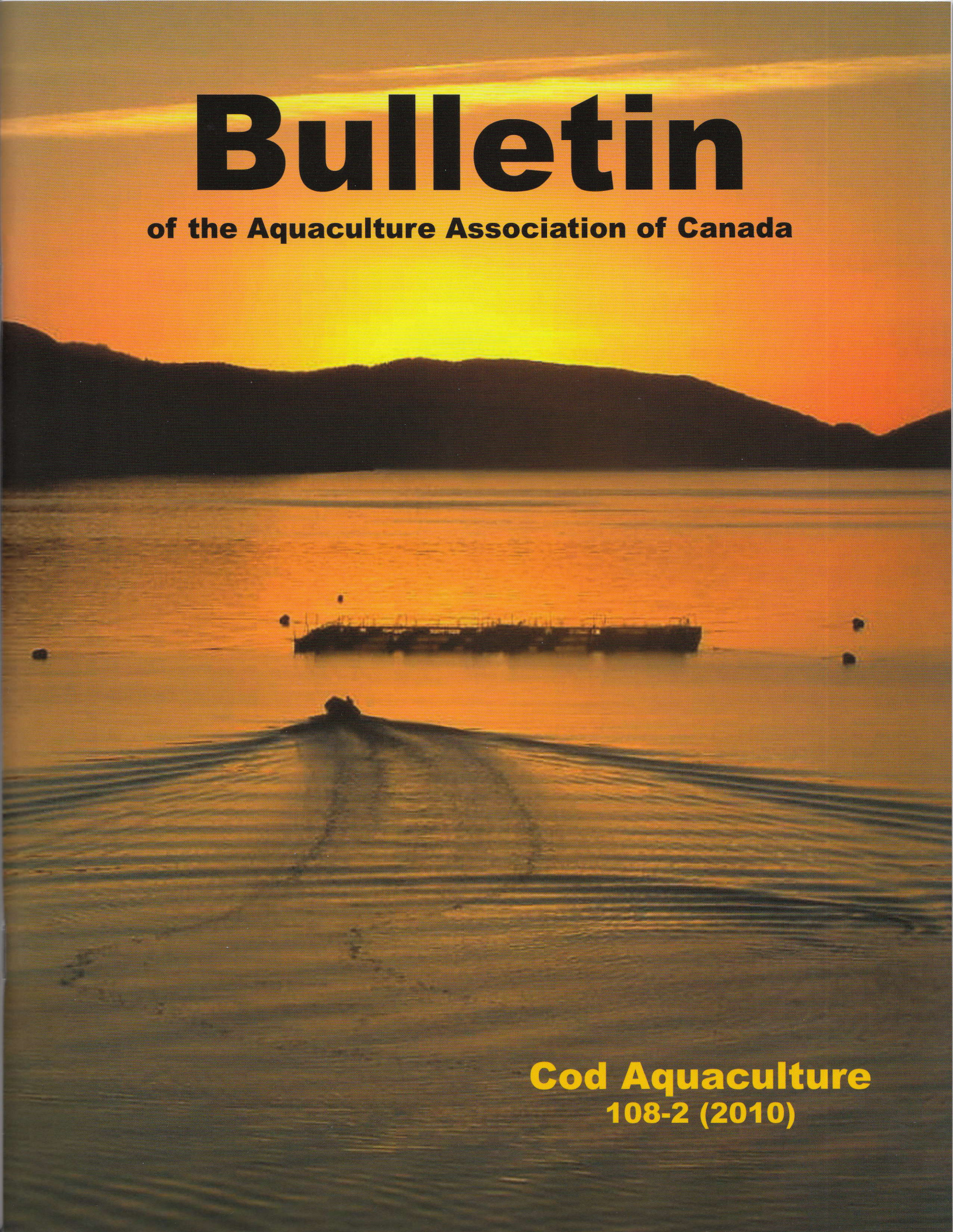
Bulletin

of the Aquaculture Association of Canada



Cod Aquaculture

108-2 (2010)

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de l'Association aquacole du Canada

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Cover: Sunrise at an Atlantic cod grow-out site in Pool's Cove, NL (Jennifer Caines photo)

Message from the President

This edition of the Bulletin contains papers presented at Aquaculture Canada 2010 on cod aquaculture. Although there was no specific session on this theme, the AAC Publications Committee felt it would make a nice package of related papers, and I hope you agree. I'd like to thank Danny Boyce for writing the Introduction to this Bulletin, and Susan Waddy and Gregor Reid for managing its editing and publication.

By the time you receive this Bulletin, I will have recently completed my term as President of the AAC. It's an interesting time to be involved in aquaculture, as we reach the historic milestone where global aquaculture production exceeds wild harvests as the principal source of aquatic foods for a growing human population. Canada is a world leader in the research and training that supports the sustainable growth of aquaculture, and the AAC contributes to this through our annual conference and through publications like this one.

I'd like to thank you for your membership in the AAC and encourage you to become more involved. We're always looking for ideas for publications, workshops and conference sessions, as well as volunteers to serve on committees and to stand for election to the Board of Directors. Help make the AAC become what you want it to be!

Tillmann Benfey

Braille net full of cod
[Danny Boyce photo]

Contents

Cod Aquaculture

Tillmann Benfey & Gregor Reid,
guest editors

Jennifer Caines photo



Atlantic Cod Farming in Canada

The AAC 2010 conference was highlighted by the quantity and quality of research that is taking place pertaining to aquaculture. The topics presented in the finfish session ranged from Production and Economics, Cultivation and Research, Egg Quality and Nutrition, to Aquaculture Genomics & Genetics. In this issue of the *Bulletin,* are 6 papers from these sessions that focused on cod.

In the past year, there seems to be some lost momentum through the reassessment of the feasibility of cod farming by commercial partners in Canada and others countries. One has to wonder where cod aquaculture is headed. Have we underestimated the production techniques and complexities of this species? Have we scaled back at a time when more research and development is required? What do we do with the gains made through the many years of R&D, especially the recent large scale Cod Genomics and Broodstock Development Project (CGP) in Atlantic Canada? The CGP has vastly increased the amount of genetic data available and has identified markers that now need to be validated. One of the most important aspects of genetic research (Genomics) is that it can greatly reduce the broodstock development timeframe by allowing scientists to identify genetic markers associated with specific traits. Will we ever as an industry in Canada benefit from the results of the CGP?

In 2001, we at Memorial's Ocean Sciences Centre submitted an application for funding that included the following sentence "Development of cod aquaculture is accelerating internationally, driven by sound market demand and depleted supply from wild stocks". How times have changed in 10 years. Farming cod is not accelerating internationally, markets are not sound and wild stocks have been returning in some areas. The current market conditions of increasing wild cod availability and associated declining prices have made the commercialization of cod unfeasible in the short term in the eyes of many.

As mentioned in the Gardner Pinfold report *Factors Affecting Viability of Cod Aquaculture in Atlantic Canada*, written for Genome Atlantic in 2010 , "The cod farming industry competes in a market dominated by supply not only from the wild stocks, but also from a supply of

whitefish generally, both wild caught and cultured. The evolution of salmon aquaculture holds some lessons for cod. Chief among these is that it takes time to meet the technical challenges of identifying and developing a suitable broodstock, understanding nutrition needs and developing low-cost feeds, and resolving such critical issues as growth, feed conversion ratios and disease". Most agree that the above noted technical challenges need to be addressed, before the appetite of commercial partners is palatable again.

However, many see cod as having long term economic potential as market conditions are variable and are based upon increasing catch rates and biomass levels.

I can say that Canada is recognized as a world leader in marine aquaculture research and development. Research and development continues despite the lack of commercial appetite that currently exists by industrial partners for cod. Recently, it has been suggested that, other than salmon, cod has the best chance of success, since we know more about cod farming than all other farmed species in the world except salmon. Cod has a strong foundation for future development and growth.

In terms of species diversification, cod is certainly a worthy candidate along with other species throughout Canada. I support an industry where species diversification is part of the portfolio. Diversification is the financial equivalent to not putting all of your eggs in one basket. We need a shared vision for the future of aquaculture in Canada. We need to maintain a vibrant and sustainable Canadian aquaculture industry that contributes to the economies of rural and coastal communities in our provinces. We need to enhance global competitiveness, productivity and environmental performances.

