feeding regime resulted in detectable waste feed being lost from the cage area.

Introduction

Control of feed cost remains one of the most potent, accessible and immediate means for a salmon grower to reduce production costs. It is widely agreed that feed expense is the largest single cost factor in producing a market salmon, comprising between 40 and 60% of the cost of production. In previous sea pen studies in British Columbia, chinook salmon had poorer growth and feed conversion performance when given unrestricted access to food via demand (fish-activated) feeders.[2] Studies in other species suggested that reduced efficiency results from a sustained high gut evacuation rate arising from high meal (feeding) frequency. Meal frequency thus appears to have implications for feed conversion efficiency.

This study was undertaken to determine the validity of the preceding explanation, by examining two of the more common meal frequencies used by British Columbia chinook growers, and to monitor whether significant feed wastage to the environment was associated with meal frequency. Use of second sea-winter chinook salmon provided the opportunity to collect data during a phase of grow-out when costs are high and when fish are prone to poor feed conversion efficiency if water temperatures decline sufficiently.[3]

Methods

Two sites were used in the study: the Department of Fisheries and Oceans Experimental Mariculture Facility at Departure Bay, Nanaimo, B.C., and an independent owner-operated farm in Sansum Narrows, Gulf Islands, near Duncan, B.C. Feed was White Crest Mills Salmon Developer I (protein 46%, fat 16%, fibre 3%, moisture <10%), fed according to the manufacturer's recommendations. Meal frequencies were once every day (ED) or once every other day (EOD). Feed was provided entirely by hand at the Sansum Narrows site and by combined hand/automated methods at Departure Bay. Disbursement of feed was such that all stocks received equivalent total amounts of feed regardless of meal frequency. Both sites had been using the diet daily for a considerable time prior to the study. Stocks at both sites were standard Big Qualicum origin, S-zero monosex chinook, obtained from the same commercial hatchery and transferred to seawater in April-May 1990. Fish were entering their second sea-winter when the study began in December 1991 and sampling was continued until late April 1992. Inventories were made by direct count.

Two sediment traps[4] were placed under each cage. Traps were also placed at two depths some distance from each cage complex to provide background data. Contents of the preservative chambers of the traps were collected regularly and the percent organic matter was determined from the difference after low and high temperature drying. An indicator dye in the preservative was used to detect loss of contents while *in situ*.

Results and discussion

Growth results were similar at the two sites. Fish on EOD feeding showed an initial small surge, but then gradually fell behind the ED feeding groups in mean weight (Fig. 1). By the end of the study (150 days), this difference had become statistically significant ($P < 0.05$) at the Sansum Narrows site, but not at the Departure Bay site. At both sites, the EOD group had considerably poorer conversion efficiencies during the latter portion of the study than the ED feeding group, even losing biomass during the mid-winter period at the Sansum Narrows site (Fig. 2). Mortality averaged 0.5% per week at Departure Bay, and 1% per week at Sansum Narrows, only increasing in mid-April near the end of the study at both sites. The mid-winter period of net loss of biomass at Sansum Narrows was not accompanied by unusual mortality. Water temperatures at both sites increased from about 8° C to 10°C during the study, with only small variations. Salinity remained 26 to 29 ppt for the duration. Table 1 summarizes conditions when the study ended.

Data from the sediment traps were highly variable and there was a tendency throughout the study at both sites for treatment values (i.e., from traps located directly under fish cages) to fall within the range of background data collected at a distance from the cage complexes during the same period. I concluded that neither of the two feeding regimes contributed appreciable organic loading to the environment surrounding the farms.

The poorer performance of the fish on the lower meal frequency would not be expected if the gut-evacuation explanation described above was true. Underfeeding was not likely a cause for the poorer performance, since the conversion rates were distinctly poorer in the EOD fish. At both sites, the same staff fed both the EOD and the ED groups. It seems likely that the fish on both meal frequencies received most if not all of the feed provided, since no significant waste was detected by the sediment traps.

In other recent work, I reported that feed conversion efficiency in chinook salmon in seawater declined rapidly below a temperature of about 9.5°C, independent of ration level.[3] For most of the present study, sea temperatures were lower than this, and the chinooks were operating metabolically in a zone where potentially several key components of the feed assimilation process were not capable of normal efficiency. While the underlying reasons are not clear, the present data suggest that at winter temperatures

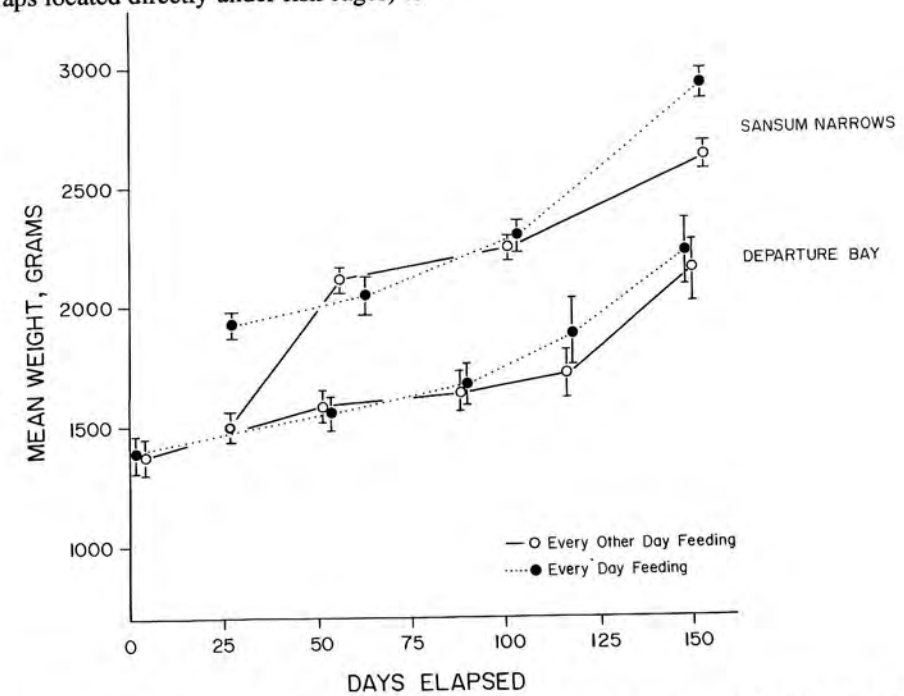


Figure 1. Increase in mean weight of chinook salmon at two rearing sites, relative to feeding frequency, during second sea-winter of growth.

Table 1. Conditions at the end of the feeding frequency study, by site and feeding pattern. ED = every day; EOD = every other day.

	Departure Bay		Sansum Narrows	
	ED	EOD	ED	EOD
Number of fish	62	68	3283	2021
Mean weight (grams)	2218	2157	2932	2627
Density (kg/m^3)	0.63	0.68	2.89	1.61
Survival (cumulative %)	79.1	86.1	87.8	82.8
Feeding rate (%/day)	0.58	0.61	0.59	0.85
Growth rate (%/day)	0.38	0.33	0.38	0.24

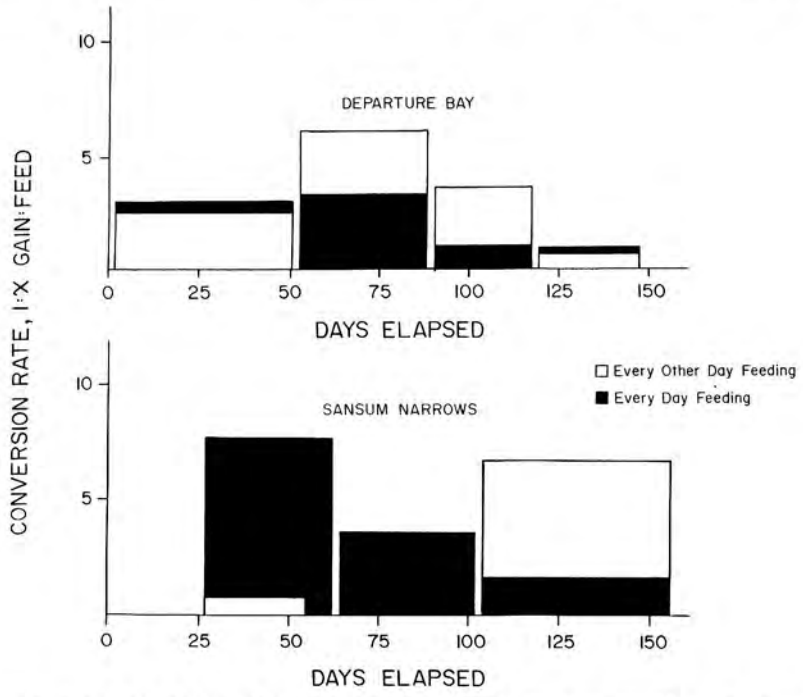


Figure 2. Conversion rate achieved by chinook salmon at two rearing sites, relative to feeding frequency, during second sea-winter of growth.

in the 7-9°C range advantage is gained from feeding cultured chinook daily.

Conclusion

Winter growth of chinook under culture conditions is affected by meal frequency in a manner contrary to what would be expected from studies published for other fish species (usually studied under temperatures closer to the mid range of their preferences). At the sea temperatures in this study, better conversion efficiency can be achieved with once daily rather than less frequent feeding.

This work was supported in part by the BC Ministry of Agriculture Fisheries and Food.

Notes and references

1. Biological Sciences Branch, Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo B.C. V9R 5K6
2. Crampton V, Kreiberg H, Powell J. 1990. In, *Proceedings Aquaculture International Congress,* p. 99-108. Aquaculture International, Vancouver, B.C.
3. Kreiberg H. 1991. *World Aquaculture* 22(1):84-85.
4. Macdonald RW, O'Brien MC, Macdonald DM. 1984. *Can. Data Rep. Hydrog. Ocean Sci.* 17(1).

Seasonal changes in food conversion ratio as an indicator of fish feeding management

Mark J. Costello,[1] *Declan T. G. Quigley*[2] *and Suzanne Dempsey*[1]

Monthly changes in fish weight and feeding were studied at four land-based freshwater Atlantic salmon (*Salmo salar*) farms in Ireland. This data was used to calculate the ratio of food given to change in fish weight (i.e., food conversion ratio, FCR). Average food conversion ratios over 12 months were 1.1–1.5 for the farms, and >1.8 during the winter. However, the feed manufacturers predicted an FCR ranging from 0.8 to 1.6 for the diets used. It was concluded that the farms did not reduce feeding rates soon enough in response to reduced water temperatures. While fish feed may not be the major cost in freshwater salmon farms, excess feeding is nevertheless an unnecessary financial loss and an environmental pollutant. Feed management may be simply and usefully monitored by calculating FCR on a regular basis in line with the changing water temperatures, fish sizes, and feeding rates.

Introduction

Several studies have shown that freshwater fish farming can have environmental impacts.[3-7] The main threats fish farms pose to water quality arise from organic matter and chemotherapeutants discharged in effluents. Fish food is the primary source of organic pollution, either directly if not eaten by the fish, or indirectly from undigested feed and metabolic waste products. Even with correct feeding, some 25–30% of a feed may be excreted as faeces.[8]

As fish are cold-blooded, their activity and appetite are positively related to temperature (within the range tolerated by the species). As digestion and gut evacuation of food are temperature dependent, fish need more frequent feeding at higher temperatures.[9,10] Food pellet size is chosen according to fish size, and while larger fish eat more than smaller fish, they eat proportionally less for their body size.[11] Additionally, fish appetite may change with changes in day length.[12] The quantity of food must therefore be adjusted for temperature and fish size, and in response to fish appetite. Feed manufacturers provide tables for predicting feeding rates for different fish sizes and water temperatures. Any stress, be it caused by a disturbance (transport, handling), presence of a predator, or disease, will reduce fish appetite. A good farmer predicts what the fish should be eating from information on fish size and water temperature, but it is also important to observe what the fish actually consume. In this way, poor appetite can be recognised and feed wastage minimised.

Correct feeding is therefore one of the most essential requirements for commercial farm management. Too little food results in poor growth and condition. Over-feeding results in food wastage, reduced food absorption by the fish and thus increased faecal waste, and poor efficiency of food conversion into fish flesh (the food conversion ratio, FCR).

Improved feed quality and farm management has resulted in significant improvements in the weight of food required to produce a given weight of fish.[9] For example, the FCR fell from 2.6 to 1.7 in Norway from 1979 to 1988.[13] A lower (better) FCR implies less feed wasted to the environment.

There has been considerable effort in improving the quality of fish diets so as to improve feed consumption, FCR, and reduce amounts of waste phosphorous (as this is a critical pollutant in freshwaters). Desirable physical qualities in feeds are that they have a low dust content and they remain intact and sink slowly in water, thus

increasing the opportunity for fish to capture them. Most modern feeds are "dry" (< 15% water) and are produced by an extrusion process such that they are more digestible and nutritious, and have the desirable physical qualities. These feeds have helped reduce the amount of nitrogen and phosphorous in fish farm effluents by about 50% since 1988.[14] However, improvements in diet quality will neither benefit the farmer or environment unless the fish are fed correctly. In this study, data collected routinely by staff from four commercial freshwater Atlantic salmon (*Salmo salar)* farms were analyzed. Monthly FCR calculated from fish growth and feeding rates were found to vary considerably, and it is proposed that this variation reflected the quality of feeding management.

