Bulletin

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Aquaculture Association of Canada

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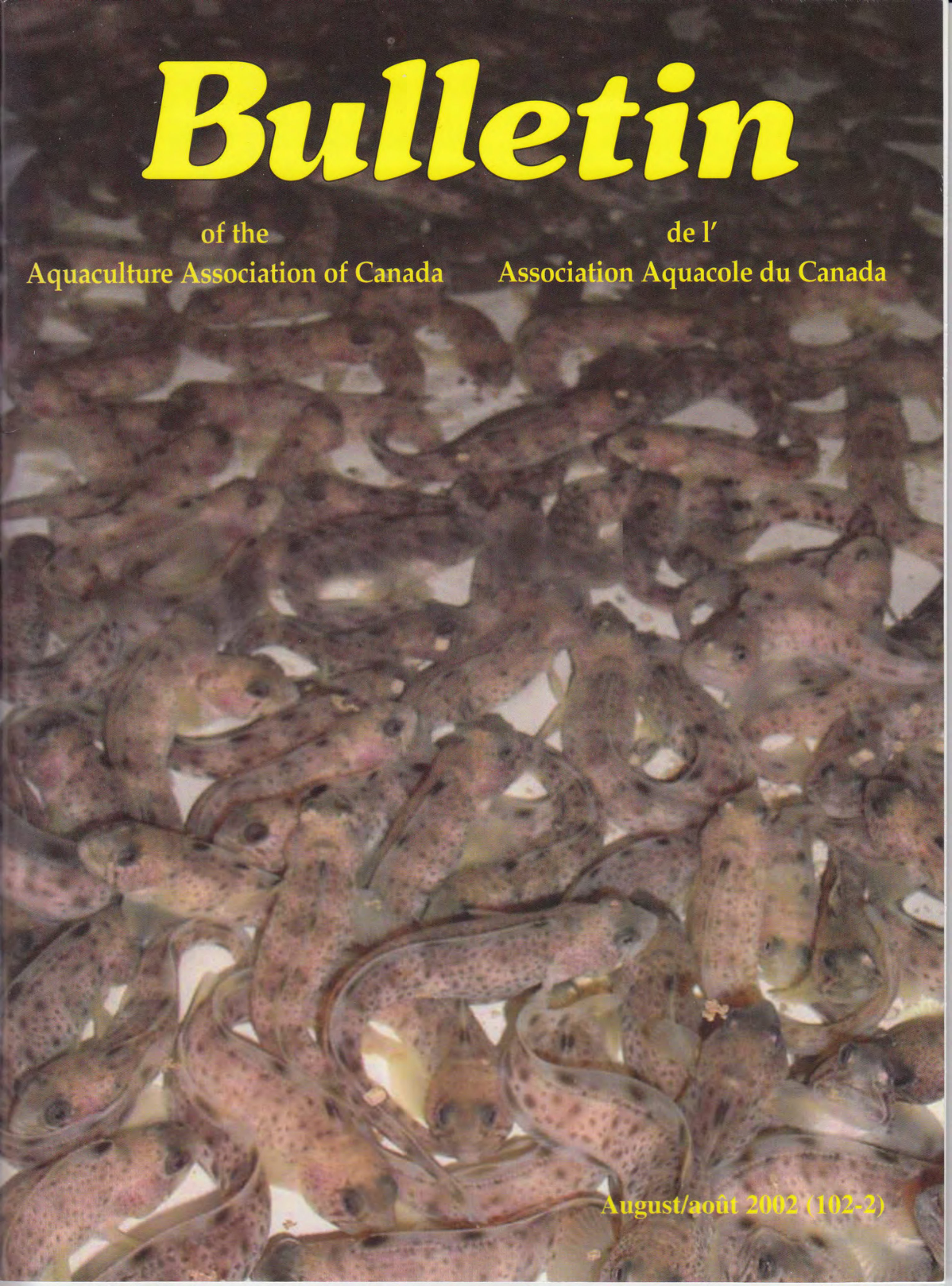

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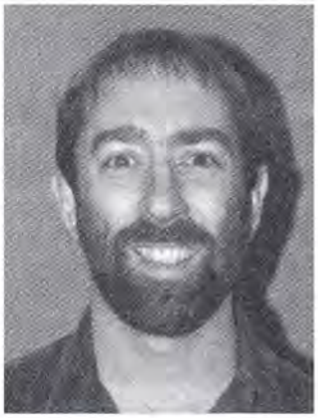

It is my honour and pleasure to take up the presidential leadership of the Aqua- culture Association of Canada, an organisation that has been actively involved with the aquaculture industry since its inception in 1984. From the time I was elected to the board of directors in 1997, I have had a first-hand view of how the organisation is actively involved in the promotion of information relevant to the burgeoning aquaculture industry through its meetings and publications. I have also seen how it has helped develop new professionals through its active student program. Several of the students who have been involved with our association have gone on to become executive board members of the AAC and of other international aquaculture organisations. As president, I will attempt to keep to the long-term objectives of our association and to maintain and strengthen our commitment to those goals.