Our industry needs to not only harvest fresh product, but to harvest fresh ideals. Many of these ideals will come from students, scientists, industry, government, not for profit organizations and other people who attended the AAC 2010 conference and presented their ideals and research. Congratulations to the AAC for another successful conference.

Danny Boyce

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Marsha Clarke

Free Amino Acids as an Indicator of Egg Viability in Atlantic Cod *(Gadus morhua)*

M Clarke, CC Parrish and RW Penney

An understanding of biochemical factors affecting marine fish egg and larval quality can be used to separate poor from high quality eggs in aquaculture, for selection of viable eggs resulting in maximum production. Levels of free amino acids (FAAs) were determined in Day 0 Atlantic cod eggs using an EZ:faast™ GC-FID Free (Physiological) Amino Acid Analysis Kit. A select number of FAAs correlated with fertilization and hatching success, and significant differences were found between higher and lower success groups for some FAAs. It is recommended that the role of those FAAs showing significant differences among Day 0 egg groups (sarcosine, thioproline, hydroxyproline, ornithine and hydroxylysine) be investigated further to understand their roles in embryo and larval development.

Introduction

An understanding of factors affecting marine fish egg and larval quality can be used to separate low from high quality eggs, thus allowing producers to select viable eggs for maximum rates of fertilization, hatching and larval survival. Several parameters measured in newly spawned eggs of marine finfish have been proposed or used as indicators of future egg viability or hatching success. For cod, these include both morphological and biochemical indices.[1-3] This study looks at the potential of free amino acids (FAAs) as indicators of egg viability and hatching success in cod using the EZ:faast™ GC-FID Free (Physiological) Amino Acid Analysis Kit. FAAs have typically been measured by extraction with 6% trichloroacetic acid (TCA) and analyses run on an automatic amino acid analyser such as the Chromaspek J180, Hilger Analytical.[4-9] The Amino Acid Analysis Kit used here was designed mainly for the analysis of blood or urine samples, and its application in analyzing fish samples, particularly eggs and larvae, is not known. While the kit does not measure taurine or arginine, it does measure a number of amino acids which have not been looked at in previous studies.

Materials and Methods

Eggs were sampled immediately after spawning, at fertilization (Day 0). Unfertilized eggs were collected for analysis along with fertilized eggs from the same batch at Day 0. Ten egg batches were used to determine FAA content. They were separated into two sampling groups (tanks): higher fertilization success (> 70% of eggs fertilized) and lower fertilization success (< 70%). Triplicate samples of 50 eggs were taken from each tank at each sampling stage, rinsed with filtered seawater, gently blotted dry and then placed in 10 mL vials. These were then filled with N_2(g), sealed with Teflon tape and stored at -80ºC

until FAA analysis.

Egg samples were ground up in 3 ml distilled water. FAAs were extracted and derivatized using an EZ:faast™ GC-FID Free (Physiological) Amino Acid Analysis Kit. A total of 100 µl of sample was mixed with 100 µl of an internal standard, norvaline (0.2 mM) and n-propanol, and passed through a sorbent tip. It was then washed with 200 µl of n-propanol, and the sorbent material ejected in an eluting medium consisting of 3:2 sodium hydroxide/n-propanol. Fifty µl chloroform and 100 µl iso-octane were added to the solution to form the organic layer containing the FAA. Finally, 1N hydrochloric acid was added to lower the pH of the solution and complete the derivatization. Samples were subsequently run on a Varian 3800 GC-FID to obtain peaks of FAA present, with the exception of taurine and arginine. Peak areas were quantified in comparison with a known quantity of internal standard to give quantitative values of FAA.

Varian 3800 GC-FID used to obtain FAA peaks.

Results and Discussion

The EZ:faast™ Kit was successful in measuring FAA levels in cod eggs and larvae. The kit is easy to use and the quick preparation of samples makes it a useful tool for performing analysis in a commercial setting. While the kit could not measure taurine and arginine levels, it did provide data for a range of FAA broader than that provided by the TCA method. Eggs from batches with high and low fertilization success showed significant differences in quantities of sarcosine, thioproline, hydroxyproline, ornithine and hydroxylysine. However, when comparing successfully fertilized eggs to those that did not fertilize, significant differences were found among the majority of the FAAs analysed as well as total FAA (Table 1). Free amino acids have been shown to be used by cod and other marine fish species during embryogenesis as a source of energy and as building blocks for important protein growth.[4,5,7,10] Without these FAAs available, it can be assumed that survival of eggs and newly hatched larvae will be limited. The results of FAA analysis on Day 0 eggs show that some FAAs may indeed be related to an egg's ability to be successfully fertilized. However, it is not certain that the difference in FAAs is a cause for differences in fertilization or a result of fertilization itself. A simple experiment to collect batches of eggs before exposure to sperm for FAA analysis preceding fertilization, and then to follow the batches through fertilization, would indi-

EZ:faast™GC-FID Free (Physiological) Amino Acid Kit, used to extract and derivitize free amino acids in cod eggs.

Table 1
Free amino acids (nmol/egg) showing significant differences (paired t-test, p < 0.05) between fertilized and unfertilized Atlantic cod eggs and in batches of higher (>70%) and lower (<70%) fertilization success.

Free Amino Acids	Fertilized Eggs	Unfertilized Eggs
Alanine, ALA	23.7 ± 1.9	11.4 ± 2.3
Glycine, GLY	9.5 ± 2.8	4.9 ± 1.3
Amino-n-butyric acid, ABA	0.82 ± 0.10	0.50 ± 0.64
Valine, VAL	13.8 ± 1.2	7.7 ± 1.1
Aminoisobutyric acid, BAIB	0.47 ± 0.12	0.27 ± 0.05
Leucine, LEU	11.1 ± 1.7	5.7 ± 1.0
Isoleucine, ILE	11.8 ± 1.0	5.8 ± 1.1
Asparagine, ASN	24.7 ± 8.9	6.9 ± 1.6
Threonine, THR	15.9 ± 2.4	7.2 ± 2.1
Serine, SER	68.5 ± 11.3	30.4 ± 10.0
Proline, PRO	4.25 ± 0.37	2.02 ± 0.4
Aspartic acid, ASP	1.22 ± 0.13	0.75 ± 0.13
Methionine, MET	2.93 ± 0.35	1.49 ± 0.35
Glutamic acid, GLU	2.36 ± 0.49	1.15 ± 0.41
Phenylalanine, PHE	2.37 ± 0.24	1.22 ± 0.29
Tyrosine, TYR	17.7 ± 3.6	6.3 ± 2.0
TOTAL	443 ± 131	227 ± 56
Free Amino Acids	**High Success Eggs**	**Low Success Eggs**
Sarcosine, SAR	0.47 ± 0.13	0.25 ± 0.03
Hydroxyproline, HYP	19.6 ± 6.1	5.0 ± 0.5
Ornithine, ORN	8.3 ± 2.5	3.6 ± 0.7
Hydroxylysine, HLY	18.4 ± 6.9	3.8 ± 1.1
Thioproline, TRP	1.30 ± 0.48	0.16 ± 0.04

cate whether FAA levels differ prior to fertilization, and can then be linked to resulting fertilization success.

The average total FAA, at 886 ± 262 nmol ind^{-1}, is more than four times the totals previously reported for Day 0 cod eggs.[4] However, the array of FAAs included in this study is larger and, while missing taurine and arginine, included eighteen FAAs not previously reported.[4] When looking at only those FAAs in the earlier study,[4] with the exclusion of taurine and arginine, the total in the current study is reduced by almost half, but still twice their value of 200 nmol ind^{-1}. The influence of egg size and volume on the higher FAA levels is not known, as egg size was not reported in the earlier study.[4] A broad array of FAAs may play a role in embryogenesis and larval development, beyond those looked at in earlier papers, including papers examining other marine fish species.[5-7,10-12]

The FAA quantities in eggs that were successfully fertilized, compared to those

which were not, showed significant differences in most individual FAAs as well as total FAA. Significant differences existed between quantities of five individual FAAs in high success *vs* low success groups, but not in total FAA. While the eggs in this case were all those from each group that were successfully fertilized, it may be that all FAAs need to meet a particular threshold for successful fertilization to occur. In the case of those FAAs that did not show a significant difference, the threshold would have to be below the lowest level in the two groups and therefore did not influence fertilization success.