Methods

As part of a review of freshwater fish farming in the Republic of Ireland,[15] we obtained data on fish weight and feeding for each month from four commercial Atlantic salmon farms. From this data, we calculated the food conversion ratio for each month as the amount of food fed divided by the change in fish weight. Each farm reared fish from egg to one year old (smolt) stage over a 15-month cycle. The farms were well established and kept records of fish growth, feeding, diseases, and other production parameters (water flow, water quality, temperature, etc.). No diseases or water quality problems occurred during the study that were considered to have compromised the analyses.

Fry were fed every 2 to 60 minutes for up to 5 seconds from dawn to dusk (in May and June) by automatic feeders. Parr and smolts were fed by hand and with automatic feeders from July until the following spring. Only "dry" feeds were used (moisture content <10%, phosphorous content <1.2%). Moist feeds are not currently used in freshwater fish farms in Ireland. Protein constituted about half, fat (lipids) about one fifth, ash and fibre about one tenth, and carbohydrates one sixth of the diets. The feeds were typical of those used by freshwater Atlantic salmon farms in Ireland. [15]

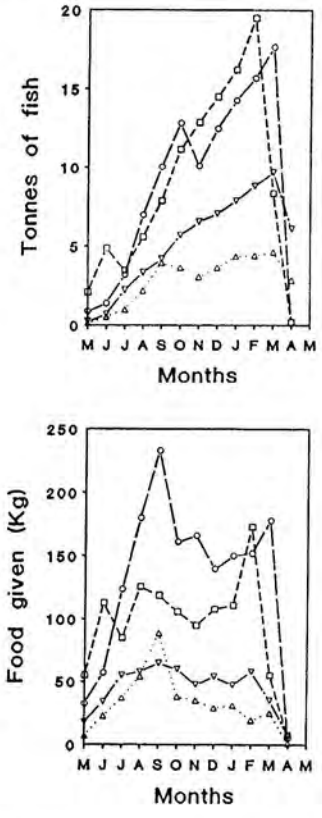


Figure 1: Monthly change in salmon biomass (tonnes) and quantity of food fed (kg), at four freshwater Atlantic salmon farms.

Results

Biomass on the farms increased from a minimum in May to a maximum the following March and April (Fig. 1a), with the greatest growth occurring from June to October. The amount of food presented to the salmon increased as biomass and temperature increased during the summer, but decreased in winter (Fig. 1b). However, the amount of food fed per weight of salmon (as % body weight per day) decreased as the salmon grew. This reflected a) the reduced requirement of food in larger fish in proportion to their body weight, b) the practice of providing an excess of food to fry to encourage them to start feeding, and c) the importance of minimising wastage as the quantity of food used increased. For each farm, the average FCR over the 12-month growing period was 1.1, 1.2, 1.3, and 1.5. However, from November to February, FCRs were >1.8 (Fig. 2).

Discussion

Experimental studies on Atlantic salmon in freshwater tanks in Norway found actual FCRs to range from 0.9 to 1.1.[9] The manufacturers of

the fish feeds used in this study stated that the FCR for their diets should range from 0.8 to 1.6. The fact that FCRs greater than 1.8 were observed on all farms in autumn and winter suggested that the farms were not feeding in accordance with fish appetite. As one would expect, the farms with the most fish (and biomass) used the most food (Fig. 1). However, the intermediate sized farms showed the poorest FCR. The poorest FCR cannot be accounted for by any possible error in recording fish weight and feed quantities. Through discussion with the farmers, it was concluded that the poor FCR was the result of failing to reduce feeding in accordance with reduced water temperatures during the winter.

Alanara and Cripps[15] found that hand feeding resulted in less food wastage than the use of automatic food dispensers because hand feeding allows the farmer to alter feed rates according to fish appetite. The results of the present study suggest that even when hand feeding, farm staff may be slow to adjust feeding rates in accordance with water temperature. Farms may calculate future feeding rates on the basis of current water temperature, but must realize that these are predictions and must be adjusted daily for changes in water temperature and fish appetite. Since it is unlikely that FCR can be calcuated daily, it is therefore critical that actual food consumption be closely observed by the farmer so as to immediately adjust the feeding rates if necessary. Feed collectors and monitors have been designed for underneath fish cages which tell the farmer when to reduce and stop feeding. The design of land-based farms should facilitate the observation of food consumption or loss.

When salmon are small, feed costs are proportionally small and automatic feeding is a common practice. Indeed, to encourage fry to feed, excess food is provided. Feed is the single largest cost in freshwater rainbow trout (ca. 40%) and marine salmonid (ca. 80%) production. In contrast, in freshwater salmon farms, where smolts are sold for further on-growing to market size in sea cages, feed costs are relatively less important (ca. 10%). Nevertheless, waste feed remains an unnecessary financial loss and environmental pollutant. The regular calculation of FCR is a useful indicator of feeding efficiency, and should be an essential element in overall feed management regimes.

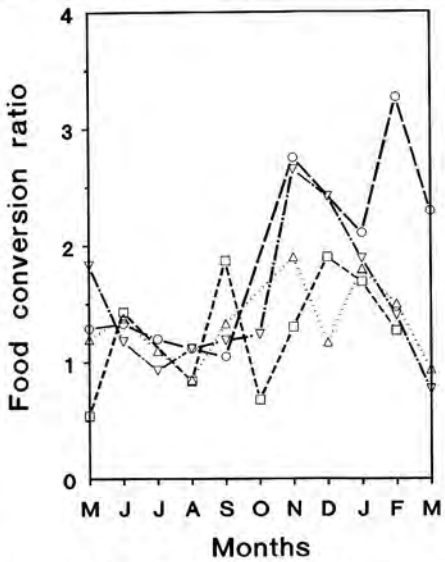


Figure 2: Monthly change in food conversion ratio at the four freshwater salmon farms.

This study was part-funded under the commission of the European Communities STRIDE *Environmental Research Sub-programme operated through the Department of the Environment. We thank the salmon farms for providing the data used in this study.*

Notes and references

1. Environmental Sciences Unit, Trinity College, Dublin 2, Ireland
2. Electricity Supply Board Fisheries Conservation, Hydrogroup, Ardnacrusha, County Clare, Ireland
3. Bergheim A, Selmer-Olsen AR. 1978. *Aquaculture* 14:267-270.
4. Carr OJ, Goulder R. 1990. *Water Res.* 24:631-638.
5. Carr OJ, Goulder R. 1990. *Water Res.* 24:639-647.
6. Trojanowski J. 1990. *Polskie Archiwum Hydrobiologii* 37:383-395.
7. Kendra W. 1991. *Trans. Am. Fish. Soc.* 120:43-51.
8. Butz I, Vens-Cappell B. 1982. *EIFAC Technical Paper* No. 41. FAO, Rome, Italy. p. 73-82.
9. Austreng E, Storebakken T, Åsgård T. 1987. *Aquaculture* 60:157-160.
10. Talbot C. 1994. *Fish Farmer* 17:48-49.
11. Talbot C. 1993. *Proc. Nutrition Soc.* 52:403-416.
12. Jørgensen EH, Jobling M. 1992. *Aquaculture* 101:135-146.
13. Seymour EA, Bergheim A. 1991. *Aquacul. Engineer.* 10:73-88.
14. Warrer-Hansen I. 1989. *Aquaculture Ireland* 40:19-22.
15. Alanara A, Cripps SJ. 1991. In, *Aquaculture and the Environment* (N De Pauw, J Joyce, eds), p. 6-7. European Aquaculture Society Spec. Publ. No. 14, Bredene, Belgium.
16. Costello MJ, Thompson E, Gillooly M, Dempsey S, Briody M, Massik Z, Quigley D, Warrer-Hansen I. 1994. *STRIDE Rept.* No. 5, Department of the Environment, Dublin, 73 pp.

Need and potential for softshell clam aquaculture in Nova Scotia

K. R. Freeman,[1] D. L. Peer[2] and C. M. Hawkins[3]

The digging of softshell clams, *Mya arenaria,* is a traditional Nova Scotian fishery currently showing signs of decline. Variability in reported landings in combination with pricing that does not appear to reflect market demand illustrates one possible effect of reportedly growing underground markets for illegally harvested product. Culture of softshell clams is one possible method of circumventing shortcomings in the fishery. Studies have shown that the fastest natural intertidal clam growth in Nova Scotia results in a harvestable shell length in 5.4 years, a time that can be reduced by holding clams in trays of sand suspended from rafts. Judicious stock selection combined with hatchery production and a grow-out system that holds clams continually submerged have the potential to provide accelerated growth and a predictable yield, both of which are presently lacking in the wild fishery.

The history of softshell clam exploitation in the Maritimes is as old as human occupation of the area itself. Commercial usage, however, only began in earnest during the mid 1800s with the harvesting and salting of softshell clams for bait in the Grand Banks fisheries.[4] With further development of fisheries markets throughout the last century and toward the present, the softshell clam became more popular as food. Consequently its value increased and harvesting escalated. Commercial harvesting of softshell clams in Nova Scotia suffers in part from the combined effects of variable market pricing and bed closures due to faecal coliform contamination. While efforts have been made to control harvesting through imposition of minimum shell lengths, policing of the fishery and recent licensing of diggers, illegal harvesting continues, likely at an accelerating rate. To protect clam stocks, size limits were introduced in the early 1970s. However, as established fisheries became controlled, underground markets developed. These uncontrolled markets are presently on the increase and reportedly are frequently supplied from closed areas.

Faecal coliform contamination is the principal reason for clam flat closures and has resulted in heightened competition amongst legal harvesters in the open areas. Also in the past year, the number of licensed harvesters has risen sharply in Nova Scotia to about 3,000. Thus, the resource is under increasing pressure, while market entry of potentially contaminated product from illegal digging raises public health concerns. One has to wonder if cases of enteric diseases such as shigellosis, salmonellosis, and possibly other enteric diseases reported in Nova Scotia[5] could be associated with the consumption of clams harvested from closed flats. One fishery officer with the Department of Fisheries and Oceans estimated that perhaps one-third of all softshell clams consumed in the Halifax metro area come from flats closed due to faecal coliform contamination. Disease risks from ingestion of contaminated clams are thus significant and will remain so while illegal harvesting and imprudent purchasing continue.

Such pressures on the resource, particularly when there is a high likelihood of considerable under-reporting of harvest, can lead to results now familiar in other over-taxed fisheries. The prevalence of illegal digging and selling is likely well known in communities adjacent to clam flats but the true extent is unknown to fisheries managers and remains the subject of speculation. Landings are reported in Nova Scotia from clam processing facilities only (Fig. 1) and indicate relatively high production from

1983 to 1992, although market prices fluctuated dramatically during that time. Given the usual inverse relationship between supply and demand price, steadily declining prices for the four years beginning in1983 would suggest proportionately rising catches. One has to wonder if the intensity and prevalence of illegal sales have not somehow affected these statistics and that actual landings far exceed those reported.

Problems associated with stock declines, quality, price instability, and conservation and protection can be addressed in part through aquaculture. In Nova Scotia, shellfish aquaculture is only permitted in leased water that meets approved bacterial standards — the lease itself bestowing on the owner proprietary rights and thus enhanced control of the product not possible in a common resource. Studies of softshell clam growth in these intertidal "commons" of Nova Scotia[6,7,8] indicate that 5.4 to 8.0 years are required to attain the current legal harvesting size of 50 mm shell length.[9] Nevertheless, this relatively long growth period from settlement to harvest may be shortened substantially through application of aquacultural techniques.

In their natural habitat, *Mya arenaria* are exposed intertidally for several hours daily and are thus deprived of food for that time. It would seem apparent that to increase growth rates to a maximum, reduction of exposure is required. As a possible way of reducing the time to reach market size, experiments were conducted in the late 1970s to examine growth of softshell clams that were continually submerged. Experimental stock was obtained in October 1977 from a commercially open bed at Three Fathom Harbour, N.S., and sorted into three shell length categories with means of approximately 8, 18, and 40 mm. The size groups each consisted of 25 animals. Each individual clam was placed in a labelled plastic cylinder containing natural beach sand and held on a wooden support tray. One tray, containing an entire set of size groups, was then suspended at a depth of 1 m from a raft anchored in Bedford Basin, an estuary receiving untreated domestic wastes from a major metropolitan area. The second tray was similarly deployed in nearby St. Margarets Bay on an uncontaminated site. Shell length and mortality were monitored approximately monthly for two years except for the St. Margarets Bay set, where data were collected for only 14 months.