Over the last 18 years many changes have occurred in the marine food production industries. In one sense we are experiencing the curse "*May you live in interesting times*". Over the course of two generations in Canada, we have seen a plateau in the overall catches from wild harvest fisheries and shifts in wealth from the pelagic and groundfish sectors to the invertebrate sector, notably crustaceans. The salmon culture industry has grown on both coasts to produce almost half a billion dollars of product in the year 2000 and has essentially entered the maturation phase of a commodity market. Trout, Arctic char, halibut, cod, haddock and other culture industries are either scaling up or are in development as the demand for food increases on a national and international basis. On the shellfish side, mussels and oysters still dominate aquaculture production, but an order of magnitude less than finfish. Several other shellfish species are being contemplated and investigated. Some groups are now looking at the concept of integrated culture using ecological principles in their systems design. Not surprisingly, considering the globally-connected world we live in, all of the above changes are being reflected to various extents on a world-wide basis.

In some ways we are simply following the course of development of terrestrial agriculture with issues such as the intensification of production, zoning, employment, environment and the social shift from a hunter-gather society to one based on farming. The big difference is the speed at which all this is happening: years vs. centuries. This is a time when there are many lessons to be learned, some new and some old.

We have just finished our 19th annual meeting and it continued to build on the long tradition of successful meetings that the AAC is known for. There was a large international contingent at the meeting in Charlottetown as the First International Mussel Forum was held within the Aquaculture Canada^{OM} framework. It was an undeniable success and a substantial amount of information was exchanged during and after the various talks. This is an aspect that I would like to encourage and continue to build on for future meetings: the collaboration of various aquaculture groups for the centralisation of information exchange. Organisations such as the AAC, the Canadian Aquaculture Industry Alliance, AquaNet, provincial aquaculture associations, governments and universities should consider co-ordinating their activities on a semi-regular basis so that integrated conferences could bring a wide diversity of information to the meeting delegates.

In the upcoming year, in addition to the preparation for the next AAC meeting in Victoria, BC (October 29-November 1, 2003) we will be working on ways to continue the development of students within aquaculture and to build bridges with other organisations. It looks to be a very busy year and I am looking forward to working with all the members of the board, the association office, and the volunteers from the membership. These are a dedicated and hardworking group of volunteers and we are lucky, as an association, to have them donate their time and talents. They are also very approachable, and so if you have any issues or ideas that are relevant to the association, please do not hesitate to contact them or myself.

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Wolffish Culture: A Productive Partnership

In early June 2002, a 2-day workshop entitled *Wolffish Culture: A Productive Partnership* was held in Rimouski, Quebec. The event was hosted jointly by government, research and industry partners from Quebec, Newfoundland and Norway. Support came from many provincial goverment sources in Quebec, including MAPAQ (Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec), DEC (Développement Économique de Canada) and SODIM (Société pour le développement de l'industrie maricole). Support was also provided by AquaNet, the National Research Council and the Newfoundland Department of Fisheries and Aquaculture. Support from Norway was provided by the Norwegian Industrial and Regional Development Fund. Workshop coordination and facilities were provided by the Université du Québec à Rimouski, the Marine Institute of Memorial University of Newfoundland, and the University of Tromsø. The Aquaculture Association of Canada supported the workshop by publishing these proceedings.

The workshop provided an opportunity for the aquaculture industry and research community to discuss various aspects of wolffish culture, including the culture strategies and technology used during the production cycle of the spotted (*Anarhichas minor*) and common wolffish (*A. lupus*). Private interests involved in wolffish cultivation present at the event were Akvaplan-niva and Troms Steinbit of Norway, Marifin Inc. of Quebec, and Atlantic

Partners in the "joys"of coordinating the workshop were Laura Halfyard, MI-MUN (left), Nathalie Le François, MAPAQ-UQAR (centre) and Helge Tveiten NFH-University of Tromsø.

The gathering of the "wolffish crew' for the First Wolffish Culture Workshop (Photo: M. Bélanger)

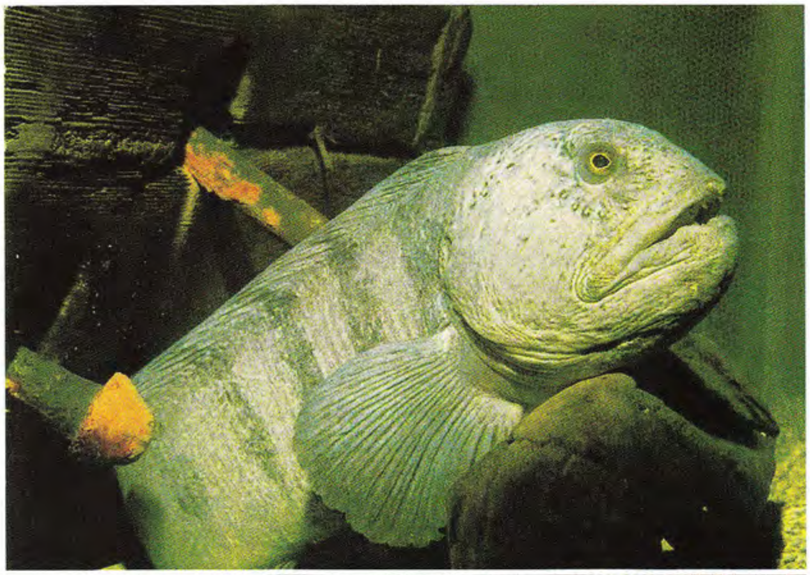
Agrifoods Ltd. of Newfoundland.