Only five of the FAAs showed significant differences between egg batches with high and low fertilization success: sarcosine, thioproline, hydroxyproline, ornithine and hydroxylysine. On average, the levels of these five in the lower success group were less than half those of the higher success group. There is little information available about the exact roles of these amino acids in fish, but it is known that hydroxylated proline and lysine are important in the formation of collagen,[13] which is important in early life stages for the development of the skeletal system and fish skin. No significant relationships were found between FAA and hatching success, indicating that FAAs do not influence hatching potential.

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NJ Rowsell

Incorporation of a Krill Protein Hydrolysate into the Feeding Regime of Atlantic cod *(Gadus morhua)* Larvae: Effect on Growth, Survival and Amino Acid Composition

NJ Rowsell and CC Parrish

An experiment was conducted to determine effects of incorporating a krill protein hydrolysate into the feeding regime of Atlantic cod larvae. Krill protein enrichment was added to lipid-rich *Artemia* and larval growth, survival, and amino acid composition were determined. Larvae were fed unenriched *Artemia* and *Artemia* enriched with AlgaMac 3010, DHA Selco, and krill protein hydrolysate in 8 different feeding regimes in triplicate over 20 days. Growth was improved in larvae fed DHA Selco/Krill Protein enriched *Artemia* on alternating days. All treatments containing krill protein produced higher specific growth rates than with the unenriched treatment, and DHA Selco/Krill Protein produced the best specific growth rate (SGR). Alanine, leucine, serine, isoleucine, lysine and valine proportions were greater in larvae fed *Artemia* enriched with AlgaMac 3010/Krill Protein and those fed DHA Selco/Krill Protein than in the unenriched treatment. Larvae fed solely AlgaMac 3010 enriched *Artemia* had higher levels of alanine than larvae fed unenriched *Artemia*. It is recommended that cod larvae be fed krill protein enriched *Artemia* on alternating days.

Introduction

The extent to which amino acid (AA) requirements are met by prey used for first feeding larvae can be estimated by comparing AA profiles in prey with those in larvae. The major essential AAs in *Artemia* nauplii are lysine (7%), arginine (5%), valine (3%) and leucine (3%), while glycine, the major non-essential AA found in zooplankton, makes up only 5% of the total free amino acids (FAA) in *Artemia* nauplii.[1] The FAA pool of pelagic fish eggs is dominated by neutral amino acids like leucine, valine, isoleucine, alanine and serine.[2] Free methionine levels in *Artemia* can be increased as much as 60 fold through enrichment with liposomes,[3] suggesting other FAAs could be enriched in live food.

The digestive tract of marine fish larvae is morphologically and functionally incomplete when they initiate exogenous feeding, so larvae, especially those from pelagic eggs, may require a supply of FAA after depletion of their own reserves.[4] In *Dentex*, a marine species with pelagic eggs, FAAs accounted for 20% of the total amino acid pool, with serine, alanine and lysine predominating. The FAA fraction underwent a considerable reduction once the larvae hatched, resembling the pattern reported for other marine species having pelagic eggs.[5] Alanine, leucine and serine dominate the FAAs during the first days after fertilization in cod.[6]

Alanine and serine together accounted for 40% of the decrease in the FAA pool during egg development, while alanine and leucine accounted for 40% of the decrease in FAA during the yolksac larvae stage.[6] Alanine, leucine, serine, isoleucine, lysine and valine, together, accounted for 75% of the disappearing FAAs and were used as an energy source early in cod development. These are the 6 FAAs that are the focus of this study.

Materials and Methods

Atlantic cod were raised to day 39 on AlgaMac 3010 enriched rotifers and were then transferred to twenty-four 30-L aquaria so that each contained 61 fish. For the first 3 days, the larvae received unenriched *Artemia* nauplii. *Artemia* were added to each aquarium 3 times daily, in amounts to keep the *Artemia* concentration in the tank water at 2000 L^{-1}. The larvae were fed enriched and unenriched *Artemia* in 8 different combinations in triplicate using DHA Selco, Spray-Dried Krill Hydrolysate (Specialty Marine Products, Vancouver), and AlgaMac 3010 (Figure 1). The experiment ended when mortalities due to the onset of metamorphosis began occurring. At the end, surviving larvae were counted. Total length (length in mm from the tip of the snout to the end of the tail fin) was measured at the beginning and at the end of the experiment. This was used to calculate specific growth rate (SGR: % day^{-1}) as $Ln (TL_2 / TL_1) \div (t_2 - t_1)$. For AA analysis, 0.5 to 2 mg samples were hydrolyzed in 1 ml of 6N HCl,[7] deproteinized with 10% sulfoslicylic

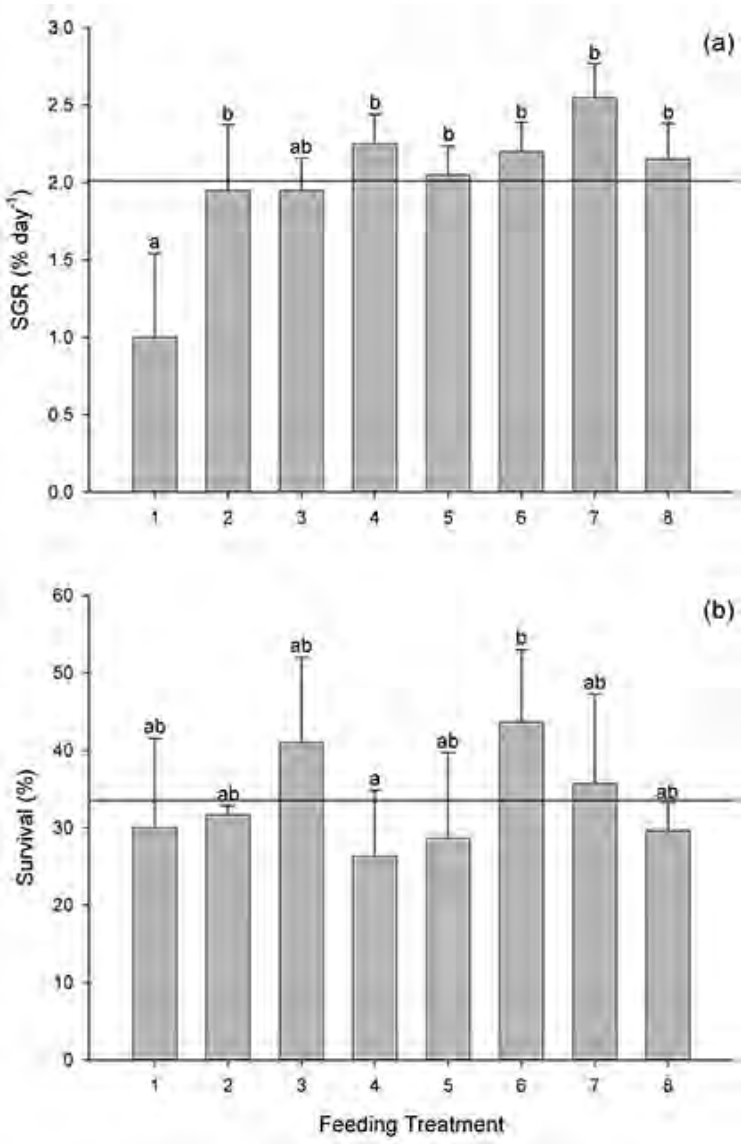

Figure 1

Growth and survival of Atlantic cod larvae (mean + sd) fed enriched and unenriched *Artemia* in eight different feeding regimes: 1) Unenriched *Artemia*, 2) AlgaMac 3010, 3) DHA Selco, 4) Krill Protein, 5) AlgaMac/DHA Selco, 6) AlgaMac/Krill Protein, 7) DHA Selco/Krill Protein, and 8) AlgaMac/DHA Selco/Krill Protein.

(a) Specific growth rates. Significant differences denoted by different letters (P<0.05). Horizontal line indicates overall mean SGR (2.01% day^{-1}).

(b) Mean survivals. Significant differences denoted by different letters (P<0.001). Horizontal line indicates overall mean survival (33.5%).

acid and analyzed on a Beckman 121 MB Amino Acid Analyzer using a single column, three buffer lithium method as per Beckman application notes.