While data analysis is still in the early stages, some initial findings appear significant. As expected, shell growth, most notably in the smaller size groups, was faster in the more eutrophic Bedford Basin. Deployment time in St. Margarets Bay, even for 14 months, was shown to produce a steeper growth curve than reported rates from several Nova Scotian Atlantic coast and Bay of Fundy clam flats. Unfortunately, conditions at both St. Margarets Bay and Bedford Basin mitigated against installation of adjacent intertidal controls. Nonetheless, com-

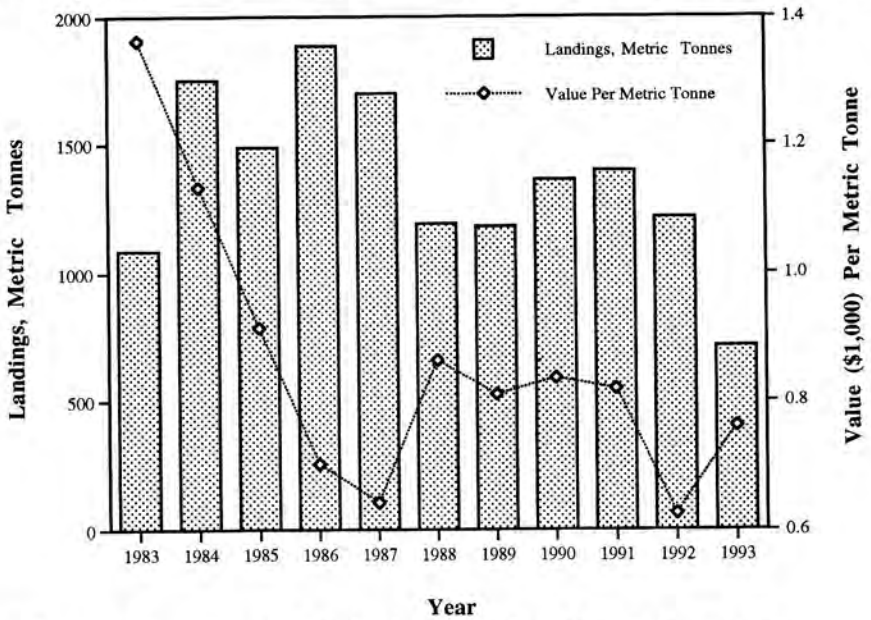


Figure 1. Reported harvest and landed value of softshell clams in Nova Scotia.

parison with other intertidal clam growth studies suggests that continuous submergence results in significant growth enhancement and would thus reduce the time to reach market size. Von Bertalanffy growth curves fitted to the data indicate time to harvest size of about 3 years for the Bedford Basin clams. While a similar analysis was attempted with the St. Margarets Bay growth data, results were inconclusive because of the relatively short deployment time of 14 months. However, the initial estimates suggested a growth time to harvest size of 4 to 5 years. While Bedford Basin does not have commercially acceptable water for shellfish cultivation, St. Margarets Bay does, and this preliminary experiment showed promise of supporting clam growth to commercial size well below three of the fastest times reported for natural intertidal growth (Fig. 2).

The reduction by roughly half in the time to reach market size by the clams grown submerged versus those grown intertidally demonstrates the aquaculture potential of this species and provides a firm biological basis for further development and experimentation. For example, the use of hatchery produced spat will eventually enable early spring outplanting and ensure maximum growth in the first year. Initial experiments in hatchery rearing of *Mya arenaria* are currently taking place in Nova Scotia and field trials to test support systems, predation control, comparative stock production, and natural spat collection began in 1995. There is some urgency in this as one long-term clam lease has been approved in the province and two experimental ones have been requested. It is hoped that these investigations will quickly lead to the development of an economical method for holding clams subtidally and eventually to a more predictable and controlled harvest through expanded aquaculture of this valuable shellfish.

The authors gratefully acknowledge the technical assistance of S. MacQuarrie, D. Roddick, and C. Hunter as well as reviews by E. Kenchington and D. Hewitt.

Notes and references

1. Department of Fisheries and Oceans, PO Box 550, Halifax, NS B3J 2S7
2. PO Box 42, Hubbards, NS B0J 1T0
3. Triton Consultants, 221 Crichton Ave., Dartmouth, NS B3A 3R8
4. Hawkins CM. 1985. The soft-shell clam. *Underwater World,* Communications Dir., DFO, Ottawa. 6 pp.
5. Health Canada. 1995. *Canadian Communicable Disease Report* Supplement. Notifiable Diseases Annual Summary, 1993. 21S1:75 pp.
6. Woo P. 1991. *Can. MS. Rep. Fish. Aquat. Sci.* 2090:iv+5 pp.
7. Newcombe CL. 1935. *Can. J. Res.* 13(6):97-137.
8. Witherspoon N. 1982. *N.S. Fish. Manuscr. Tech. Rep.* Ser. 83-01:66 pp.
9. *Maritime Provinces Fishery Regulations,* Feb. 24, 1993: Sect. 43 (softshell clams).

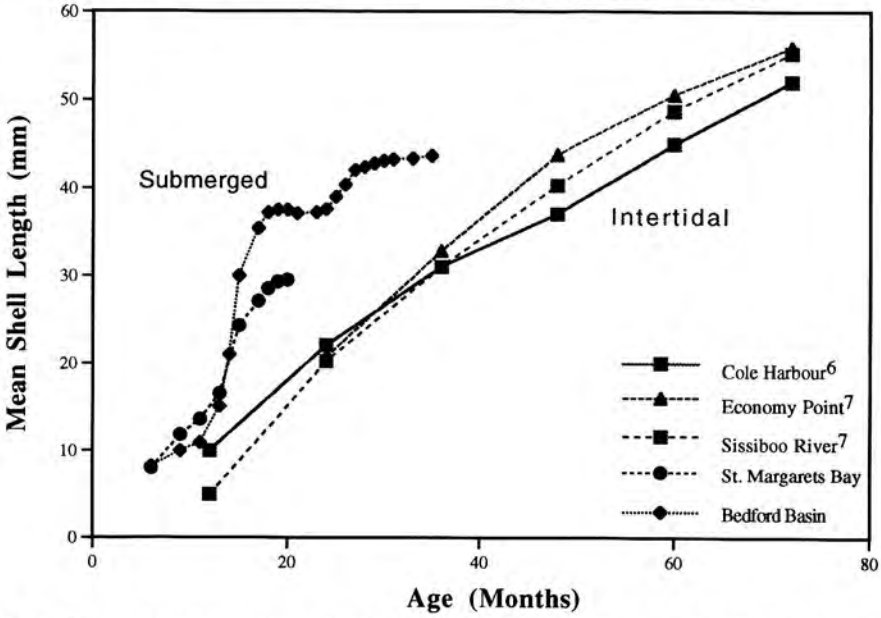


Figure 2. Shell growth comparison of intertidal and submerged softshell clams in Nova Scotia.

Experimental raft culture of the green mussel, *Perna viridis* (L): spat collection, transfer and growth

W. M. Indrasena [1,2] *and T. B. Wanninayake* [2]

The green mussel, *Perna viridis* (L), a commodity in demand among the local and tourist communities in Sri Lanka, occurs only in Trincomalee Bay on the northeastern coast. The consumption of green mussels is therefore limited to neighbouring fishing communities. Coir ropes, polypropylene ropes and barnacle-coated asbestos panels were used to collect spat that were cultured in Trincomalee Bay, the more sheltered Clappenburg Bay, and the high salinity Puttalam lagoon on the northwestern coast. In each location growth was monitored monthly in spat cultured in lantern nets at three depths. Spat collection was a difficult task as suitable collectors must be set on rafts at the time of onset of settlement. Spat grown at the mid-water level grew faster than those at the surface and bottom, reaching a length of 60 mm in 5 months.

Introduction

The most common species of edible mussels in Sri Lanka are the brown-lipped mussel, *Perna perna,* and the green-lipped mussel, *P. viridis.* Brown-lipped mussels are widely distributed along rocky shores from the western to the southern coast and they have been successfully introduced and cultured in Puttalam lagoon on the west coast.[3] However, of the bivalve species in Sri Lanka, *P. viridis* is in greatest demand from local communities and tourists. This species is found only in Trincomalee Bay on the northeast coast of Sri Lanka so consumption is limited to the neighbouring fishing communities and tourists. During the southwest monsoon season, waters on the northeastern coast are calm and fishermen harvest wild mussels by diving. These mussels are occasionally sold in the nearby villages. Since the supply depends on this natural wild population, the harvesting season is short and production is insufficient to fulfil the requirements of the neighbouring communities. The research reported in this study was carried out with the objective of increasing mussel production by collecting spat and transferring them to suitable areas for grow-out.

Materials and methods

Spat collection

Preliminary studies on spat collection were done in Thambalagam Bay on the northeastern coast of Sri Lanka using floating rafts constructed of empty oil drums coated with anticorrosive paints and fixed to a light wooden frame (4 m x 4 m). The rafts were anchored in the deep water of the bay where heavy spat settlement occurs naturally. Three different types of collectors were used to collect mussel spat: coir ropes (5 cm diameter) were used as 15 cm long pieces (6 to 10 pieces per rope) attached to a strong polypropylene rope at 15 cm intervals, uncoiled polypropylene rope pieces were used as brushes, and barnacle coated asbestos panels as strings. The collectors were checked for spat settlement, spat were counted, and collectors were replaced every fortnight.

Growth

Spat collected from Thambalagam Bay were grown in trays similar to Japanese lantern nets either in the bay itself or in the more sheltered Clappenburg Bay in Trincomalee inner harbour. One hundred spat were grown in each tray in

triplicate at three different depths: surface (just below the water level), mid-water (about 1 m below the surface tray), and near the bottom (about 1 m below the mid-water). Later, some spat were introduced into Puttalam lagoon in the northwestern coast, where the salinity fluctuates annually around a mean of 40 ppt. The growing mussels were cleaned and measured monthly. Linear growth curves were statistically compared with analysis of covariance via multiple regression using indicator variables.

Hydrobiological Data

Seasonal varability of salinity, temperature, and secchi disc visibility was determined from fortnightly samples. In addition, a quantitative and qualitative analysis of bivalve larvae, zooplankton, and phytoplankton in the bay was done. The condition index of mussels in the wild stock was studied monthly as a ratio of cooked-dried meat weight to internal shell volume.

Results and discussion

The seasonal settlement of green mussel spat on different substrata showed at least two major peaks per year, one from April to June and the other from November to January (Fig. 1). The peak in April was very sharp and clear in the first year of the study. Settlement was not as pronounced in the second year, indicating the pattern of settlement varies between years. During the peak settlement season in Thambalagam Bay, coir ropes had the significantly highest numbers of spat followed by barnacle coated plates/shells and polypropylene ropes, respectively. After the introduction of mussels into Clappenburg Bay, heavy settlement of spat on dead barnacles underneath the floating drums of the rafts and some abandoned shells was also noticed, indicating the possibility of propagating green mussels after the introduction of the spat to suitable sites in the Trincomalee inner harbour. The mussels seem to have a broad spawning period — from April to August — with settlement being varying with the type of cultch. The induction of spawning during this time may be due to the sudden changes in salinity in the bay.

Thambalagam Bay, a part of Koddiyar Bay, is vulnerable to large fresh water influxes from the Mahaweli estuary that reduce the salinity from 33 ppt to 0–5 ppt during the torrential rains of the northeast monsoon season. Even if the condition index of the mussels did not fluctuate very much, the condition of mussels in the wild population was at its maximum just before the dramatic drop in salinity in November, and both surface and bottom salinities of the bay remained around 5 ppt from November to February and increased up to 30 ppt in March. The salinity increased gradually after that. Both surface and bottom water temperature remained at about 30°C (± 2°C) year-round, so temperature probably did not trigger spawning as it does in temperate mussels. Bivalve larvae, containing large numbers of D-shaped and veliger mussel larvae, were also at peak densities from October to December and from April to June.

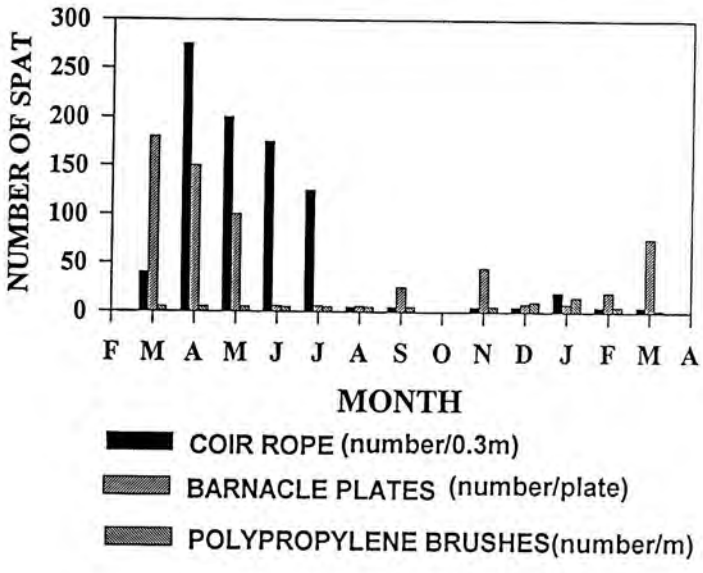


Figure 1. Seasonal variation of the settlement of mussel spat (*Perna viridis*) on coir ropes, barnacle coated asbestos panels, and polypropylene brushes in the Thambalagam Bay of Trincomalee Bay.