The workshop provided a forum for presentation and discussion of various marketing issues including the European and North American market niches for wolffish fillets and leather products, potential names for the product, problem of fat deposition in the tissues, consumer preferences for taste and texture, and marketing strategies.

A banquet and seafood demonstration provided insight into the potential of this high quality white-fleshed product as a high-end restaurant food and as a quality leather product. Marketing strategies will need to delve into these various options to increase the economic returns of wolffish culture, particularly since the culture technology is, at present, primarily reliant on expensive land-based systems.

During the past 20 years, research into culture methods has enabled the successful completion of the full life-cycle of wolffish in captivity. Issues that need to be addressed in the future include: differences in the culture performance of the spotted and common wolffish, and the refinement of culture techniques for the various life stages to improve efficiencies and to reduce the timeline of the production cycle. Growth needs to be improved through developments in feeds and feeding methods, by optimizing environmental conditions, and by monitoring health risks. Improvements in broodstock nutri-

Common wolffish *(A. Lupus)* (Photo: Memorial University)



Spotted wolffish (Photo: Memorial University)

tion, genetics and reproductive strategies should also increase egg quality and survival rates.

Much of the technology used for wolffish culture has been adapted from existing salmonid and marine finfish technology, particularly raceway and land-based tank systems. The financial feasibility of culturing wolffish in the various rearing systems, however, needs to be investigated to improve information available to investors. Also, the industry will need to develop in a controlled manner to keep the marketing, hatchery production and grow-out phases synchronized. Private sector investment will also be required to promote the development of this industry.

Wolffish culture shows promise for regions of Canada and Norway that have high-quality seawater and the necessary infrastructure support. Of particular interest are suitable locations with the space for land-based systems, relatively cold temperatures, and a labour force to support the industry and foster economic growth. This workshop provided an opportunity for representatives of industry, government and research to discuss the various aspects of wolffish culture and to plan collaborative activities for the continued growth of this emerging industry.

— *Laura C. Halfyard*
Marine Institute, Memorial University

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Evaluation of the Potential of Marine and Anadromous Fish for Cold-water Mariculture in Quebec

Nathalie Le François, Hélène Lemieux and Pierre Blier

Concern about overexploitation of wild aquatic resources, slow recovery of the groundfish fisheries, and the need to encourage the diversification of the mariculture industry in Quebec (Canada) provides a strong incentive to explore the potential of marine and anadromous fish species for cold-water mariculture. Starting from a list of over 40 indigenous fish of potential commercial interest, a biotechnical review was initiated. Technical sheets for each species were produced and aquaculture-based selection criteria were developed for the three approaches to aquaculture development (complete production cycle (egg to egg), on-growing, and stock enhancement). On the basis of the required biological parameters, species were ranked according to their degree of suitability for each type of culture. The final classification analysis for fish cultured through the complete production cycle positioned the Atlantic wolffish as the top candidate species (score of 91%), followed by spotted wolffish and Arctic charr (scores of 87%). Growth rate, optimal temperature for growth, minimal lethal temperature, duration of the weaning period, larval size, and feed requirements were the determining criteria. The inclusion of four species of cultured salmonids (salmon, trout, Arctic charr, and brook charr) in the evaluation procedure allowed the validation of the method and comparison among species of their suitability for mariculture.

Introduction

A wide array of marine fish species (n = 47) found in the coastal environment of Quebec were evaluated for their potential use in diversifying the aquaculture industry.(1) This paper is presented in the form of a case study of a cold-water maritime region with underdeveloped aquaculture potential. There has been a pronounced decline in landings from the traditional fishery in Quebec, so there is a need for economic diversification in the maritime regions of the province. Quebec's 12 000 km coastline is characterized by extreme temperature conditions and extensive ice coverage. Mariculture activity is currently limited to cultivation of mussels and scallops. A few commercial and research initiatives on marine fish culture have been attempted, including the on-growing of juvenile cod and winter flounder taken from the commercial fishery, and the on-growing of cultured brook charr juveniles in seawater. Presently there are no marine fish culture facilities in operation. There is, however, a healthy freshwater salmonid culture industry producing rainbow trout, Arctic charr, and brook charr. The emerging mariculture industry in Quebec will be able to rely on the high-caliber research facilities and solid scientific expertise that exist in the province.

The provincial government, in collaboration with the aquaculture industry and research and university representatives, developed a strategic plan for aquaculture development in 2000.(2) Part of the plan involved the evaluation of the potential of marine finfish species for mariculture development in Quebec. The objective of the evaluation was to conduct a versatile, objective, large-scale study examining three principal mariculture production strategies: complete production cycle (egg to egg), stock enhancement, and on-growing of juveniles. The evaluation involved the use of well-defined criteria appropriate for Quebec's environmental and economic situation to (i) identify the species with the most potential for cultivation, (ii) clearly assess the potential for risk associated with a given species, (iii) identify R&D needs, and (iv) provide the basis for concerted action by the financial, government and research communities to develop marine fish mariculture in the province.