Results and Discussion

The best growth occurred when DHA Selco and Krill Protein enriched *Artemia* were fed to the larvae on alternating days (Figure 1a). Growth, expressed as the increase in total length from the start of the experiment to the end of the experiment, was also significantly greater (P=0.010) in larvae fed this combination. Actually, all larvae fed Krill Protein alone or in combination with other *Artemia* enrichments produced above average growth rates and significantly higher SGRs than in the unenriched treatment. Growth is primarily an increase in body muscle mass by protein synthesis and since fish larvae have very high growth rates, they have a high dietary requirement for amino acids.[(2)]

The larvae fed *Artemia* enriched in DHA Selco alone was the only treatment that did not produce SGRs that were significantly different from that in the unenriched treatment. The larvae fed unenriched *Artemia* produced significantly lower SGRs than all other treatments, with the exception of the DHA Selco treatment.

Larvae fed AlgaMac 3010/Krill Protein enriched *Artemia* on alternating days produced significantly higher survival (44%, P<0.001) than larvae fed Krill Protein enriched *Artemia* alone (Figure 1b). Separately, AlgaMac 3010 resulted in 32% survival and the Krill Protein treatment gave 26% survival. This suggests

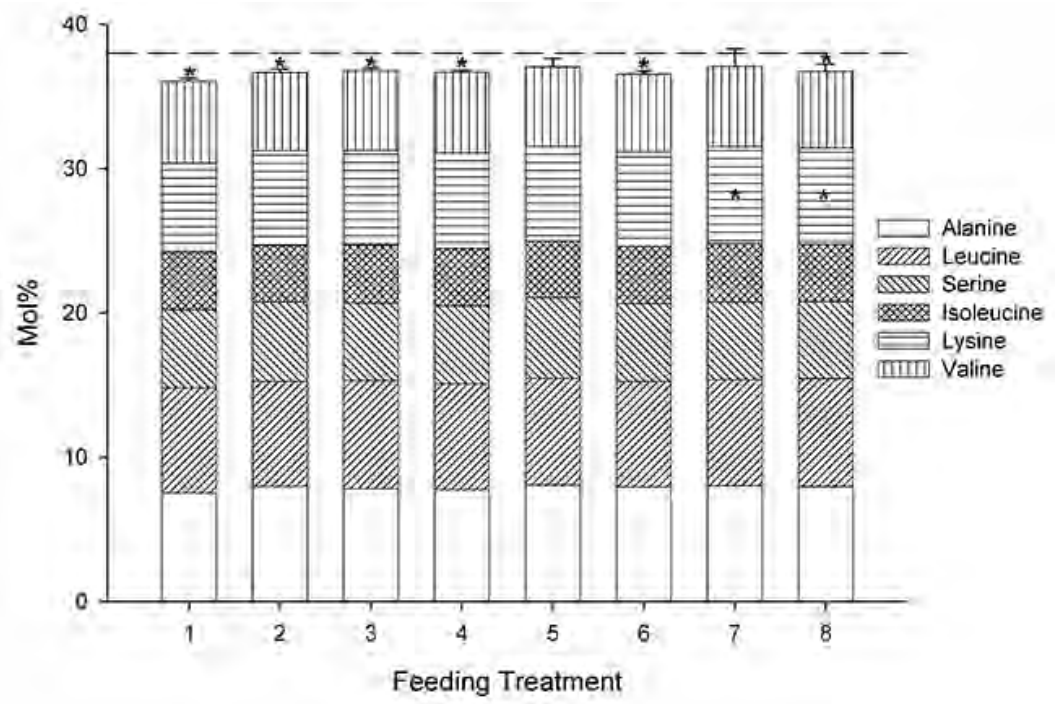

Figure 2

Free amino acid proportions (mol%). Feeding treatments as in Figure 1 caption. Horizontal line indicates total mol% of these FAAs in larvae at the start of the experiment (38%). All treatments, except 5 and 7, were significantly different from this starting value (P<0.05). Lysine in treatments 7 and 8 was significantly different from the proportion in the unenriched treatment (P<0.05). Error bars are standard deviations for the sum of the six selected amino acids.

that adding protein to an enrichment regime already high in lipids may contribute to improved survival in cod larvae; however, larvae fed *Artemia* enriched with Krill Protein alone produced a significantly lower survival, one even lower than in larvae fed unenriched *Artemia.*

Alanine, leucine, serine, isoleucine, lysine and valine accounted for approximately 37% of the larval total in all treatments (Figure 2). The horizontal line depicts the amount of the six amino acids in larvae at the start of the experiment. All larvae, except those fed AlgaMac/DHA Selco and DHA Selco/Krill Protein enriched *Artemia* contained less of these FAAs at the end of the experiment ($P<0.05$).

Lysine was higher in larvae fed DHA Selco/Krill Protein and AlgaMac/DHA Selco/Krill Protein enriched *Artemia* than in those fed unenriched *Artemia* ($P < 0.05$). In herring larvae, significantly more lysine was retained in the body after AA assimilation than glutamate, suggesting that the larvae were preferentially using glutamate over lysine as an energy substrate.[8] The increase in this essential AA with DHA Selco/Krill Protein enriched *Artemia* coincides with the best growth rate and the 3rd highest survival among the 8 treatments.

AlgaMac 3010 and Krill Protein enriched *Artemia* fed on alternating days produced the best survival but the treatment fed all three enriched *Artemia* on alternating days produced a poor survival. This suggests that feeding one protein-rich component followed by two lipid-rich components is not optimal. As a result of the improved survival in the AlgaMac/Krill Protein treatment as well as the improved growth rate in the DHA Selco/Krill Protein treatment, a suggested feeding regime would be: Day 1 – AlgaMac 3010, Day 2 – Krill Protein, Day 3 – DHA Selco, Day 4 – Krill Protein, then repeat.

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Thanks to the late Joe Brown who started this whole project off and to Jonathan Moir, Bent Urup and Laura Halfyard for advice and support.

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N King

Newfoundland Commercial-Scale Atlantic Cod Hatchery Production Technology Project—Live Feed Component

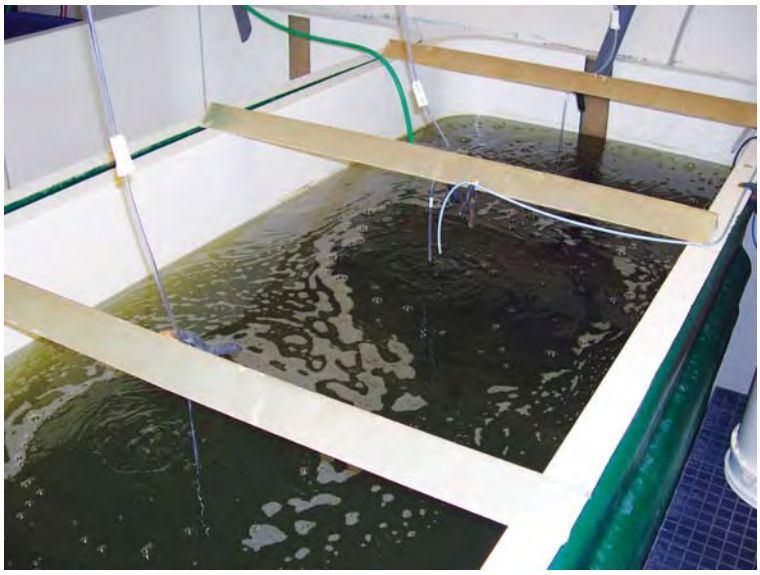
N King, R Healey, D Tucker, S Hann Haley and D Boyce

Hatchery production has traditionally been one of the biggest bottlenecks to overcome in marine fish farming. Marine fish larvae are extremely sensitive to environment, nutrition and husbandry practices, and mortality rates greater than 60% are generally realized across the industry. Experienced hatchery managers understand very well how continually optimizing protocols is essential to guaranteeing the health of their fry and subsequent farm performance. Indeed, hatchery production of marine fish like cod is a perpetually evolving process, and as new solutions to problems arise, the technology to produce marine juveniles is always advancing. With hatcheries in North America so far removed from the nucleus of modern hatchery technology in the Mediterranean and Northern Europe, it is critical to extend international collaboration in order to gain exposure to the latest information and technology. As an example of this, Skretting Marine Hatchery Feeds (MHF) integrated into a team of professional consultants from abroad to assist Ocean Science Center—Dr. Joe Brown Aquatic Research Building (OSC-JBARB) staff in their effort to scale-up cod hatchery production in support of industrial demonstration of cod farming in Newfoundland, Canada. Our objective was to apply a new live feed culture and enrichment program, and coordinate the supply of these feeds to the larval fish culture department.