The growth of mussels

cultured in Thambalagam Bay, Clappenburg Bay, and in Puttalam lagoon are shown in Figure 2. The mussels cultured at the mid-water level had the highest growth rate in both Thambalagam Bay (Trincomalee Bay) and in Clappenburg Bay in Trincomalee inner harbour. This growth rate was significantly higher than in mussels grown either near the surface or the bottom ($p<0.1$). However, the mussels grown in the mid-waters of both Clappenburg and Thambalagam Bays reached the commercial size of 65 mm in 6 months. There were no significant differences in the growth rates of mussels grown in Thambalagam Bay and Clappenburg Bay at each depth. The mussels transplanted to Puttalam lagoon had poor initial growth, possibly due to severe environmental stress from the high salinity (40 ppt). Further studies are required, however, to conclude whether Puttalam lagoon is suitable for commercial culture of green mussels.

The rapid growth of the mussels introduced to Clappenburg Bay suggests the possibility of transplanting green mussel spat to more sheltered sites of Trincomalee Bay for commercial culture. The mortalities caused by predation could be prevented by transplanting spat initially in small meshed (1/2 cm x 1/2 cm) trays and transferring them to larger mesh trays after approximately two months. The trays were fouled mainly by barnacles that must be removed at least monthly. Hydroid coelenterate colonies and sponges were destroyed by exposing them to the hot sun for half-hour intervals followed by dipping in fresh water for about 5 minutes after every exposure to the air.

In conclusion, mussel spat can be collected using coir ropes and barnacle coated plates and they can be introduced to more sheltered waters where the salinity fluctuates around 33 ppt yearround. The sudden decrease in the condition index of green mussels in the wild can be used to forecast peak spawning seasons. This will allow for the increased deployment of suitable collectors for large scale commercial culture. The most suitable depth for growing mussels appears to be about 2 m below the surface of the water, as the highest growth rates were obtained at this level.

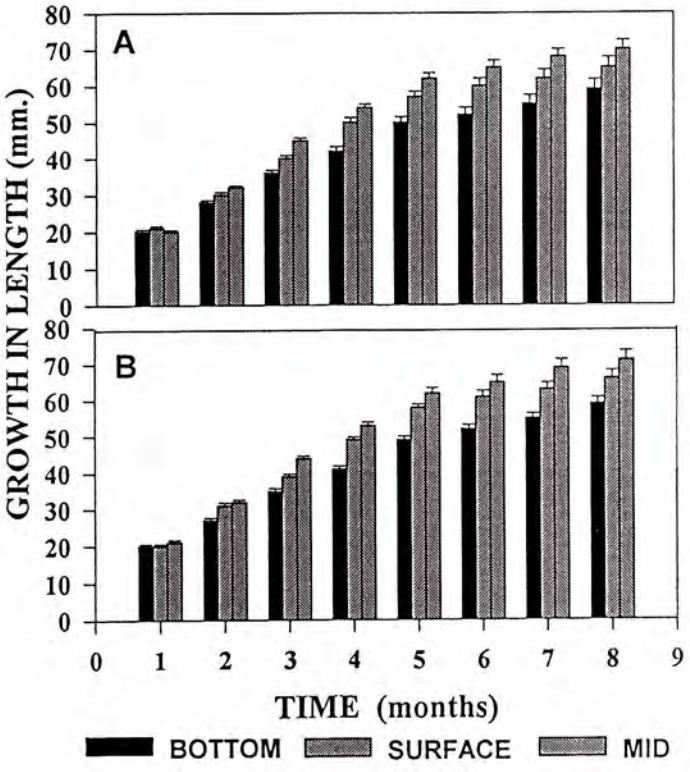


Figure 2. Growth of green mussel, *Perna viridis*, cultured in Thambalagam Bay (A) and Clappenburg Bay (B) at surface (n=100), mid (n=100), and bottom water (n=100) levels.

Financial support for this project by the IDRC is gratefully acknowledged.

Notes and references

1. Dept. Food Science &Technology, Technical University of Nova Scotia, PO Box 1000, Halifax, NS B3J 2X4
2. NARA, Crow Island, Mattakkuliya, Colombo 15, Sri Lanka
3. Indrasena WM, Wanninayake TB. 1994. *Bull. Aquacul. Assoc. Canada* 94-2:33-35.

Introduction of oyster stick culture to the intertidal waters of Sri Lanka

W. M. Indrasena,[1,2] *T. B. Wanninayake*[2] *and U. E. F. Fernando*[2]

There is considerable potential for growing oysters in the tropical waters of Sri Lanka. Raft culture of oysters has been successful in the deep waters of Trincomalee Bay, but an alternative culture method is required for the vast intertidal areas of bays and lagoons. In this study, oyster spat were collected from the deep water of Snug Cove in Trincomalee Inner Harbor, using old oyster shells on floating rafts. Spat collected were then grown in the intertidal region of Snug Cove on about 1700 hardwood stakes driven into the mud (exposing about 30 cm of the stake). Oyster shells with attached spat were affixed to some of the stakes, while other stakes carried just oyster shells. The growth of spat on randomly selected stakes was monitored monthly as were the presence of fouling organisms, pests, and predators. Relevant hydrobiological data were also collected. High numbers of spat were collected and about 30 to 40 oysters were grown on each stake. Oysters had an average length increase of 0.6 to 0.8 mm per month during the first three months, but then the growth rate gradually declined. Predation by gastropod drillers and fouling by unwanted oyster species were common.

Introduction

The culture of edible oysters in Sri Lanka has not reached commercial levels due to insufficient natural production and low demand from local communities. However, the exploitation of wild oysters is increasing dramatically because of demand from the rapidly developing tourist industry. Picking oysters from the rocky shores in the neighbourhood of tourist areas is a common activity for the local people, resulting in the overexploitation of some species, particularly during the peak tourist seasons. In addition, local fishing communities have responded to the demand from the rapidly increasing export market for these shellfish.

A variety of methods have been used on an experimental scale to grow oysters in both intertidal and deep waters of Sri Lanka. Studies on the settlement of oyster spat and growth of *Crassostrea madrasensis* and *C. belcheri* using racks in the intertidal waters of Trincomalee Bay on the eastern coast and floating rafts in the deep waters, indicated that the oysters in these warm waters spawn year round and there is heavy spat settlement in some sheltered coves.[3] The oysters have an average growth rate of 1 cm per month during the first six months, reaching the commercial size of 10 to 12 cm in one year. *C. madrasensis* cultured in the estuarine waters of the northwestern coast as well as in the high salinity of Puttalam lagoon have similar growth patterns.[4] The growth of wild *Saccostrea cucullata* (Born) and *S. commersalis* on the rocky shores of the western and southern coasts of Sri Lanka is significantly lower than that of *C. madrasensis* and *C. belcheri.*

Raft culture has been shown to be an ideal method for culturing oysters, but it is expensive and rafts can be deployed only in deep waters. Racks are relatively inexpensive and are ideal for shallower areas providing they remain submerged and that maximum tidal amplitude does not exceed 1 meter. In this study, stick culture was examined as an alternative method for the

intertidal culture of oysters, especially in areas completely exposed during low tide.

Materials and Methods

Spat settlement was monitored fortnightly in the deep waters (9-10 m) of Trincomalee Bay using 10 cm x 10 cm asbestos panels on a floating raft (5 m x 5 m). In the intertidal region, spat settlement was studied using bamboo splits as well as old oyster and clam shells on stakes. The shells, with and without spat, were individually fixed to the hardwood stakes (46 x 2.5 x 2.5 cm) and the stakes were driven into the mud, exposing about 30 cm of the stake. About 1700 stakes were spaced at approximately 61-cm intervals covering an area of 49 m x 12 m of the intertidal region of Snug Cove in Trincomalee Inner Harbor. Growth of spat on the shells in the intertidal region was studied monthly. The condition index of oysters collected from the wild stock was measured monthly and hydrobiological parameters such as salinity, temperature, qualitative and quantitative analysis of phytoplankton, zooplankton, and bivalve larvae, and secchi depth visibility were monitored bi-weekly.

Results and discussion

The studies on the settlement of various species of oyster spat in Trincomalee Bay was previously reported.[3] There was considerable settlement of oyster spat in Snug Cove year round with peaks occurring from May to July and December to January (Fig. 1). In the deep waters, the highest spat settlement occurred during January with an average of 548 spat per plate being collected. Settlement declined rapidly thereafter. A similar pattern of settlement was observed on bamboo splits and sticks in the intertidal region. However, settlement on the bamboo splits are indicative only of the peak settling season as it is obvious that the veliger larvae do not regard the bamboo as being the same cultch substratum as old oyster shells. Because of this, stakes with shells can be introduced in large numbers during this time in the intertidal region and spat collected on oyster shell in the deep water can also be cultured on stakes in the intertidal region. The common species of oysters settling in this area were *S. commercialis*, *S. cucullata*, *C. madrasensis*, and *C. belcheri*. The early stages of spat of both species of *Crassostrea* and *Saccostrea* are extremely difficult to identify and separate until they reached a size of at least 1-2 cm in length.

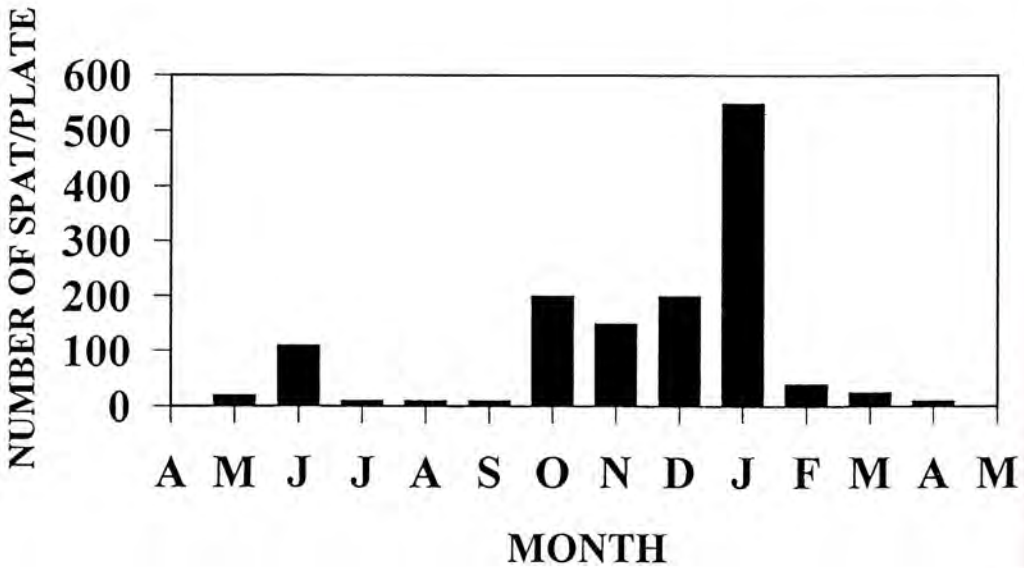


Figure 1. Seasonal variation of oyster spat settlement in the sub-tidal waters of Snug Cove, Trincomalee Inner Harbour.

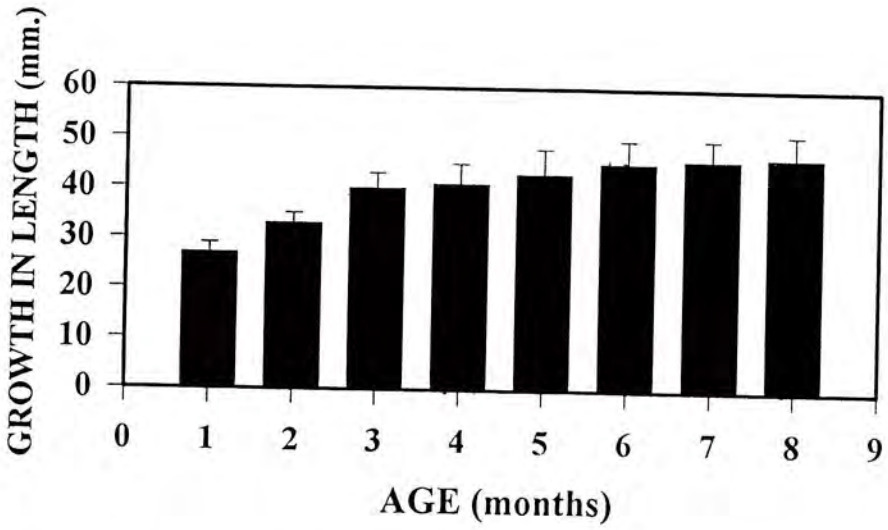


Figure 2. Growth of *Saccostrea cucullata* settled on old oyster shells and held on stakes in the intertidal region of Snug Cove.

However, *S. cucullata* was the most common type of oyster settling on the stakes.