Material and Methods

The first step in developing the strategic plan for aquaculture development was the compilation of a list of 47 fish species based on the description of the demersal fish assemblage for the east coast of North

America[3] and completed using the reference book *Atlantic Fishes of Canada.*[4] The list was reviewed and refined by several mariculture and fisheries experts. The list was not exhaustive, but was comprised of a wide array of fish species in order to clearly test the outcomes of the proposed selection procedures. Technical sheets containing relevant information on critical aspects of interest in assessing the potential of the fish for each type of aquaculture was developed for each species. The species were then subjected to a selection procedure that involved two stages of selection, followed by a final classification of the suitability of the species for mariculture. The first stage of selection used simple, unequivocal and discriminating criteria of primary importance in Quebec's environmental, technical and economic context. In this first selection, a species was either rejected or directed toward a specific mariculture scenario in which the species was subjected to a second stage of selection.

The species that appeared most suitable for each of the production strategies (complete cycle, on-growing, or stock enhancement) were submitted for a closer evaluation of their potential using criteria specific to each strategy. At this second stage of selection, the species was either rejected or submitted to a final classification procedure. The final step identified species showing the strongest mariculture potential. Each criteria had a maximal score that considered its relative importance in a given culture situation and its prospects for development. Three fixed range of values were used for each criteria and the total score was then reported in terms of percentage. A lack of information about the species did not influence the final score, but allowed the rapid identification of areas of knowledge that were inadequate and could benefit from research efforts.

Results and Discussion

The final ranking of species deemed suitable for the complete-cycle production strategy (the only scenario presented in this paper) identified the Atlantic wolffish and the spotted wolffish as the marine fish species with the most potential for culture in Quebec (scores of 91% and 87% , respectively). The spotted wolffish *ex aequo* with Arctic charr (87%). The small difference in the score between the two wolfish species is likely attributable to the difference in the R&D effort expended on the two species: 10 to 15 years for Atlantic wolffish and 5 to 7 years for spotted wolfish. Despite its lower ranking, the spotted wolffish displays a faster growth rate than the Atlantic wolffish.

Wolfish are the only marine fish species that ranked higher than most salmonid species, and this occurred despite their short history as aquaculture species and the lack of refinement of aquaculture practices. The main characteristics that favour wolffish for mariculture are their high tolerance of cold temperature, rapid growth under culture conditions (fish reach 2.5 kg in 18 months at 6°to 10°C), resistance to disease and suboptimal water quality, tolerance of high rearing densities (100 kg/m^2), feed conversion rates that are similar to salmonids, low complexity of the rearing technology (similar to salmoniculture), no requirement for live prey because the juveniles are robust and there are no larval stages, high flesh yield (45-50%), no directed fisheries, decline of natural populations worldwide, and the availability of Norwegian expertise in both the private and research sectors. Commercial production in Norway will reach 10 tonnes in 2002.

After the selection procedure and prioritization of the species, the following research priorities were identified for wolffish:

1. Technology transfer
2. Broodstock collection and juvenile production
3. Nutrition and reproductive physiology
4. Optimization of growth
5. Enhancement of prospects for profitability
6. Market analysis
7. Adapting technologies used with other species to lower production costs

The evaluation program was developed in collaboration with Norway (Akvaplan-niva, the University in Tromsø and the Norwegian Institute of Fisheries and Aquaculture Research), Newfoundland (Marine Institute and Ocean Science Centre of Memorial University of Newfoundland) and Fisheries and Oceans Canada (Maurice Lamontagne Institute).

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Marketing Issues and Opportunities for Farmed Wolffish in the United States and Europe

H. Johnson and L.C. Halfyard

Market information suggests that farmed wolffish fillet products would be ranked high compared to many other marine species. Wolffish is a lean white-fleshed fish with a market niche similar to Dover sole, halibut, monkfish and sea bass. Value-added fillets would be the primary product, but secondary products (e.g., fish leather) and improvements in farming efficiencies should increase the financial returns. Development of marketing strategies should include defining the name of the product, promoting the product to up-scale restaurants, and distinguishing farmed wolffish from the wild product.

Introduction

Aquaculture accounts for approximately 20% of the total world supply of seafood products and is projected to increase to ~30% by the year 2025. Fish consumption in the United States is about 7 kg per capita and the population is presently about 274 million. Seafood imports now exceed the net domestic landings and Canada is the lead supplier of seafood to the US marketplace, with value-added processing increasing (e.g., cooked and smoked fillets).

The US seafood situation will be affected by long-term supply constraints, periodic oversupply, and an increased reliance on aquaculture products. Population growth will drive demand for aquaculture products and seafood, but environmental groups will also apply pressure on the aquaculture industry. The EU seafood market presently has a 3.1 million trade deficit and, with the decline in supply of North Atlantic groundfish species, there is increased interest in cultured species. In Europe and some high-end sectors of the US market, chefs are familiar with wolffish from the traditional fishery. Approximately 50,000 metric tons of wild wolffish product comes into the European and American marketplace from Iceland, Russia, the United States, Canada, and other countries.