After 25 years of commercial fish production, rotifers and *Artemia* remain as the most important live feeds for start feeding marine fish larvae. Typical survival rates of fry rarely exceed 60% during the live feed stage of production.[1] Additionally, nutritional deficiencies and disease risks stemming from live feed can lead to certain deformities and affect production success.[2-7] During the 2008-2009 cod production season at JBARB, high mortality rates of larvae coincided with the live feed period of the existing cod rearing protocol (rotifers 10 to 30 days post-hatch [dph]; *Artemia* 30 to 55 dph). Mortality varied among larval rearing tanks, but inevitably resulted in the complete termination of all tanks between 20 and 45 dph. Together with RSP Services, a UK-based consultancy group, Skretting MHF was invited to assist the JBARB team in improving their facilities and protocols.

In the live feed room of JBARB, Skretting MHF worked together with staff to apply Ori-Go products, Ori-Culture and Ori-Green, to rotifer production and live feed enrichment. The objectives were to 1) improve the efficiency of the live feed

Rotifer culture tank

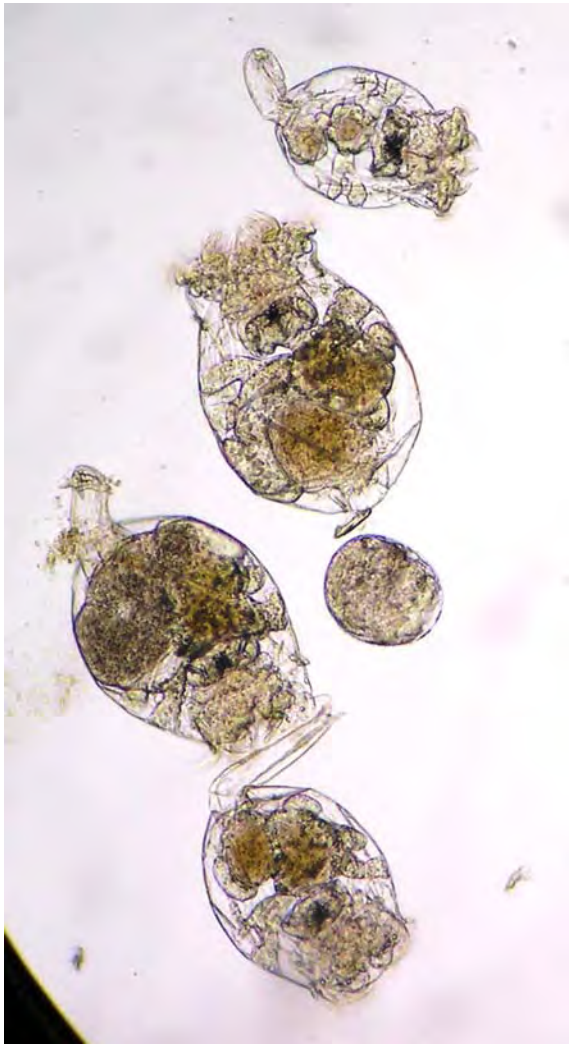


program by reducing labour and updating equipment and control systems, 2) improve the hygiene of the live feed by implementing a new diet and culture program, 3) improve the quality and nutritional stability of the live feed by creating a standard routine program, and 4) intensify the live feed production within current infrastructure to meet demands of a commercial cod hatchery producing 1 million fish.

The first action of Skretting MHF was to host a Live Feed Workshop to update all staff on recent innovations and critical factors that affect quality and stability of live feeds during production. Next, during the later part of the 2009 production season, we performed a complete audit of live feed procedures and equipment that included a real-time assessment of condition and performance of rotifer cultures, rotifer enrichments, *Artemia* hatch and *Artemia* enrichment. During the audit, we found daily inconsistencies in rotifer culture performance that were mostly due to oxygen instability related to feed type (yeast-based) and batch feeding method. We also found some indicators that rotifers were underfed (empty stomachs, hour-glass shaped lorica) and damaged due to under-sized harvesting equipment. With regard to *Artemia*, the audit revealed daily inconsistency in survival rate and enrichment uptake. Our aim in the following period was to design and implement a program to address these issues.

Prior to the start of the 2010 production season, we outlined live feed protocols, built/installed equipment and demonstrated every aspect of the proposed program within the JBARB facility. Rotifer production tanks were reconfigured according to Ori-Cul-

A rotifer culture sample with minimal suspended debris, thereby allowing quicker rotifer concentration and shorter washing time.



Healthy rotifers

ture specifications. A new rotifer harvester (200 L) was built to harvest up to 3 billion rotifers in less than 1 h with no physical damage to the plankton. A PT4 oxygen/temperature control system was installed to maintain dissolved oxygen levels in the culture tank at 8 to 9 mg/L. We installed a feeding system that provided a continuous drip of Ori-Culture from a chilled container at a rate of 16 L per 24 h using a peristaltic pump. Finally, within the larval culture room, a cold storage system was implemented to maintain temperature of enriched rotifers and *Artemia* at 5º to 6ºC.

During cod production, two rotifer enrichments were performed each day (morning and afternoon). A morning enrichment using Ori-Green (250 ppm) for 3 h was fed to the larvae at 9 am. The afternoon enrichment, consisting of two separate tanks (one fed Ori-Green and one fed mixed-algae concentrate), was started at 11 am and harvested at 2 pm. These "pm" enriched rotifers were fed to the fish at 5 pm, and then cold stored for overnight feeding. *Artemia* were hatched for 22 h. Nauplii were rinsed and stocked into enrichment tanks at 2 pm and provided with a single dose of Ori-Culture (200 ppm) to initiate feeding prior to the enrichment period. *Artemia* were enriched with Ori-Green (400 ppm) for 12 h between 8 pm and 8 am. Lastly, we created a quality checkpoint to assess enriched rotifers and *Artemia* for mortality, enrichment uptake, hygiene (i.e., flocs, sediments, ciliates) and physical stress.

During the 2009/10 cod production, Skretting MHF held weekly reviews of live feed records with the staff via conference call and provided a site visitation during the rearing period when problems occurred in the past.

Enriched *Artemia*

The successful outcome of this project was in part due to the high quality, high productivity and stable performance of the live feed production areas. We effectively intensified the rotifer production cycles resulting in population growth from 600 rotifers/mL (day 0) to 1600 rotifers/mL (day 4) which is excellent for such a large strain rotifer (mean lorica length 260 μm!). Thus, the JBARB live feed room supplied more than 3 billion rotifers/d, which is double the level achieved in 2008/09. *Artemia* production was increased from <500 million (2008/09) to more than 900 million by adding some tank volume, but also increasing enrichment density from 300 *Artemia*/mL to 500 *Artemia*/mL. Finally, this project demonstrated that two staff can effectively operate this live feed room in a research-oriented environment that is now capable of supporting production levels in excess of 1 million cod.

Acknowledgements

We would like to acknowledge the funding sources for the Cod Demo Project at Ocean Sciences Center: Atlantic Canada Opportunities Agency (ACOA), NL Department of Fisheries and Aquaculture (DFA), Department of Fisheries and Oceans Canada (DFO), Newfoundland Aquaculture Industry Association (NAIA) and Memorial University of Newfoundland (Dr. Joe Brown Aquatic Research Building).