The growth of *S. cucullata* cultured on stakes in the intertidal region of Snug Cove is shown in Figure 2. *S. cucullata* grew rapidly in the first three months. Growth subsequently declined and after four months the growth rate was slow, possibly due to a density effect. The oysters reached a mean length of 48 mm after eight months. Previous studies of *C. madrasensis* and *C. belcheri* grown in the intertidal area of a different part of Trincomalee Bay indicated that these species reached a marketable size of 11 cm in 12 months. In comparison, *S. cucullata* had a significantly slower growth rate in this study. However, oysters growing in the intertidal region face severe environmental conditions because they are exposed to the hot sun for several hours every day during low tide. Not only are the oysters stressed by the increase in temperature but they are also without food for long periods of time. Recently settled spat were also vulnerable to desiccation during low tide and to predation by crabs and drillers. However, once oysters reach a size of 2 to 4 cm, the shells were hard enough to tolerate high temperatures, as well as predation to a certain extent, and about 30 to 45 oysters grew well on each stake. The shells grew longitudinally as there was not enough space to increase in width due to crowding. Also, the oysters grew upwards increasing in height and internal shell volume, resulting in higher meat contents. However, rapidly growing commercial species such as *C. madrasensis* and *C. belcheri* can be collected from other areas of Trincomalee Bay and transplanted to the intertidal regions using the present stick culture technique for commercial culture.

In conclusion, stick culture was an ideal method for the vast intertidal areas of the enclosed bays, coves, lagoons and at least 30 to 45 oysters per stake could be grown yielding 51,000 to 76,000 oysters from an area as small as 6,400 square feet. Introduction of spat of *C. madrasensis* and *C. belcheri* could be better than *S. commercialis* and *S. cucullata* for commercial purposes as far as growth is concerned.

Financial support for this project by the IDRC is gratefully acknowledged.

Notes and References

1. Department of Food Science and Technology, Technical University of Nova Scotia, Halifax NS B3J 2X4
2. NARA, Crow Island, Mattakkuliya, Colombo 15, Sri Lanka

3. Indrasena WM, Wanninayake TB. 1986. *Proc. First Asian Fish. Forum,* p. 56-61. ICLARM, Manila, Philippines.
4. Indrasena WM, Wanninayaka TB. 1994. *Bull. Aquacul. Assoc. Canada* 94-2:30-32.

Comparative collection of scallop spat using different types of artificial substrate

G. Jay Parsons,[1,2] *Shawn M. C. Robinson,*[1]
Jim D. Martin[1] *and Ross A. Chandler*[1]

One of the major impediments to the growth of the giant sea scallop aquaculture industry in Atlantic Canada is the shortage of spat supply. Key components of successful collection of wild spat are: knowledge of the distribution and abundance of larvae, and development of methods that maximize collection of settling spat. The objective of this study was to evaluate the efficacy of three artificial substrates used at different quantities in artificial collectors. Monofilament gill netting at the highest quantity used (4.1 m^2) realized the greatest spatfall. However, the optimum substrate type and quantity depends on a number of factors including cost and durability of substrate material, degree of fouling, labour involved in handling and sorting spat, and settlement, growth, and survival of spat.

Introduction

Giant sea scallop, *Placopecten magellanicus*, culture is an expanding commercial industry in Atlantic Canada with considerable potential.[3] The biological feasibility of producing market-sized scallops within two to three years has been demonstrated[3,4] but early reports of the economic feasibility were not favourable, partly because of the costs associated with the collection of wild spat.[5]

One of the major impediments to the growth of the industry in Atlantic Canada is the shortage of spat.[6] Spat for scallop culture can come from either hatcheries or the wild, but aquaculturists currently rely on wild spat obtained using artificial collectors consisting of "onion" bags filled with monofilament gill netting or other materials. There is a high demand for scallop spat, so it is essential that the collection of wild spat be maximized.

The factors that influence spat settlement on artificial collectors have been widely studied and include timing, depth, and location of collector deployment; type, quantity, and thickness of substrate material; and presence of biofilm and other biological cues.[3,7,8]

The overall aim of this project was to improve the economic feasibility of scallop aquaculture by examining ways of maximizing wild spat collection. The objective was to evaluate the efficacy of artificial substrate materials and quantities on giant sea scallop settlement.

Materials and methods

The study was conducted in Passamaquoddy Bay, New Brunswick, on a scallop aquaculture site located just north of Tongue Shoal. This area has been monitored regularly over the last eight years for larval settlement and is known to have a consistent supply of larvae and spat.[3]

Scallop settlement was evaluated using standard "onion" bag collectors. Three mesh substrates (monofilament gill netting, Netron™, and Dupont "blue") each at six quantities (calculated as surface area — 0.5, 1.0, 2.1, 2.6, 3.1, and 4.1 m^2) were compared. Monofilament gill netting is commonly used for spat collection in Canada and can be obtained commercially or from the fishery. The second material, Netron™, imported from Japan through a North American distributor, has not been widely used for giant sea scallop spat collection, but has been used elsewhere. The final material, Dupont

"blue", is a new product for scallop aquaculture and was produced in Canada.

Each treatment had three replicates. Spat bags were deployed on 2 September 1994 at the beginning of the spat settlement season and were retrieved between 14-24 November at the end of the season. Spat bags were suspended three meters off the bottom by SCUBA divers. At retreival, divers placed individual spat bags into plankton nets. The bags were washed and spat were sorted, identified, and enumerated. A subsample of up to 100 spat from each sample was measured.

Results

Giant scallop spat settled on all the collectors. There were, however, significant differences in spat settlement among both the type and quantity of substrate material. Collectors containing monofilament gill netting had significantly greater numbers of spat (mean of 570 spat per bag) than those containing Dupont "blue" (mean of 410 spat per bag) or Netron™ (mean of 406 spat per bag) (Table 1). There was no significant difference between the numbers of spat settled on the Netron™ and Dupont "blue" substrates. There was a significant difference among the various quantitities of substrate material (ANOVA, $p<0.05$) (Table 1). Generally, collectors with more mesh had greater numbers of spat.

Even though the spat collectors were only deployed for a few weeks, there were significant differences in the size of spat (shell height) in collectors with different quantities of substrate. There was no significant difference in spat size among the three substrate types (ANOVA, $p< 0.05$) (Fig. 1). Generally, spat were smaller in the collectors with the greatest amount of substrate material.

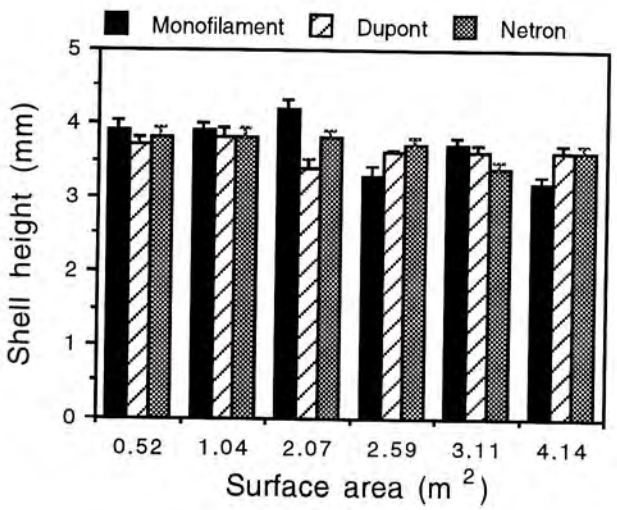


Figure 1: Mean shell heights of giant scallop spat grown on different substrate types and quantities off Tongue Shoal.

Table 1. Mean giant sea scallop spat (number per onion bag) collected on different substrate types and quantities off Tongue Shoal.

Substrate Area (m^2)	Number of spat per onion bag		
	Monofilament gill netting	Dupont "blue"	Netron™
0.52	290.7	215.3	206.6
1.04	424.7	319.7	329.0
2.07	640.7	398.3	370.0
2.59	630.3	431.0	432.7
3.11	664.7	484.0	470.7
4.14	769.3	571.7	552.7
Mean	570.1	410.2	406.3

Discussion

The results in this spat settlement study contrast with those obtained by Naidu et al. [7] who found that spat settlement was reduced at higher quantities of monofilament substrate. Naidu et al.[7], however, left their spat collectors out for a period of one year. It is possible that spat mortality was related to substrate quantity and that onion bags with greater amounts of monofilament had reduced rates of water flow (hence reduced food availability). Also, compaction of the netting in the collectors with the highest quantity of gill net may have led to higher mortalities.

Our results demonstrated that monofilament gill netting at the highest quantity used (4.14 m^2) realized the greatest spatfall. However, the optimum substrate type and quantity for scallop aquaculture depends not only on the efficacy of settlement, but also on cost, labour for handling and sorting spat, substrate durability, growth and survival of spat, and fouling. Monofilament gill netting can be readily obtained from the fishery (or at least it once was). However, there are costs associated with acquiring and transporting the monofilament. Netron™ has to be imported and Dupont "blue" is a new product being produced in Canada. Netron™ has about 1.9 times more surface area for an equivalent length than Dupont, so this needs to be considered if Dupont "blue" is bought by length. Labour to pack the onion bags should not be much different among substrate types. However, Dupont "blue" comes in a tightly wound strand and has to be stretched apart before it is stuffed into the spat bags. This extra labour could add costs to the initial use of Dupont "blue". There are also some indications that labour involved with the sorting of spat from the monofilament is higher than that with Netron™ and Dupont "blue". [8,9]

Monofilament gill netting does not maintain its shape in the spat bag and thus could limit spat survival and growth. Also, monofilament can be difficult to handle when sorting spat from the collectors. Netron™ has not been widely used for giant scallop spat collection, but preliminary trials suggest that it has "memory" and maintains its shape in the spat collector. Dupont "blue" is a similar type of mesh and will also maintain its shape. Because it is produced in Canada, it will possibly be less expensive than Netron™.

There were no differences in the growth of spat with the different substrate types. However, the study was only conducted during the settlement period and there were differences in spat growth among bags containing different quantities of substrate. This suggests that even though spat settlement was highest in the bags with the greatest amount of substrate material, growth and possibly survival may be compromised.

Several other factors, which may have an impact on the cost benefit analysis of the various substrates, were not considered in this study. These include differences in levels of fouling among substrates. Again, there is some evidence that the monofilament may attract a greater number of fouling organisms compared to Netron.™ [9] The long-term durability of the different materials also needs to be considered. If, of course, any of the above factors change over time (e.g., cost of materials), the scallop aquaculturist would have to re-evaluate the performance to determine which is the best material for their particular circumstances.

We thank S. Ford, K. Longmire, J. Baltzer, D. Robichaud, W. Huber, Capt. W. Minor, Dr. P. Dabinett, Hillsburn Basin Scallop Group Ltd., A. Jeffs of Dupont Canada, Great Maritime Scallop Trading Co. Ltd., and Atlantic Fisheries Adjustment Program of Department of Fisheries & Oceans, Scotia-Fundy.

Notes and references

1. Department of Fisheries and Oceans, Biological Station, St. Andrews, NB E0G 2X0
2. Aquaculture Unit, Marine Institute of Memorial University, PO Box 4920, St. John's, NF A1C 5R3
3. Dadswell MJ, Parsons GJ. 1991. In, *An International Compendium of Scallop Biology and Culture* (SE Shumway, PA Sandifer, eds.), p. 300-306. World Aquaculture Society, Baton Rouge, LA.
4. Dadswell MJ, Parsons GJ. 1992. *J. Shellfish. Res.* 11:299-305.
5. Wildish DJ, Wilson AJ, Young-Lai W, DeCoste AM, Aiken DE, Martin JD. 1988. *Can. Tech. Rep. Fish. Aquat. Sci.* 1658:iii+21 pp.
6. Dabinett P, Couturier C. 1994. *Bull. Aquacul. Assoc. Canada* 94-3:8-11.
7. Naidu KS, Cahill FM, Lewis DB. 1981. *J. World Mariculture Soc.* 12:165-171.
8. Parsons, J. Unpublished data.
9. Vigneau S., L'APPIM, QC. Personal communication.

Physiological effects and retention of domoic acid in the oyster, *Crassostrea gigas*, fed the toxic alga *Pseudonitzschia pungens* f. *multiseries*

J. N. C. Whyte, T. O. Jones, N. G. Ginther and L. D. Townsend[1]

Crassostrea gigas exposed continuously to domoic acid from *Pseudonitzschia pungens* f. *multiseries* displayed a stress response characterized by haemolymph acidosis, hypoxia and hypercapnia resulting from shell closure caused by high accumulation of toxin. A marked increase in number and activity of haemocytes also resulted from exposure to the alga. When exposed to pulsed feeding, toxin retention reflected availability of the toxin and changes to uptake-release equilibrium in the tissue rather than feeding inhibition. Clearance of toxin from the oyster tissue was complete in 54 h.

Introduction

In 1987 domoic acid in *Pseudonitzschia pungens* f. *multiseries* was identified as the neurotoxin responsible for food poisoning associated with consumption of cultured mussels.[2] The toxin was first recorded in British Columbia on the west coast of Vancouver Island in 1992.[3] Bivalve fisheries were closed when the sentinel species, *Mytilus californianus*, contained 98 µg/g toxin, a level well above the allowable 20 µg/g. Physiological effects of accumulated domoic acid in invertebrates have received limited attention. The Pacific oyster, *Crassostrea gigas*, has adapted successfully to an ecological niche with extremes of temperature, salinity, and exposure to air. These environmental stressors cause marked changes in the chemistry and phagocytic activity of oyster haemolymph.[4] Stress responses in the oyster caused by assimilated domoic acid and tissue retention of the toxin are presented in this report.