Wolffish Markets

The four Ps of marketing are product, price, place, and promotion. Questions to be asked include: What product does the wolffish substitute for, in what form will the market want wolffish, what will the market pay for wolffish, what volume will the market absorb, where will wolffish be sold, and how should wolffish be promoted? Generally the American consumer prefers a seafood product that is skinless, boneless and tasteless! Wolffish is a lean, pearly white fleshed fish with a mild sweet taste and firm texture that can substitute for Dover sole, halibut, monkfish or sea bass. The marketing niche for wild wolffish is about US$4.50 per pound ($9.90 per kilogram) (Fig. 1), which is comparable to the results of a recent European wolffish marketing study indicating wolffish would sell for $9-$12 per kilogram.

Preferred products for the US market will probably be fresh boneless fillets, graded by size. There may be a market for a smaller fillet than is currently being obtained from wild fish (which often produce fillets too large for single-serving portions). A smaller fillet size could also reduce the production cycle for farmed wolffish.

Over the past 10 years there has been a substantial drop in the price of farmed salmon due to increased global production and availability, as well as reduced operating costs on the farms. It would be expected that cost reductions in wolffish farming could be gained by reducing operating costs and improving feed management strategies.

Identification of the different marketing niches for wolffish products would probably initially be directed to high-end restaurants in both Europe and the United States. Up-scale supermarkets, supermarket chains, and fish markets may be other venues for select products. Finally, value-added products may be developed for certain sectors. Issues that must be addressed to successfully market wolffish include clearly defining the name of the product, setting up direct sales organizations, promoting to up-scale restaurants, and creating a 'buzz' in the seafood sector. Some confusion may exist as to how to promote wolffish and what name to use (e.g. wolffish, Atlantic wolffish, striped

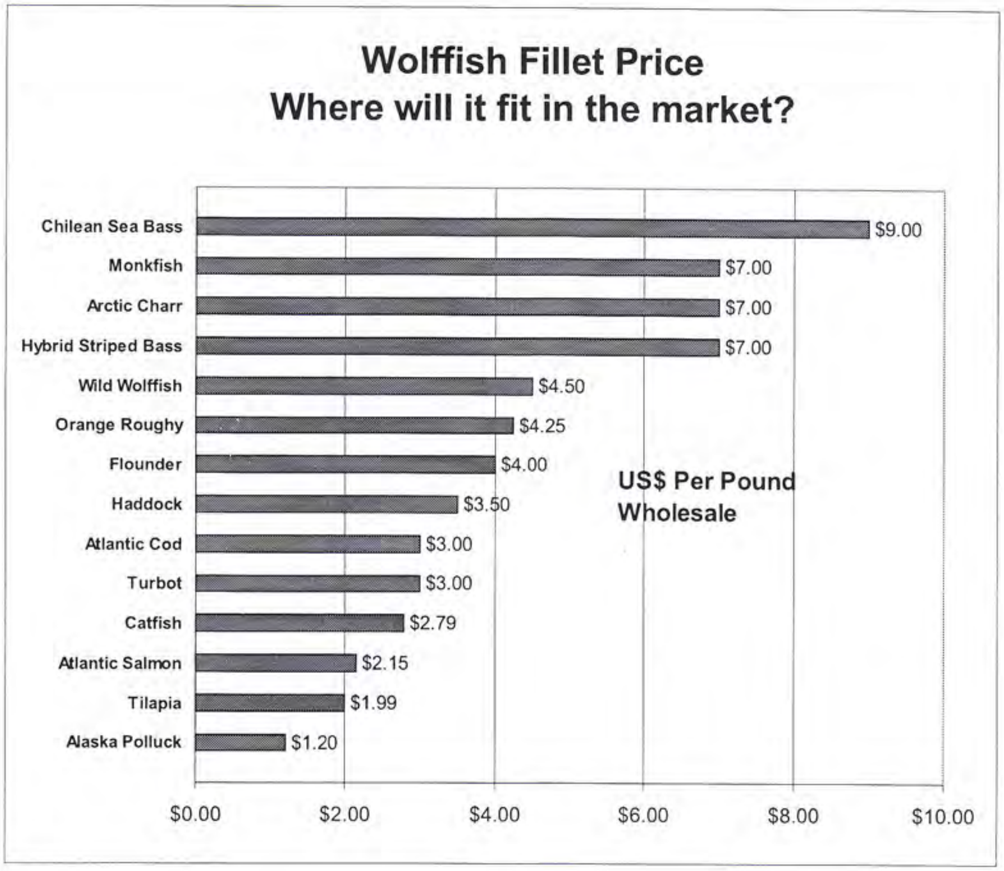

Figure 1. Various seafood products and US market prices compared with the expected fillet price(US$ per pound) for wolffish.

wolffish, ocean catfish, spotted wolffish, and loup de mer). Other examples of this problem have been addressed by developing a distinct name for the product, such as Patagonian toothfish for the Chilean sea bass and goosefish for monkfish. Some of this marketing effort on wolffish is already being done by an European company, Aquanor, which is marketing wild wolffish as 'Loup de mer', but care must be taken to distinguish farmed wolffish from wild product.

It is recommended that the aquaculture industry develop incrementally to match market demands to production volumes. Also, reducing production costs by improving feed conversion rates, growth rates, and fillet yield will help alleviate risks when market prices fluctuate. Farmed wolffish products must be differentiated from wild wolffish, but generic promotion similar to that used by the US catfish industry would probably foster greater market growth.