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R. Prickett

Successful Partnerships for a Sustainable Future — Juvenile Cod Production at the Dr. Joe Brown Aquatic Research Building (JBARB) 2009-2010

R Prickett, D Boyce, J Monk, M Drake, B Armstrong and C Canning

Low numbers of cod juveniles during the 2008/2009 production runs at the Dr. Joe Brown Aquatic Research Building (JBARB), prompted collaboration with RSP Services Ltd, as a means to identify system limitations and improve production. Several modifications were made to the existing system. These included improved filter media (to reduce clogging), and the addition of protein skimmers, ozone treatment, and oxygen monitoring equipment. Significant adjustments were also made to egg incubation, live feed, and larval rearing protocols, to reduce the potential for contamination. As a result, over 900,000 weaned larvae were counted at the first grade (approximately 100 mg average size) from 2.6 million larvae stocked, giving an average survival of 35% (range 15% to 60%), and no production tanks were lost during the run.

Following low production numbers during the 2008/2009 production run, RSP Services Ltd., a company with several years of commercial cod hatchery experience in Europe, were invited to assist the Dr. Joe Brown Aquatic Research Building (JBARB) team in improving its facilities and protocols. The aim was to train and instruct staff in the latest hatchery technology relevant to producing commercial quantities (500,000 +) of cod juveniles per production run for the aquaculture industry in Newfoundland.

The work began in May 2009, when RSP Services Ltd., together with the JBARB technical staff, reviewed the water supply system. Work was carried out during the summer of 2009 and included a thorough cleaning of the existing pipe-work using detergents and disinfectants, followed by rinsing with clean fresh water and leaving to dry. In addition, the sand in the existing sand filtration system was replaced with a novel media (Alternative Filter Media; AFM) that had better filtration properties and did not clog like conventional silica sand. Finally, two protein skimmers were added to the system on a loop from the header tank in order to remove fine solids and dissolved organic matter. Ozone treatment was also added to the skimmers to improve their efficiency but this system was not used in the main production run due to a shortage of time for properly testing its effects on small larvae. Other equipment, which was purchased but not used, included a

recirculation system for 2 x 6 m^3 larval tanks. Oxygen supply and monitoring equipment was also purchased for the live feed room and the first-feeding tanks.

Also during the summer period, RSP Services Ltd., together with the Newfoundland and Labrador Commercial Atlantic Cod Farm Demonstration Project staff and Mr. Nick King of Skretting, reviewed the egg incubation, live feed and larval rearing protocols used in the past and proposed recommendations for limited changes only where appropriate. Some of these involved minor physical changes to the systems used, such as the addition of a second egg collector to help reduce faecal contamination of spawned eggs, modifications of the egg incubation tank water inlet and outlet systems to reduce bacterial contamination of eggs and larvae, and better harvesting equipment for the live feed systems.

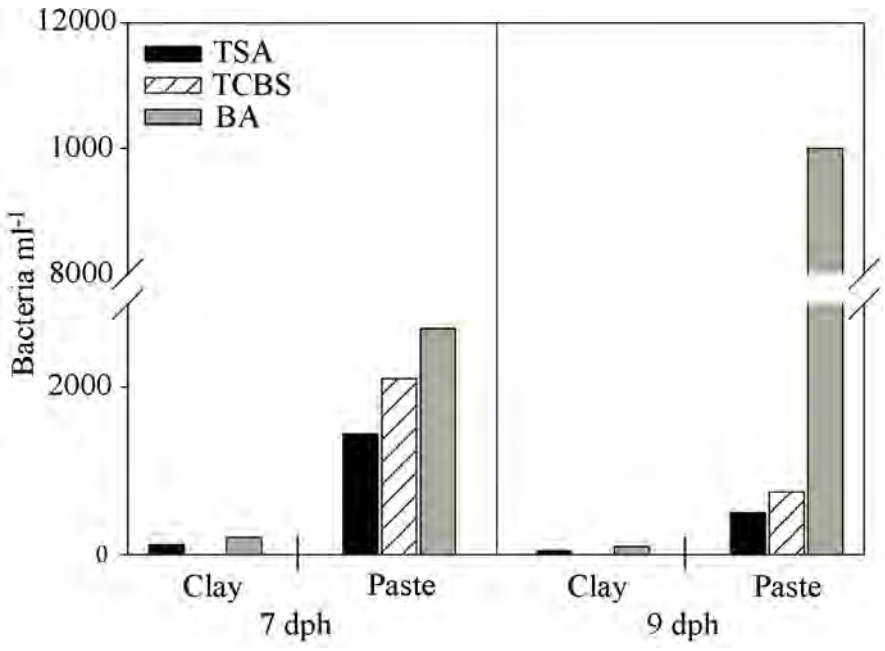
Family breeding tanks at JBARD

Some procedural protocol changes were tested prior to stocking the main production batch; these included stocking first-feeding tanks with eggs instead of larvae (stocking larvae was better), using clay in place of live algae or algal paste (clay was as good if not better), and disinfecting *Artemia* with Chloramine T prior to feeding (reduced bacterial levels for up to 5 hours after treatment) and at a later period, improving the weaning protocols.

When the modifications to the system were finally ready, the 18 first-feeding tanks (12 x 3 m^3 and 6 x 6 m^3) were stocked with larvae over a period of 1 week (October 21st to 27th, 2009). This was designed to produce a single batch of juveniles of uniform size primarily to satisfy the client's requirements. Although this put a greater pressure on inputs such as rotifers and *Artemia*, it helped maintain the same level of biosecurity throughout the system.

Cod fry

The addition of clay instead of algae in the first-feeding tanks during the live feed and weaning phases proved to be a major success in terms of growth, swim bladder inflation, survival and later weaning success. Monitoring of bacterial levels in the larval tanks showed a significant reduction in bacterial numbers compared to controls receiving algal pastes, and was prob-



Bacterial counts from tank water samples taken 7 and 9 days post-hatch (dph). Culture media used were trypticase soy agar (TSA; a general media for all types of bacteria), thiosulphate citrate bile salts agar (TCBS; *Vibrio* specific agar) and blood agar (BA; sensitive to *Aeromonas* bacteria).

ably the main reason for these improvements.

Over 900,000 weaned larvae were counted at the first grade (approximately 100 mg average size) from 2.6 million larvae stocked, giving an average survival of 35% (range 15% to 60%), and no tanks were lost during the run. There was also a notable improvement in larval quality compared to previous batches, with few deformities and a high tolerance to stress during handling.

Apart from the innovative protocols and techniques used during this run, the results also proved conclusively that the facilities and staff at the JBARB are capable of producing commercial quantities of quality marine finfish juveniles and demonstrates the potential for the facility to become a major marine finfish research centre for aquaculture on Atlantic Canada's east coast.

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Gynogenesis and the Genetic Basis of Sex Determination of Atlantic Cod *(Gadus morhua)*

Jessica Whitehead

JA Whitehead, TJ Benfey and DJ Martin-Robichaud

The objective of this research is to use gynogenesis (uniparental maternal inheritance) to identify the sex determining mechanism in Atlantic cod. After first adjusting spermatocrit to 15%, several milt dilutions and UV doses were examined for their efficacy at excluding the paternal genome in developing embryos following activation with irradiated spermatozoa. A 1:10 dilution exposed to a 189 to 266.8 mJ/cm^2 UV dose and a 1:20 dilution exposed to a 113.4 mJ/cm^2 reduced spermatozoan motility by roughly 50%. Genotyping showed that all ova activated with these spermatozoa were haploid gynogens, with the 1:10 dilution and 113.4 mJ/cm^2 UV exposure producing the largest number of survivors. This protocol was used to activate development in larger numbers of eggs which were then pressure treated to restore diploidy. Once these fish are large enough to determine their sex, the sex ratio of the gynogenetic populations will be compared to control groups. If only females result from gynogenesis, then female homogamety can be supported. A sex ratio similar to that of the controls, on the other hand, may suggest female heterogamety.

Introduction

Atlantic cod *(Gadus morhua)* has recently received attention as an alternative to Atlantic salmon *(Salmo salar)* to diversify the aquaculture industry in Atlantic Canada. However, trials conducted by New Brunswick's Cooke Aquaculture Inc. found serious challenges preventing the advancement of cod culture. The most prominent of these is pre-harvest sexual maturation, with all fish becoming fully sexually mature before reaching harvest size. Sexual maturation reduces flesh quality due to use of energy and essential nutrients (lipids and proteins) by the developing gonads rather than their storage in muscle tissue.[1] Triploid Atlantic cod females experience dramatic suppression of ovarian development, allowing for greater overall energy investment into flesh compared to diploids which invest positively to their ovaries and negatively to their flesh (Figure 1) and often experience high mortality prior to harvest. This characteristic has generated interest for the culture of all-female triploid stocks of Atlantic cod.