Materials and methods

P. pungens f. *multiseries* was grown in 500-L plastic bags (17 ± 0.5°C) and harvested at early stationary phase.[5] Oysters, shell height 13.0 ± 0.8 cm, were acclimated to experimental water temperatures and fed *Thalassiosira pseudonana* for 2 wk prior to experimentation. In the first experiment, oysters in aerated flow-through aquaria (16.0 ± 0.8°C) were exposed to 335×10^3 cells/mL (1.8 pg/cell) at a continuous flow of 50 mL/min for 48 h. Haemolymph was sampled (n=10) at 0, 4, 8, 36, and 48 h and PO2, PCO2, and pH determined.[6] In the second experiment, oysters held in flow-through aquaria (21.0 ± 0.8°C) were exposed to 335×10^3 cells/mL (1.6 pg/cell) at a continuous flow of 50 mL/min for 48 h. Haemolymph samples (n=10) taken at 0, 4, 8, 12, 24, and 48 h were analyzed for cell count and chemiluminescence (CL) activity.[7] In the third experiment, oysters held in 3 upwellers linked to a header-tank with recirculating water were fed algae (180×10^3 cells/mL) every 6 h for a total of 120 h. Toxin clearance with continuous flushing of filtered seawater lasted 120 h. Oysters (n=10) collected at intervals during all three experiments were analyzed for toxin.[8]

Results

Body burden of domoic acid in oysters exposed continuously to the alga increased to 36.3 µg/g after 4 h, declined to 29.1 µg/g on a further 4 h of exposure, and by 36 h was 9.9 µg/g. The data regression curve, $r^2=0.70$, indicated a maximum of 42.1 µg/g after 14 h (Fig. 1a). Haemolymph PO2 in oysters declined steadily from a maximum of 85.1 mmHg at 0 h to 50.1 mmHg after 8 h exposure and continued to

decline to a minimum of 35.2 mmHg at 48 h (Fig. 2a). Haemolymph PCO2 increased significantly from 1.52 mm Hg at 0 h to 11.50 mmHg after 8 h exposure, then declined to 3.84 mmHg by 36 h (Fig. 2a). During the same period haemolymph pH declined from 7.35 at 0 h to a minimum of 6.79 at 8 h, but recovered to 7.20 and 7.19 after 36 and 48 h exposure.

Oysters exposed to algae continuously at 21°C increased in domoic acid from 0 to a near maximum of 0.73 µg/g after 8 h (Fig. 1b). The regression curve, r^2 = 0.86, indicated a slow assimilation in toxin to 48 h (Fig. 1b). Haemocyte number and haemocyte CL increased significantly within the first 4 h of exposure to a maximum of 5.12×10^6 cells/mL and 4.25×10^6 counts per minute (cpm), respectively (Fig. 2b).

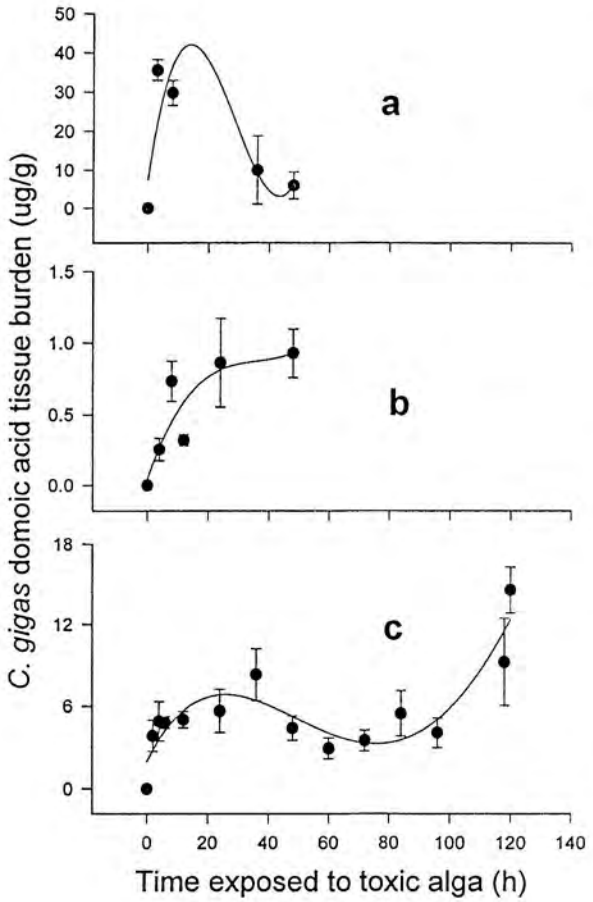


Figure 1. Mean (± 1 SE) domoic acid levels in *C. gigas* exposed to *P. pungens* f. *multiseries* continuously at 16°C (a), continuously at 21°C (b), and in a pulsed manner at 16°C (c).

Haemocyte CL declined to control levels (0 h) 1.88×10^6 cpm after 12 h of exposure, but cell number remained significantly elevated after 48 h. Pulsed feeding of oysters provided the toxin retention curve, $r^2 = 0.86$, represented by Fig. 1c. All algae were cleared from the header tank within 2 h of addition. Algae fed for 108 h contained 2.1 pg/cell and for the remaining time 6.7 pg/cell. Toxin accumulation peaked at 6.9 µg/g after 25 h, declined to 3.3 µg/g after 77 h, then increased again to 14.4 µg/g after 120 h. Rate of toxin uptake was 0.44 and 0.51 µg/g/h for the first and last hour of exposure. Clearance of toxin from the oyster was best represented by a third order regression curve, $r^2 = 0.90$ (Fig. 2c). Loss of domoic acid was 0.65 µg/g/h during the first 6 h of clearance. After 24 and 48 h the clearance rates were 0.25 and 0.08 µg/g/h, respectively. Further toxin depletion resulted in no detectable domoic acid in the tissue after 54 h when the rate was 0.01 µg/g/h. No mortalities occurred during intoxication or detoxication.

Discussion

Within 4 h of continuous exposure to *P. pungens* f. *multiseries*, shells of the oyster closed tightly in a typical stress response. During this period the body burden of toxin had reached 26 µg/g. Shell closure caused a decrease in haemolymph PO2 and pH, and an increase in PCO2 from impeded gas exchange at the gill surface. Decline in haemolymph PO2 has been observed on shell closure of the clam *Mya mercenaria* when subjected to salinity stress.[9] Increased acidity is a function of metabolic conversion of sugar to succinic acid under anaerobic conditions.[9] Compensation for the acidosis after 8 h exposure was achieved by increased bicarbonate buffering, providing increased PCO_2, from dissolution of calcium carbonate in the shell matrix.[10] Observed changes in haemolymph pH and gases confirmed the stress response in the oyster from high levels of accumulated domoic acid.

Toxin in the oysters exposed to algae at 21°C peaked at only 0.86 ± 0.31 µg/g after 48 h exposure and no shell closure was evident. This low assimilation re-

sulted from the expected leaching of toxin from the stationary phase algal cells on transfer from 17 to 21°C water. Even at these low toxin levels the increases in circulating haemocytes and phagocytic activity indicated an immune response in the oyster. Similar responses in oysters, *C. gigas*, *C. virginica*, and *Ostrea edulis*, have resulted from other stressors such as changes in salinity, temperature, and exposure to air.[4]

To simulate variable cell concentrations that oysters are exposed to from blooms subjected to currents and tides in the nearshore, a pulsed rather than a continuous feeding regime was used. Toxin levels were higher in oysters on continuous exposure, in agreement with results from feeding trials with *Mytilus edulis* and *P. pungens* f. *multiseries* that indicated constant feeding provided higher values for domoic acid uptake.[11] Pulse fed oysters displayed an initial peak uptake of toxin that was later reduced as the rate of clearance became faster than uptake. As the oyster shells remained open during the experiment, a change in the retention equilibrium of the water soluble toxin rather than feeding inhibition was implied. By contrast, toxin retention in *Mytilus californianus* indicated a constant accumulation of domoic acid with extended pulse feeding.[12] Toxin accumulation increased immediately when an algal batch higher in cellular toxin was supplied. Degree of intoxication was therefore a function of toxin availability either through cell concentration or cellular toxin levels. Data also suggested that a threshold toxin level for feeding inhibition existed between 10 and 30 µg/g of intoxication. Loss of 71.9% toxin on 24 h clearance suggested that the oyster can clear domoic acid significantly faster than the 17 and 27% losses observed for the mussels, *M. edulis* and *M. californianus*.[11,12]

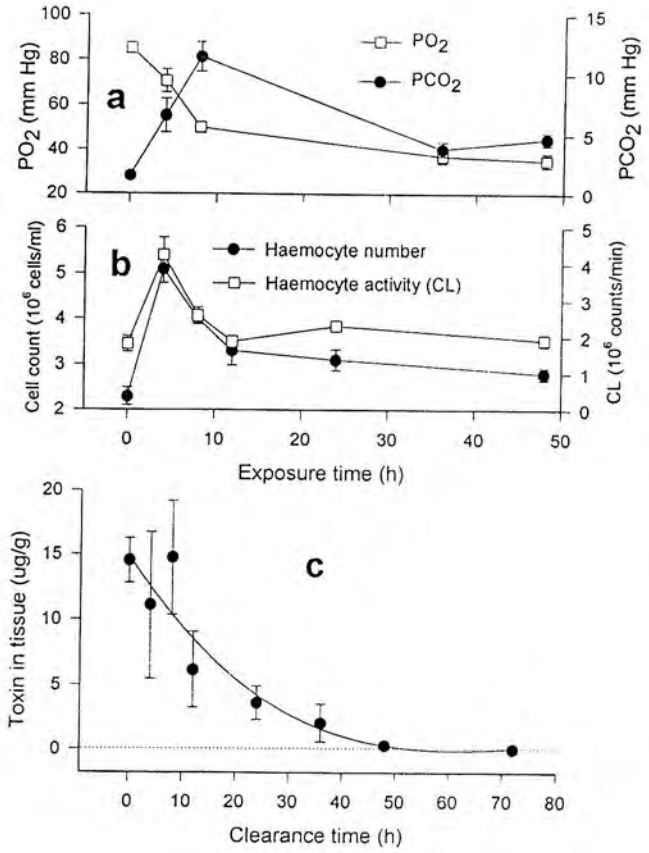


Figure 2. Mean (± 1 SE) PO_2 and PCO_2 (a), haemocyte number and haemocyte activity (CL) (b) in haemolymph of *C. gigas* exposed to *P. pungens* f. *multiseries*, and domoic acid levels in oyster tissue with time of clearance (c)

Notes and references

1. Fisheries and Oceans Canada, Pacific Biological Station, 3190 Hammond Bay Road, Nanaimo, BC V9R 5K6
2. Todd ECD. 1993. *J. Food Prot.* 56:69-83.
3. DFO Inspection Branch. 1992. *Summary of Marine Toxin Records in the Pacific Region,* 25 pp.
4. Fisher WS, Auffret M, Balouet G. 1987. *Aquaculture* 67:179-190.
5. Whyte JNC, Ginther NG, Townsend LD. 1995. *J. Appl. Phycol.* 7:199-205.
6. Cameron JN. 1971. *J. Appl. Physiol.* 31: 632-634.
7. Bachère E, Hervio D, Miahle E. 1992. J. *Dis. Aquat. Organ.* 11:173-180.
8. Quilliam MA, Xie M, Hardstaff WR. 1991. *Instit. Mar. Biosci., Natl. Res. Counc. Can., Tech. Rep.* No. 64, 52 pp.
9. Crenshaw MA, Neff JM. 1969. *Am. Zool.* 9:881-885.
10. Truchot JP. 1988. *Amer. Zool.* 28: 55-64.
11. Wohlgeschaffen GD, Mann KH, Subba Rao DV, Pocklington R. 1992. *J. Appl. Phycol.* 4:297-310.
12. Whyte JNC, Ginther NG, Townsend LD. *1995. In,* Harmful Marine Algal Blooms (P Lassus, G Arzul, E Erard, P Gentian, C Marcaillou eds), p. 531-537. Lavoisier Publishing, Paris.

Aquaculture Association of Canada
Charter by Letters Patent

WHEREAS an application has been filed to incorporate a corporation under the name

THE AQUACULTURE ASSOCIATION OF CANADA
L'ASSOCIATION AQUACOLE DU CANADA

THEREFORE the Minister of Consumer and Corporate Affairs by virtue of the powers vested in her by the Canada Corporations Act, constitutes the applicants and such persons as may hereafter become members in the corporation hereby created, a body corporate and politic in accordance with the provisions of the said Act. A copy of the said application is attached hereto and forms part thereof.