There appears to be a market for wolffish in the United States but it needs to be developed. American seafood lovers like variety and uniqueness and wolffish would provide another choice on the menu. In his presentation, Chef Roy Butterworth highlighted the importance of promotion of the product to restaurants and particularly to new culinary chefs who like to try different products. Supermarket demonstrations, in-store cooking classes and special banquet/dinner presentations will help increase knowledge of wolffish in the American and European markets.

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Cultured Wolffish *(Anarhichas minor)* — A Potential Species for Exclusive Restaurants?

Roger Richardsen and Jan A. Johansen

A market test of cultured wolffish *(Anarhichas minor)* was done within a selected range of exclusive restaurants in Norway, Germany and France. The study indicated that spotted wolffish has good market potential. Respondents appreciated the freshness and long shelf-life of the fish. The firm texture of the fish was regarded as both an advantage and a disadvantage: the product can withstand a lot of pre-treatment and heating in the kitchen, but the flaky texture of cod and other white fish species was preferred by chefs. The large amounts of mucus (slime) on the skin and the visible fat in the fillets lowered the chef's overall positive impression of wolffish. Therefore, the industry still needs to develop feeding and production strategies to fully meet the needs of the market. The most interesting market segments for wolffish should be the hotel and restaurant sector, which could function as a gate opener to the consumer in the long run.

Introduction

The spotted wolffish *(Anarhichas minor)* is a marine species considered to have potential for aquaculture. Research and development have succeeded in demonstrating the fish is suitable for intensive fish farming. Spotted wolffish thrive and grow rapidly at low temperatures, and coastal areas of the North Atlantic seem appropriate for farming this species. The first wolffish cultured in northern Norway will be marketed in 2002.

The coastal population in Norway previously considered the spotted wolffish to be a low value species, but this has changed considerably during the last decade. Today the fish is a common item on menus in Norwegian restaurants, and future supplies from the farming industry could provide advantages in the marketing of the species. The market price for wild-caught spotted wolffish varies seasonally and the supply is very unstable,[1] due to the fact that the fish is mainly taken as a by-catch in cod fisheries.[2] The fluctuating catch causes supplies to be unpredictable for chefs and other customers who require a reliable supply of fresh product. The majority of wild-caught spotted wolffish is marketed frozen, so the sensory quality of the product does not satisfy quality-conscious customers such as chefs in white-tablecloth restaurants.[3] Fresh spotted wolffish would meet these demands and would be appreciated by customers.[1,4]

Methods of Analyses

The purpose of the market/product test study was to identify the market position for farmed spotted wolffish in a few European markets by evaluating the perception of professional chefs of the sensory quality, price, and convenience of wolffish.

The spotted wolffish used in this test was cultured by Troms Steinbit AS, Northern Norway. The fish were shipped gutted, head on, and in sizes that varied between 3.5 and 5.5 kg. Each participating restaurant received a 20-kg box of wolffish, fresh chilled in ice for further preparation and testing. The restaurants also received a questionnaire for evaluating the product. All together, 32 white-tablecloth restaurants in Norway (13), France (10), and Germany (9) participated in the study. The restaurants were recruited by the Norwegian Seafood Export Council and chosen because of their requirement for high-quality products. The chefs were interviewed in depth 1-2 weeks after receiving the test material.

Perception of Sensory Quality

As shown in Figure 1, the chefs consider whole, gutted spotted wolffish as a very fresh fish. Further, the profiles show that a high average score was given on each product attribute except for the natural presence of slime (mucus). The results from the interviews indicate that the relatively high quantity of slime on the fish confused chefs with little or no experience in preparing the product. They considered the slime to be an indicator of low quality, so a lower score was given. The interviews indicated that chefs experienced in preparing wolffish associated the high slime content with freshness and quality. In addition, experienced chefs commented that the slime was different from mucus that appears on fish that have begun to deteriorate. In general, the overall evaluation of the profile analysis indicates that the chefs liked the quality of the fish.

As Figure 2 indicates, the Norwegian chefs were satisfied with the quality of a prepared meal of wolffish. But further analyses indicated that two product attributes lowered the overall impression of the sensory quality of the fish. Firstly, more than half the respondents perceived the taste of wolffish as being too neutral, lacking a distinctive fish taste and having taste elements of crustaceans. Secondly, some chefs considered the consistency or texture of the flesh as "too closed" or firm. Those chefs preferred a more open or flaky texture, which provides an opportunity to add spice, juice, and/or sauces to the fish before serving.

Estimates of Price and Product Categories

Table 1 indicates the price chefs were willing to pay for whole gutted and fillet products from spotted wolffish. Be aware, however, that these indicative prices are at the consumer level and are not a farm gate price. Half the chefs preferred whole, gutted product to fillets. Time-saving preparation is the main argument in favor of fillets, while the desire for quality control guides the preference for whole product.