The first step in developing single-sex stocks for any species is to determine the genetic basis of sex determination,[2,3] as it is the heterogametic individuals that determine the sex of progeny. Species with male heterogamety follow the mam-

malian system of XY-male and XX-female and those with female heterogamety follow the avian system of ZW-female and ZZ-male. Gynogenesis is a process commonly used to uncover the genetic basis of sex determination, whereby inheritance of the paternal genome is prevented from contributing to offspring.[2] The result is progeny that have inherited only the maternal genome and are termed haploid gynogens. The diploid condition is restored using pressure shock to prevent extrusion of the second polar body.[4] Pressure treatment for polar body retention has previously been used for producing triploid cod.[5] The sex of gynogenetic offspring can be used to determine if the mother is homogametic (producing only female offspring) or heterogametic (producing male and female offspring).[3] However, although male and female progeny provides evidence of female heterogamety, it does not eliminate the possibility that other factors influence sex determination.[4] The objective of this research is to use gynogenesis to determine the genetic basis of sex determination of Atlantic cod, as a first step for producing single-sex stocks for aquaculture.

Materials and Methods

The paternal genome was prevented from contributing to offspring by exposing spermatozoa, diluted in artificial seminal plasma (Mounib's Medium[6]), to UV light. All milt samples were diluted to a constant density (15% spermatocrit ≈ 4.9 x 10^9 spermatozoa/mL) to produce an effective and repeatable UV treatment. Microscopic observation of activated spermatozoa following UV treatments was conducted to assess sperm motility to determine the relationship between milt dilution (1:10 to 1:80) and UV dose (0 to 756 mJ/cm^2). To test the success of UV treatments, irradiated spermatozoa were used to activate development in ova ("activation" is used in place of "fertilization" because the latter indicates paternal genetic contribution to ova). A proportion of the resultant embryos were sampled >2 days post-activation (p.a.) for genotyping and the remainder raised until hatch

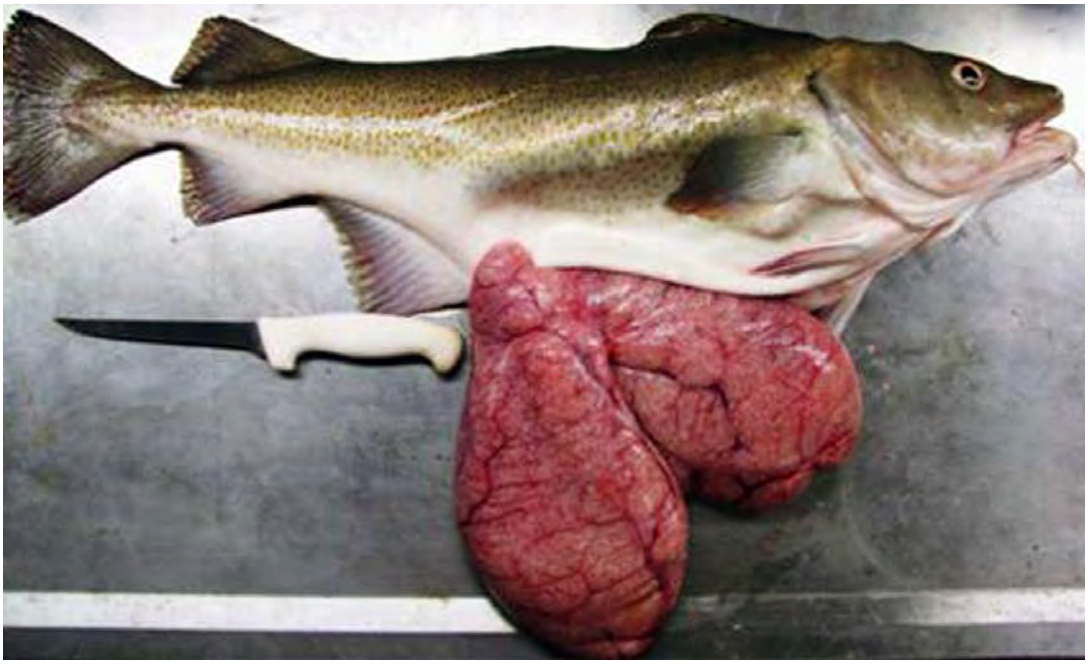

Figure 1
Ripened ovaries excised from a diploid female Atlantic cod.
[Photo: Nathaniel Feindel]

(18 to 21 days p.a.). Genotyping was used to identify offspring with solely maternal inheritance (gynogens) and those with both paternal and maternal inheritance, using six microsatellite DNA markers known to have multiple alleles in cod. Gynogenetic larvae had small, misshapen bodies at hatch (Figure 2) and died early in development. The lowest UV dose that provided the largest yield of haploid gynogens was chosen as the optimum UV treatment for large-scale production of diploid gynogens, with diploidy restored (Figure 3) by hydrostatic pressure shock (5 minutes at 8500 psi, beginning 180°C-minutes p.a.[5]).

Results

Initial UV treatments resulted in spermatozoa motility declining from 100% to 20%, 5% and 0% at 1:20, 1:40 and 1:80 dilutions, respectively, when exposed to a UV dose of 756 mJ/cm^2. Further tests confirmed that 50% motility was retained when a 1:10 dilution of spermatozoa was exposed to a UV dose of 189 to 266.8 mJ/cm^2 or a 1:20 dilution exposed to 113.4 mJ/cm^2. Genotyping showed that all ova activated with spermatozoa diluted 1:10 or 1:20 and exposed to UV doses of 113.4 to 302.4 mJ/cm^2 were gynogens. The highest yield of haploid offspring was produced from the 1:10 dilution exposed to a UV dose of 113.4 mJ/cm^2. This protocol combined with pressure shocking, plus control treatments, was scaled-up and successfully produced large numbers of viable presumptive gynogens and control larvae that are currently being reared until large enough to be sexed.

Figure 2
Haploid Atlantic cod larvae with characteristics of haploid syndrome: small, stunted body.

Discussion and Conclusions

When these fish are approximately nine months old they will have reached a size at which they can be sexed in order to determine sex ratios of the gynogenetic and

Figure 3
Diploid Atlantic cod larvae.

control groups. Blood samples will be taken from each fish to confirm ploidy by measuring erythrocyte DNA content via flow-cytometry and genotyping will be used to confirm gynogenetic status. If UV treatments were not completely effective, some triploid offspring will be present within the gynogenetic population; ploidy analysis will allow for exclusion of triploids from the sex ratio analysis. If gynogenesis gives rise to an all-female population, female homogamety will be confirmed, meaning that males likely contain the sex determining genetic information. If a combination of males and females results from gynogenesis then female heterogamety can be supported, although female heterogamety is not the only explanation for male and female progeny resulting from gynogenesis since there may be additional factors that affect offspring sex.

Acknowledgements

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Sex control in Atlantic cod *(Gadus morhua)*

Song Lin

S Lin, TJ Benfey and DJ Martin-Robichaud

Pre-harvest sexual maturation, a major environmental and economic constraint to aquaculture operations, commonly occurs in both sexes of Atlantic cod *(Gadus morhua)*. Production of female triploids would alleviate this problem because they are sterile. The objective of this project is to develop endocrine manipulations to produce monosex stocks of cod as the first step to producing monosex triploids, using 17α-estradiol (E2) and 17α-methyldihydro-testosterone (MDHT) applied to fish diets during the labile period of gonadal differentiation. Dietary treatments of 5, 10 and 20 ppm E2 and 0.67, 2 and 6 ppm MDHT were administered to fish as they grew from 17 ± 2 to 43 ± 1 mm standard length (SL). The highest and lowest MDHT doses resulted in a significantly higher male ratio compared to controls. The female ratio in all E2 groups did not differ from controls. Histological investigation of gonadal development showed that undifferentiated and differentiated gonads were observed by 11 mm and 13 mm SL, respectively, so it is likely hormone treatments were not administered before sexual differentiation had already commenced.