TO: THE MINISTER OF CONSUMER AND CORPORATE AFFAIRS OF CANADA

-I-

The undersigned hereby apply to the Minister of Consumer and Corporate Affairs for the grant of a Charter by Letters Patent under the provisions of Part II of the Canada Corporations Act constituting the undersigned, and such others as may become members of the Corporation thereby created, a body corporate and politic under the name of

THE AQUACULTURE ASSOCIATION OF CANADA
L'ASSOCIATION AQUACOLE DU CANADA

The undersigned have satisfied themselves and are assured that the proposed name under which the incorporation is sought is not the same or similar to the name under which any other company, society, association or firm, in existence is carrying on business in Canada or is incorporated under the laws of Canada or any province thereof or so nearly resembles the same as to be calculated to deceive and that it is not a name which is otherwise on public grounds objectionable.

-II-

The applicants are individuals of the full age of twenty-one (21) years with power under the law to contract. The name, the place of residence and the calling of each of the applicants is as follows:

Charles B. Schom, Associate Professor, 116 Elizabeth Avenue, St. Andrews, N.B.
David E. Aiken, Research Scientist, Windridge, St. Andrews, N.B.
John M. Anderson, Executive, RR#1 Lower Bayside, St. Andrews, N.B.
Joël de la Noüe, Director, 1082 Dijon, Ste. Foy, Quebec
Terence R. Jackson, Executive, Island View Drive, R.R.#3, Manotick, Ontario

The applicants shall be the first directors of the Corporation.

-III-

The objects of the Corporation are:

(a) To foster an aquaculture industry in Canada, to promote the study of aquaculture and related science in Canada, to gather and disseminate information relating to aquaculture, and to create public awareness and understanding of aquaculture (it being intended that the term "aquaculture" used herein shall be interpreted as encompassing all aspects of marine, brackish and freshwater plant and animal husbandry);

(b) To promote, support and encourage the educational, scientific, and technological development and advancement of aquaculture in Canada;

(c) To gather and disseminate technical and scientific information on aquaculture development in Canada and throughout the world;

(d) To conduct seminars for the presentation, exchange and discussion of information, findings and experiences on all subjects and techniques related to aquaculture;

(e) To encourage the teaching of all phases of aquaculture and the training of aquaculturists in accredited colleges and universities in the field of aquaculture;

(f) To encourage private industry and government agencies, both provincial and federal, to support education, research and development.

Notwithstanding the foregoing, no substantial part of the activities of the Corporation shall be lobbying, the carrying on of propaganda, or otherwise attempting to influence legislation, and the Corporation shall not participate in or intervene in (including the publishing or distribution of statements) any political campaign on behalf of a candidate for public office. The Corporation shall not carry on any other activities not permissible for a nonprofit organization that is exempt from federal or provincial income tax, or by an association to which contributions are deductible under the appropriate statutes. Notwithstanding any other provision of these Articles, this Association shall not, except to an insubstantial degree, engage in any activities or exercise any powers that are not in furtherance of the purposes of the Corporation.

-IV-

The operations of the Corporation may be carried on throughout Canada and elsewhere.

-V-

The place within Canada where the head office of the Corporation is to be situated is the Town of St. Andrews, in the Province of New Brunswick, Canada.

-VI-

In the event of dissolution or winding-up of the Corporation all its remaining assets after payment of its liabilities shall be distributed to one or more organizations in Canada operated primarily for educational or scientific purposes as shall qualify for exemption from federal and provincial income tax, as the Board of Directors may determine.

-VII-

The Bylaws of the Corporation shall be those filed with the application for letters patent until repealed, amended, altered or added to.

-VIII-

The Corporation is to carry on its operations without pecuniary gain to its members and any profits or other accretions to the Corporation are to be used in promoting its objects except that the Corporation shall be authorized to pay reasonable compensation for services rendered and to make payments and distributions in furtherance of its corporate objects set forth herein.

-IX-

(a) The officers of the Corporation shall be a PRESIDENT, a PRESIDENT ELECT, a VICE PRESIDENT, a SECRETARY, a TREASURER, and such other officers as may be provided in the Bylaws.

(b) The names of the persons who are to serve as officers of the Corporation until the first meeting of the elected Board of Directors are:

OFFICE	NAME
President	John M. Anderson
Vice President	David E. Aiken
*Second Vice President	Joël de la Noüe
Secretary	Charles B. Schom
Treasurer	Terence B. Jackson

*The office of Second Vice President shall be a temporary office, to expire at the time of the first Annual Meeting of the Corporation

(c) The officers shall be elected at the annual meeting of the Board of Directors or as provided in the Bylaws.

Dated at the City of Halifax, in the Province of Nova Scotia, this 26th day of January, 1984.

Bylaw Number One

BEING a Bylaw relating generally to the transaction of business and the affairs of The Aquaculture Association of Canada (hereinafter sometimes referred to as "The Association").

Article One — Name and Purpose

Section 1.01 **Name.** The name of the Association shall be THE AQUACULTURE ASSOCIATION OF CANADA — L'ASSOCIATION AQUACOLE DU CANADA, as described in the Letters Patent.

Section 1.02 **Purpose**. The purposes and activities of the Association shall be as described in the Letters Patent.

Article Two — Membership and Dues

Section 2.01 Eligibility for Membership. Any individual or institution may become a member in good standing of the Association by having a genuine interest in the purposes of the Association, by paying the appropriate dues and by adhering to the rules and regulations laid down in the various sections and subsections the Letters Patent, the Bylaws and the Policy, Rules and Regulations of the Association.

Section 2.02 **Classes of Membership**. There shall be four classes of membership in the Association: (a) Individual; (b) Student; (c) Corporate; and (d) Sustaining.

a) *Individual* membership shall be available to all persons.
b) *Student* membership shall be available to any individual registered as a student at any educational institution recognized as such by the Board of Directors.
c) *Corporate* membership shall be available to any company or corporation. Membership shall be listed in the company or corporate name, and the company or corporation shall designate one person who will exercise the rights and receive the benefits of membership in the Association.
d) *Sustaining* membership shall be available to individuals, companies and agencies who shall be designated Sustaining members. Under a Sustaining membership two individuals may be designated to receive all rights and privileges of full membership in the Association.

Section 2.03 **Rights, Benefits, Privileges.** All rights, benefits and privileges of membership in the Association shall extend equally to all classes of membership except as specifically excluded or abridged by subsections (a) through (d) hereof.

Section 2.04 **Dues**. Dues shall be paid annually by or for all members except those for which dues are specifically exempted by the Bylaws of the Association.

Section 2.05 **Amount of Dues**. The amount of dues to be paid by each class of membership shall be decided by the Board of Directors.

Section 2.06 **Expulsion**. Any member may be expelled from the Association by vote of a majority of the members in attendance at a special or general meeting called for that purpose and for which proper written notice has been given at least 30 days in advance of such meeting.

Section 2.07 **Resignation**. Any member may resign from the Association by giving written notice to that effect to the Board of Directors.

Article Three — Meetings, Voting, Elections

Section 3.01 **Annual Meeting**. The annual meeting of the membership will be held at the time and place set by the Board of Directors of the Association. The Secretary shall serve by mail a written notice thereof not less than 90 days previous to such meeting, to each member at his address as it appears on the records of the

Association. Said notice shall contain a description of any proposed or contemplated amendments to the Letters Patent.

Section 3.02 **Motions Requiring Expenditure of Funds**. Motions that direct or require the expenditure of funds of the Association shall not be accepted from the floor at an annual business meeting of the general membership. Such expenditures shall only be authorized by the Board of Directors after evaluation by the Finance Committee.

Section 3.03 **Eligibility to Vote**. In all matters of business on which the membership is entitled to vote, Individual members, Student members and the designated individuals of both Corporate and Sustaining members shall be eligible to cast one vote.

Section 3.04 **Elections**. Elections shall be held annually for the number of Directors specified in Section 6.02 of this Bylaw . At the Board meeting immediately preceding the annual general meeting the Board of Directors shall elect, from among the directors who will serve following the annual general meeting, a President-Elect, Vice President, Secretary and Treasurer.

Section 3.05 **Voting**. Voting for Directors on the Board shall be by mail. At least three (3) months prior to the next annual meeting a ballot containing a list of approved nominees plus provision for write-in candidates should be mailed to the membership. Election results will be announced by the Secretary at the regular annual business meeting.

Section 3.06 **Quorum**. A quorum for the transaction of business at any meeting of the members shall be ten (10) members.

Section 3.07 **Other Business**. Other business matters may be voted on by the general membership at the annual business meeting or by mail ballot to the membership from the Board of Directors.

Article Four — Officers of the Association

Section 4.01 **Officers of the Association**. The officers of the Association shall be members eligible to hold office and shall be: a) President; b) President Elect; c) Vice President; d) Secretary; and e) Treasurer.

Section 4.02 **Elections**. All officers shall be elected for a period of one (1) year, more or less. The President Elect shall, upon completion of his term, accede to the Presidency. Therefore elections in accordance with Section 3.04 shall be for President Elect, Vice President, Secretary and Treasurer. The President Elect will be installed as President at the close of the regular annual business meeting.

Section 4.03 **Term**. Individuals elected to the office of Vice-President, Secretary or Treasurer may be re-elected to serve two (2) or more consecutive terms.

Section 4.04 **Nominations**. Nominations for office shall be made by an Election Committee as described in Section 7.01(a) of this Bylaw.

Section 4.05 **Vacancies**. In the event that any officer resigns or otherwise leaves a position vacant the Board of Directors shall, by majority vote, appoint a member of the Association to fill the remainder of Director's two-year term and shall elect a Director to fill the remainder of the Officer's term.

Section 4.06 **Removal**. Any officer of the Association may be removed by a vote of at least two-thirds (2/3) of the members of the Board of Directors present at a meeting for which written notice has specified the intent to consider such removal.

Article Five — Duties of Officers

Section 5.01 **President**. The President shall be responsible for the conduct of business and the organization of the Association. He shall preside over all annual, special and Board meetings. He shall make such appointments as are authorized in the Bylaws, and shall exercise such other functions and responsibilities as may be determined from time to time by action of the members of the Association or the Board of Directors of which he is Chairman. The President is authorized to, from time to time, appoint ad hoc committees from the membership of the Association to perform various duties as in the discretion of the President may

seem appropriate. The President shall also serve as ex officio member of all committees.

Section 5.02 **President Elect**. The President Elect shall, at the end of his term, accede to the presidency. During his term as President Elect he shall perform the duties of the President in the absence of the President, he shall serve as Chairman of the Time and Place Committee, and he shall chair or appoint a chairman for the Arrangements Committee.

Section 5.03 **Vice President**. The Vice President shall act in the capacity of President Elect in his absence or, in the absence of both the President and the President Elect, shall perform the duties of the President until a new President Elect has been elected. The Vice President shall also serve as chairman of the Awards Committee.

Section 5.04 **Secretary**. The Secretary shall be responsible for recording the minutes of Association business meetings and meetings of the Board of Directors, for ensuring that membership and mailing lists are maintained for the Association, for ensuring that a current record of policy, operational actions, bylaws and resolutions is maintained, for notifying the Minister of Consumer and Corporate Affairs of any modifications to Association bylaws, for ensuring that all corporation filing requirements are complied with, for ensuring that bylaw changes are communicated to the membership, for certifying documents issued by the Association, and for maintaining custody of the seal of the Association.

Section 5.05 **Treasurer**. The Treasurer shall be responsible for maintaining documented accounts of all receipts, shall arrange for the payment of bills and the receipt of funds, and shall report on the financial status of the Association upon request of the President or the Board of Directors of the Association. At the time of each annual meeting, or as requested by the President, the Treasurer shall provide the Board of Directors with a properly audited and detailed financial report. The Treasurer shall also serve as Chairman of the Finance Committee.

Article Six — Board of Directors

Section 6.01 **Board of Directors**. The Board of Directors shall consist of the President, the immediate Past-President, eight (8) or more directors who are elected from the membership and delegates from affiliated organizations as defined in Article Eight.

Section 6.02 **Term**. Three (3) or more Directors shall be elected annually to the Board of Directors to serve two-year terms.

Section 6.03 **Responsibility**. The Board of Directors shall be responsible for the management of the business affairs of the Association.

Section 6.04 **Quorum**. Business may be conducted at any meeting of the Board of Directors at which the elected members present and eligible to vote constitute a quorum, which for these purposes is a majority of the elected membership of the Board of Directors.

Section 6.05 **Compensation**. No elected Officer, Director or appointed committee member may receive compensation for services rendered to the Association. Certain travel expenses may be defrayed when authorized by the President with the concurrence of the Treasurer, or when authorized by the bylaws of the Association. Clerical and other necessary operating expenses may be paid by the Association when so authorized by the President.

Section 6.06 **Auditors**. The Board of Directors shall be responsible for selecting an auditor to review the financial reports of the Association and to carry out any auditing functions required.

Section 6.07 **Borrowing**. The Directors may from time to time:

a) Borrow money on credit of the Association;
b) Limit or increase the amount to be borrowed;
c) Issue debentures or other securities of the Association;
d) Pledge or sell such debentures or other securities for such sums and

such prices as may be deemed expedient; and

e) Secure any debentures, or other securities, or any other present or future borrowing or liability of the Association, by mortgage, charge or pledge of all or any currently owned or subsequently acquired real and personal, moveable and immoveable property of the Association.