Choice of Future Product and Market Strategy

Our results indicate that chefs perceive the spotted wolffish as a product with strong attributes, some of which will be of major importance when cultured wolffish are introduced into the market place. A majority of the respondents considered the relatively high amount of slime/mucus on the skin to be a negative attribute. Experienced chefs recognized that mucus is typically present on extremely fresh, chilled spotted wolffish. This characteristic of fresh wolffish should therefore be communicated to avoid misconceptions about the quality of the product. One of the strongest product attributes of the fish seems to be the extreme length of the shelf life. Exclusive restaurants consider freshness one of the most important product attributes and this feature of wolffish should be emphasised in market communications.

Further, the relatively high amount of visible fat had a negative effect on the overall positive impression of wolffish. Farmers may have to reduce the fat content, either by starving the fish prior to slaughtering or by reducing the fat content of the feed. The market research indicated that chefs generally find that all

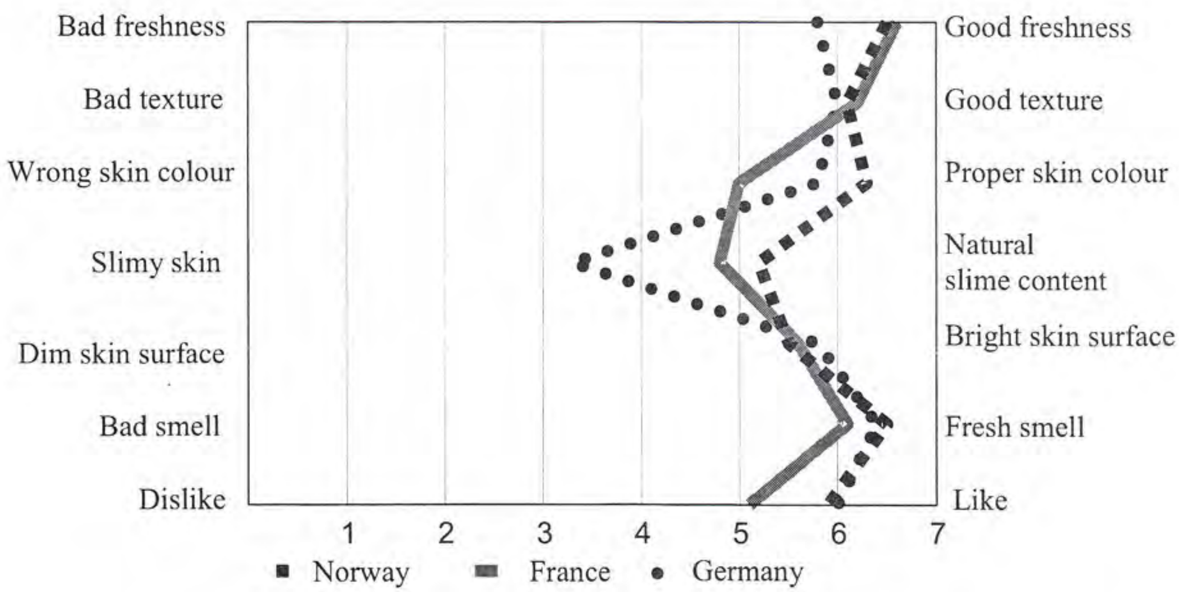

Figure 1. Profile analysis of whole, gutted spotted wolffish

Table 1. Estimates of the prices chefs are willing to pay for spotted wolffish.

	Price per Kilogram of Product		
Product	**Norway**	**Germany**	**France**
Gutted with head on	US$6.40	US$7.40	US$7.40
Fillets	US$8.00	US$10.80	US$11.60

farmed fish are too fatty. A change in the feed formula would probably be a good approach to produce a cultured fish that is similar to wild-caught fish.

Another finding of the study was that cultured wolffish lack the characteristic taste of wild-caught fish. Some of the chefs evaluated the neutral taste as an element of uncertainty when they considered whether they would buy the fish in the future. This point could, however, be a strong positive attribute in market areas such as the United States. The only way to alter the perceived taste of the flesh is to change the feed formula by including ingredients that are part of the natural food of the species.

Several respondents evaluated the texture of the product as firm compared with seabass *(Dicentrarchus labrax)* and Atlantic cod *(Gadus morhua)*. However, some chefs considered the firm structure of the fillet as an advantage if the fish are being barbecued or exposed to a long lasting heat treatment such as during banquets. The firm texture of the flesh appears to be a product strength and should be emphasised in future market promotions.

Farmers should be aware that chefs have an underlying negative attitude to fish farming and the quality of farmed fish. Fish farmers, trade associations, and exporters have a considerable challenge to improve the customer's trust of the industry. Information on cultured products and communication to the public should be based on factual information and documentation of product quality and safety in the food production chain.