Introduction

As an alternative to Atlantic salmon *(Salmo salar)*, Atlantic cod aquaculture could reach the same production level as salmon in 15 to 20 years.[1] A major constraint facing commercial cod culture is pre-harvest sexual maturation resulting in increased mortality, reduced fillet quality, extended market time, and release of fertilized eggs from domesticated stocks into the wild. Several approaches, including temperature and photoperiod manipulation, selective breeding and triploidy, have been tested to address the early maturation problem in cod.[2]

Triploidy has been shown to sterilize female Atlantic cod resulting in little or no ovarian development, but substantial development of testes still occurs.[3] As a result, techniques to produce all-female triploids are being studied. All-female production can be accomplished by gynogenesis or direct and indirect hormonal feminization.[4] Gynogenesis is not a suitable strategy for cod at this time since the genetic mechanism of sex determination is currently unknown in this species. However, the phenotypic sex of gonochoristic teleosts can be influenced by direct and indirect hormonal feminization. Direct feminization involves direct exposure of fish to exogenous estrogen via diets or immersion. “Indirect feminization” refers to the crossing of regular females with “neomales” to yield all-female populations. Neomales are sex-reversed females that display male phenotypic characteristics, and are produced by direct masculinization using androgen treatments.

The objective of this research is to develop effective endocrine treatments for successful sex reversal of both sexes of Atlantic cod to produce monosex stocks as the first step to producing all-female triploids. The most effective time to administer hormones is also known as the labile period of gonadal development, when the gonads are the most sensitive to hormones. This occurs in early stages of gonadal development, prior to any indication of gonadal sex differentiation.[5] It is possible to use evidence from histological descriptions of early gonadal development to narrow in on this critical developmental stage, and then rely on the efficacy of hormonal treatments to determine the stage of gonadal differentiation most receptive to hormonal influences. Chiasson et al.[6] reported that gonadal development in Atlantic cod begins when they are 18 mm total length (TL) and is completed by 35 mm TL. The experiments described here were designed to expose larval cod to exogenous hormones during this interval.

Materials and Methods

Cod larvae were reared from eggs at the St. Andrews Biological Station. During development, samples of larvae were collected, measured (SL) and fixed in 5% buffered formalin for histological evaluation of gonadal development. When the remaining larvae reached an average size of 17 mm SL they were transferred into twenty-one 500 L tanks at the density of 500 fish per tank. They were fed hormone treated diets during the growth interval between 18 and 45 mm SL, with triplicate groups fed 5, 10 and 20 ppm for the estrogen 17α-estradiol (E2) and 0.67, 2 and 6 ppm for the non-aromatizable androgen 17α–methyldihydrotestosterone (MDHT). Hormones were incorporated into diets by soaking them in hormones dissolved in ethanol and allowing the diets to dry. Fifty fish per tank was sampled at the end of the treatment period to confirm their size. The remaining fish were reared until they reached 150 mm SL; at this time 60 fish per replicate were sexed and the sex ratio of each treatment group

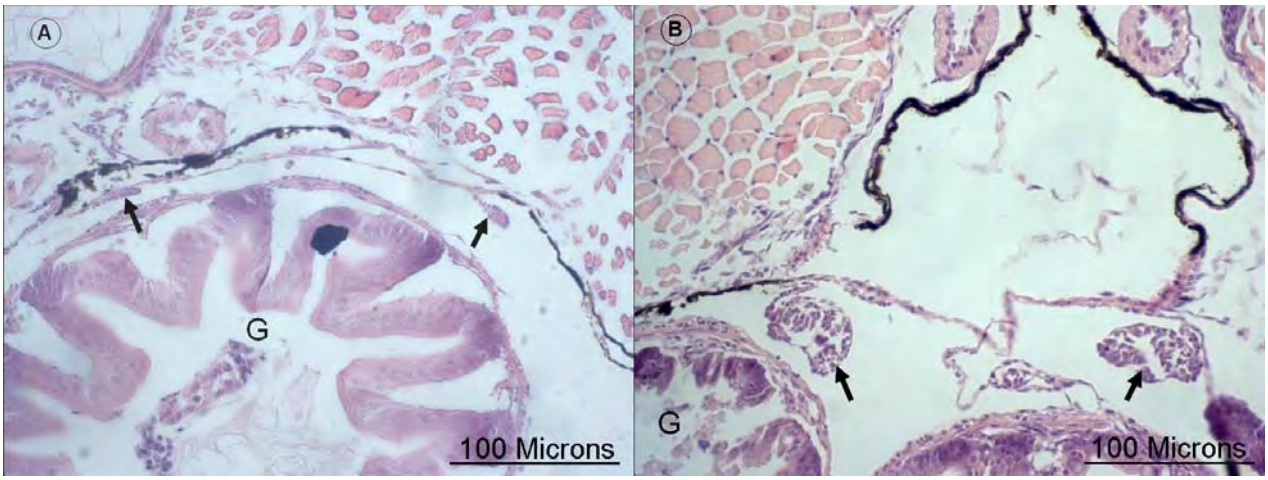
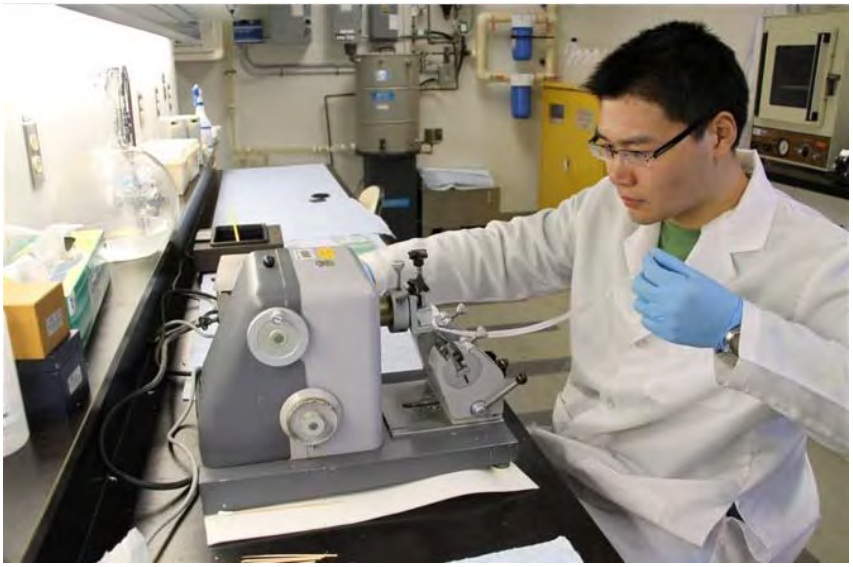


Figure 1

Transverse section through the gonads of Atlantic cod larvae. (A) Undifferentiated gonads (black arrows; 11 mm SL larva at 58 days post-hatch [dph]). (B) Presumptive ovaries (black arrows; 13 mm SL at 65 dph). G = gastrointestinal tract.

determined. Standard histological procedures were used to evaluate gonadal development of Atlantic cod reared from 7 to 144 mm SL under routine culture protocols. Paraffin embedded transverse sections (7 µm) were dehydrated and stained with hematoxylin and eosin and then gonadal development was evaluated microscopically.



Checking sex differentiation by histology

Results

No significant difference in growth was observed among treatment groups. The percentage of females was 55% in the control group, 46 to 51% in the E2 treatments and 41 to 53% in the MDHT treatments. Although the lowest and highest doses of MDHT resulted in a significantly higher male sex ratio of 53% and 59%, respectively, compared to the control, the shift in sex ratio was not proportional to hormone dose. Histological evaluation indicated that undifferentiated and differentiated gonads were present by 11 and 13 mm SL, respectively (Figure 1). This is earlier than reported in the previous study.[6]

Fish kept for further breeding

Discussion and Conclusions

Theoretically, the sex ratio should be proportional to the amount of hormone presented in fish diets, which was not observed in this experiment. One explanation for this outcome, supported by histological evaluation of gonadal development, is that gonadal differentiation had already commenced when fish reached 17 mm SL and that hormone treatments were therefore administered too late in development. Therefore, assuming the labile period is before larvae reach 13 mm SL, further experiments will expose larval cod to treated diets prior to this stage. Moreover, results from this first experiment may indicate that the dosages used were too low to alter sexual differentiation. In order to test this possibility, dosages for the second year of experimentation will also be increased.

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