Section 6.08 **Removal**. Any elected Director of the Association may be removed from office by a vote of at least two-thirds (2/3) of the members of the Board of Directors present at a meeting for which written notice has specified the intent to consider such removal.

Section 6.09 **Parliamentary Authority**. The rules contained within The Modern Edition of Robert's Rules of Order shall be employed to conduct business at meetings of the Board of Directors of the Association in all cases where they are not inconsistent with the Bylaw.

Section 6.10 **Board Meetings.** Business may be conducted via teleconference or video conference at any meeting of the Board of Directors called for that purpose and at which a properly constituted quorum is present.

Section 6.11 **Vacancies**. In the event that any Director of the Association resigns or otherwise leaves a position vacant, the Board of Directors may by majority vote, appoint a member of the Association to fill the remainder of the Director's two-year term.

Article Seven — Committees

Section 7.01 **Committees**. All activities and recommendations of standing committees are subject to approval of the Board of Directors. Except as noted below, the President shall annually appoint Association members in good standing to the following standing committees.

a) **Election Committee**. The Election Committee shall consist of the Past President as Chairman, the President Elect, and such other members as may be appointed by the Chairman. The Election Committee shall be responsible for all matters pertaining to the conduct of the annual election, including the slate of nominees, the ballot and the tabulation of votes cast by the membership. The Election Committee shall provide the Board of Directors with two (2) nominees for each elected office at least four (4) months before the next annual meeting of the Association. Before listing any individual as a nominee, the Election Committee shall obtain the consent of that individual to accept nomination and to stand for election.

b) **Finance Committee**. The Finance Committee shall consist of the Treasurer as chairman and three (3) or more appointed members. Pursuant to sections 5.05 and 6.06 of this Bylaw the committee shall recommend an auditor for approval by the Board of Directors, and shall ensure that a properly audited financial statement is prepared. The Finance Committee shall also be responsible for evaluating the financial aspects for proposed projects and activities of the Association.

c) **Rules Committee**. The Rules Committee shall consist of a chairman and two (2) or more additional members to study the Letters Patent, Bylaws, Policy, Rules and Regulations of the Association and recommend necessary changes.

d) **Time and Place Committee**. The Time and Place Committee shall consist of the President Elect as chairman and three (3) or more additional members to select the time and place of the next unscheduled annual meeting of the Association.

e) **Arrangements Committee**. The Arrangements Committee shall consist of a chairman plus three (3) or more members, all of whom are to be appointed by the President Elect. This committee shall make all necessary arrangements for the annual meeting identified by the Time and Place Committee under Section (d) above.

f) **Program Committee**. The Program Committee shall consist of a chairman plus three (3) or more members, all of whom are appointed by the President Elect. It shall be the duty of this committee to assemble a program of scientific, technical, educational and other activi-

ties for the annual meeting over which the President Elect will preside as President.

g) **Publications Committee**. The Publications Committee shall consist of a chairman, the Editor-in-Chief of Association publications, and such other members as the President may deem appropriate. This committee shall be responsible for the various publications of the Association.

h) **Awards Committee**. The Awards Committee shall consist of the Vice President as chairman and such other members as the President may deem appropriate. The committee shall be responsible for recommendation and administration of the various awards of the Association.

i) **Student Affairs Committee**. The Student Affairs committee shall consist of a chairman plus three (3) or more members. This committee shall encourage the active participation of students in the affairs of the Association and recommend appropriate activities and pursue those approved by the board to promote the development of aquaculture education.

Article Eight — Affiliates

Section 8.01 **Purpose**. It is the policy of the Association to encourage other aquaculture organizations in Canada to affiliate with the Aquaculture Association of Canada in order to promote communication within the aquaculture industry and related disciplines.

Section 8.02 **Eligibility**. Any Canadian aquaculture organization with 25 or more individual members and the general objective of enhancing the aquaculture industry in Canada may make application to the Board of Directors of the Association, who shall render a decision after considering the application in the context of the stated purposes and objectives of the Association.

Section 8.03 **Delegates**. Each organization affiliated with the Association shall be entitled to name one (1) delegate to the Board of Directors of the Association. It shall be the function of this delegate to represent the views of his organization and to convey information between his organization and the Association.

Section 8.04 **Rights of Members of Affiliates**. Members of affiliates shall pay AAC member rates for AAC meetings and any publication sold to Association members. Members of affiliates may present papers at annual meetings. Such other benefits as shall be provided from time to time by the Board of Directors.

Section 8.05 **Membership List**. Each affiliated organization shall annually submit to the Secretary of the Association an accurate and current list of members, complete with such supplementary information as the Board of Directors of the Association may require.

Section 8.06 **Dues**. For each of its individual, student, institutional and other types of member in good standing an affiliated organization shall annually remit to the Association such membership dues as are established by the Board of Directors under authority of Section 2.05 of this Bylaw. These funds shall be transferred to the Association by such date as may be set by the Board of Directors of the Association, but not later than the start of the annual business meeting of the Association.

Section 8.07 **Constitution**. An affiliated association is responsible for the adoption and amendment of its own constitution and Bylaws, the election of its own officers and directors, and the conduct of its own affairs.

Section 8.09 **Termination** of Affiliation. Affiliate status once granted to an organization shall remain in effect until such time as that organization terminates its affiliation by written notice to the Board of Directors, is dissolved, fails to meet its obligations to the Association under this Bylaw, or is rescinded by two-thirds (2/3) vote of the Board of Directors of the Association present at a meeting for which proper notice has been given and at which a quorum exists.

Article Nine — Association with the World Aquaculture Society

Section 9.01. **Purpose**. The Association shall enter into Association with the World Aquaculture Society for the purpose of communication with aquaculturists on a worldwide scale.

Section 9.02. **Terms of Association**. The terms and conditions of association are negotiated with the World Aquaculture Society and are the subject of a formal agreement.

Article Ten — Amendments

Section 10.01 **Amendments**

a) **Amendments to a Bylaw**. The provisions of this Bylaw may be amended, altered or rescinded by a majority vote of those members of the Board of Directors present at a regular meeting for which proper notice has been given, or any special meeting called for that purpose. No amendment or repeal of any part of any Bylaw of the Association shall be enforced or acted upon until the approval of the Minister of Consumer and Corporate Affairs has been obtained so long as such procedure remains a requirement of the Canada Corporations Act. The Secretary shall ensure that all such changes to a Bylaw are communicated to the membership at the earliest opportunity.

b) **Petition for Change in Bylaw**. A petition for change in a bylaw can be submitted to the Board of Directors at any time by ten percent (10%) or more of the membership. The Board of Directors shall review the proposed changes and offer them, with recommendation, to the membership for majority vote in accordance with Section 3.07. Any changes in a bylaw so approved shall be passed by the Board of Directors and submitted for the approval of the Minister of Consumer and Corporate Affairs, if so required.

Article Eleven — Execution of Documents

Section 11.01 **Signatures**. Contracts, documents or any instruments in writing requiring the signature of the Association other than cheques and negotiable instruments shall be signed by any two of the President, the President Elect, the Vice President, the Secretary and the Treasurer. Cheques and negotiable instruments shall be signed as provided by the Policies, Rules, and Regulations. All contracts, documents, cheques and instruments in writing so signed shall be binding upon the Association without any further authorization or formality. The Directors may, from time to time by resolution, appoint such further or other officers on behalf of the Association to sign contracts, documents and instruments in writing generally, or to sign specific contracts, documents and instruments in writing. The seal of the Association when required may be affixed to contracts, documents and instruments in writing signed as aforesaid.

Article Twelve — Indemnification of Directors and Officers

Section 12.01 **Indemnification**. Each Director and Officer of the Association and his executors, administrators and assigns, shall from time to time and at all times, be indemnified and saved harmless out of the funds of the Association from and against all cost, charges and expenses whatsoever which such Director or Officer sustains or incurs in or about any action, suit or proceeding which is brought, commenced or prosecuted against him for or in respect of any act, deed, matter or thing whatsoever made, done or permitted by him in or about the execution of the duties of his office, and also from and against all other cost, charges and expenses which he sustains or incurs in or about or in relation to the affairs thereof except such costs, charges or expenses as are occasioned by his own willful neglect or default.

Article Thirteen — Miscellaneous

Section 13.01 **Gender**. In all cases in the Letters Patent and Bylaw the use of one gender is a matter of convenience and shall be interpreted and understood as applying equally to both sexes.

Section 13.02 **Endorsements**. No member, committee chairman, Director or Officer of this Association shall use the seal, logo or name of this Association to endorse, condemn or express an evaluation of any product or service of any firm or individual.

Section 13.03 **Seal**. The seal of the Association shall be in such form as shall be prescribed by the Board of Directors.

Calendar

•**British Trout Farming Conference,** 4-6 September 1996, Sparsholt College, England. Main focus is on the papers presented, but a small trade show is held as well. Information: Shaun Leonard, Department of Fish, Game and Wildlife Management, Sparsholt College, Winchester, Hampshire S021 2NF (fax 01962 776587).

•**5th Canadian Workshop on Harmful Marine Algae,** 11-13 September 1996, St. John's, Newfoundland. To promote exchange of new and unreported information and to plan for future research. Program includes oral and poster presentations, review of relevant work by different agencies, and workshop sessions, including one on harmful marine algae and aquaculture site management. No registration fee. Information: M.A. Paranjape, Ocean Ecology Division, DFO, Box 5667, St. John's, NF Canada A1C 5X1 (tel 709 772-6184; fax 709 772-3207; e-mail mparanjape@ nflorc.nwafc. nf.ca)

•**Aquaculture Nutrition and Feed Management Short Course,** 15-20 September 1996, Corpus Christi. Outline: Nutrition of warm and cold water species: shrimp, catfish, tilapia, redfish, salmon and trout; Feeds formulation; Feeding and management practices; Tours of research, hatchery, production and processing facilities. Contact: Ed Lusas, Food protein R&D Center; College Station, Texas USA 77843 (tel 409 845-2741; fax 409 845-2744).

•**Sea Fare International 96,** Las Vegas, 26-27 September 1996. Information: Sea Fare Expositions, Inc., 5305 Shilshole Ave. NW, Suite 200, Seattle, WA USA 98107 (tel 206 789-6506; fax 206 789-9193).

•**Marketing and Shipping Live Aquatic Animals and Plants, Industry Conference and Exposition,** 13-15 October 1996, Seattle, Washington USA. Aimed at individuals, companies and agencies involved in the growth, harvesting, processing, regulating and shipping of live aquatics. Oral and poster presentations and commercial displays of products and services. Information: Nor'Westerly Food Technology Services, 2743 56th Avenue SW, Seattle, WA USA (tel 206 938-0676; fax 206 933-7937; e-mail 103243.675@compuserve.com).

•**The Health of Coastal Ecosystems through Shellfish Restoration—An International Conference**, 20-23 November 1996, Crystal Sands Crowne Plaza Resort, Hilton Head Island, South Carolina. Themes: Remediation/Pollution Abatement; Habitat Restoration; and Stock Enhancement/Aquaculture. Program will consist of invited oral presentations and contributed posters. Information: Elaine Knight, S.C. Sea Grant Consortium, 287 Meeting Street, Charleston, SC (tel 803 727-6406; fax 803 727-2080; e-mail knightel@musc.edu).

•**First Mote International Symposium on Marine Stock Enhancement,** 21-23 November 1996, Mote Marine Laboratory, Sarasota, Florida. Contributed papers welcome. Information: Mote Symposium, Center for Professional Development, Florida State University, Tallahassee 32306-2027 (slampman@ mailer.fsu. edu).

•**World Aquaculture 97,** 20-23 February 1997, Washington State Convention Center, Seattle, USA. Theme: Linking Science to Sustainable Industry Development. Information: World Aquaculture '97, 21710 7th Place West, Bothell, WA USA 98021. For questions about registration or the trade show call 1 206 485-6682 or fax 1 206 483-6319.

•**California Aquaculture Association,** Annual Conference and Trade Show, 20–22 March, Fresno Hilton Inn, Fresno, California. Information: CAA , P.O. Box 1004, Niland, CA 92257 (tele619 359-3474).

•**Aquaculture Canada 97,** the 14th annual meeting of the Aquaculture Association of Canada, 10-13 June 1997, Radisson Hotel, Quebec City, Canada. Information: AAC, Box 1987, St. Andrews, NB Canada EOG 2XO (tel 506 529-4766; fax 506 529-4609).

Aquaculture Canada '95

Steering Committee:

Al Castledine, Chairman

Neil Bourne

Linda Townsend

Christine Hodgson

Bill Heath

Bill Pennell

Dave Mitchell

Warren Nagata

Committee Chairpersons:

Program Committee — Neil Bourne

Local Arrangements Committee — Christine Hodgson

Advertising Committee — Bill Heath

Ways and Means Committee — Al Castledine

Trade Show Committee — Linda Townsend

Thanks to:

Denise Mason

Tracy Michalski

Marianne Flett

Jenny Dawson-Coates

Jerry Kelly