This study indicates that spotted wolffish has good market potential. The product seems to be ranked on a high level, comparable to product like farmed Atlantic salmon *(Salmo salar)*. The most interesting market segments should be the hotel and restaurant sector,

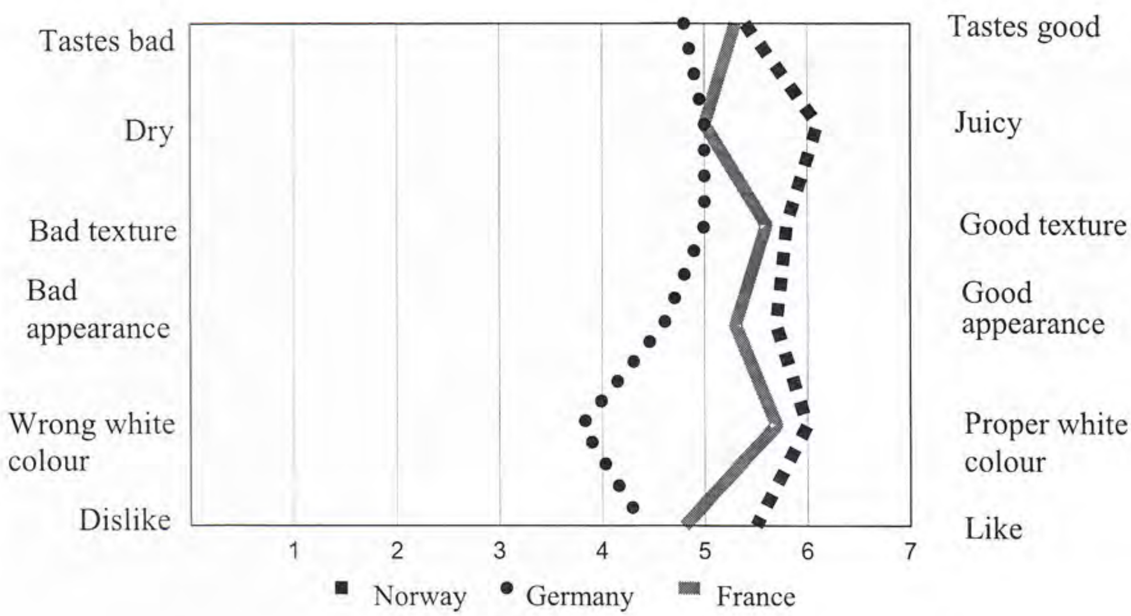

Figure 2. Profile analysis of prepared meal from spotted wolffish

which could function as a gate opener to the consumer in the long run. The quality attributes indicate a particular potential as a "banquet fish" which could be a strong driver of market demand.

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“Getting to Know Your Product”

L.C. Halfyard

“Getting to know your product” was one of the themes of the wolffish workshop, which was highlighted by an excellent demonstration by Chef Roy Butterworth, an award-winning Canadian chef from New Brunswick. Fresh Norwegian farmed wolffish, produced by Tomma Marin, was filleted, prepared and presented in a superb dinner featuring roasted wolffish.

Other marketable products from wolffish include leather items made from the skin, value-added products such as smoked wolffish, and possibly biomolecular products.

Chef Roy Butterworth indicated that the high-end restaurant sector is receptive to seafood products that are unique, mild tasting and very white. He also felt that wolffish showed promise for the banquet service sector since it has a high level of stability when cooked, which is especially critical as delays often cause concern for the chef about the the potential for overcooked or dry dinners!

Opportunities to promote wolffish include demonstrations to various culinarians, especially those in high-end restaurants, and supermarket in-store cooking classes.

Farmed Norwegian spotted wolffish flown fresh from Tomma Marin, Norway (M. Bélanger photo).

Lively discussions of wolffish product by the Norwegian, Canadian, and US crew. L to r: Laura Halfyard, Helge Tveiten Inger Andreassen, Howard Johnson, and Lars Olav Sparboe (M. Bélanger photo).

The beauty of wolffish leather products was sported by Norwegian researcher Sigrun Espelid from FiskeriForskning, who also enjoyed receiving one of the first servings of roasted wolffish served with wild mushrooms, port wine balsamic syrup, sweet potato and summer savory crisp (L.C. Halfyard photo).

Chef Roy Butterworth from New Brunswick preparing and serving farmed spotted wolffish to Joe Brown from Memorial University of Newfoundland, one hungry researcher who enjoyed the five-star gourmet meal! (M. Bélanger, photo)

The Quebec wolffish crew and 'the slaves' who ran around doing numerous things during the workshop! (L to r: Jean Claude Blais, Marifin Inc., Bruno Archer, Marifin Inc., Nathalie Le François, Simon Lamarre, and Delphine Ditlecadet) (M. Bélanger photo).

After dinner wolffish joke … What do you get when you feed a wolffish?The finger!!! (L to r: [illegible] Sparboe, Inger-Britt Falk-Petersen, .

Extraction of High-Value Biomolecules to Diversify Mariculture Production of Wolffish

Nathalie Le François and Pierre Blier

The profit margin of aquaculture operations based exclusively on flesh production is vulnerable to fluctuations in market prices and feed, labor and/or energy costs, an obstacle to new finfish mariculture initiatives. This project proposes to evaluate the economic potential of extracting high-value biomolecules from cultivated fish to enhance the profitability of aquaculture. This type of information could encourage interest from private investors in developing the mariculture potential of Quebec's maritime regions. The Atlantic and spotted wolffish (*Anarhichas lupus* and *A. minor*) have been identified as marine fish species with high potential for cultivation in Quebec's environmental conditions. We propose to evaluate and compare both species for seasonal and size-related variations in 1) production of antifreeze proteins, 2) content and properties of antimicrobial polypeptides, and 3) content and kinetic properties of digestive enzymes.

Introduction

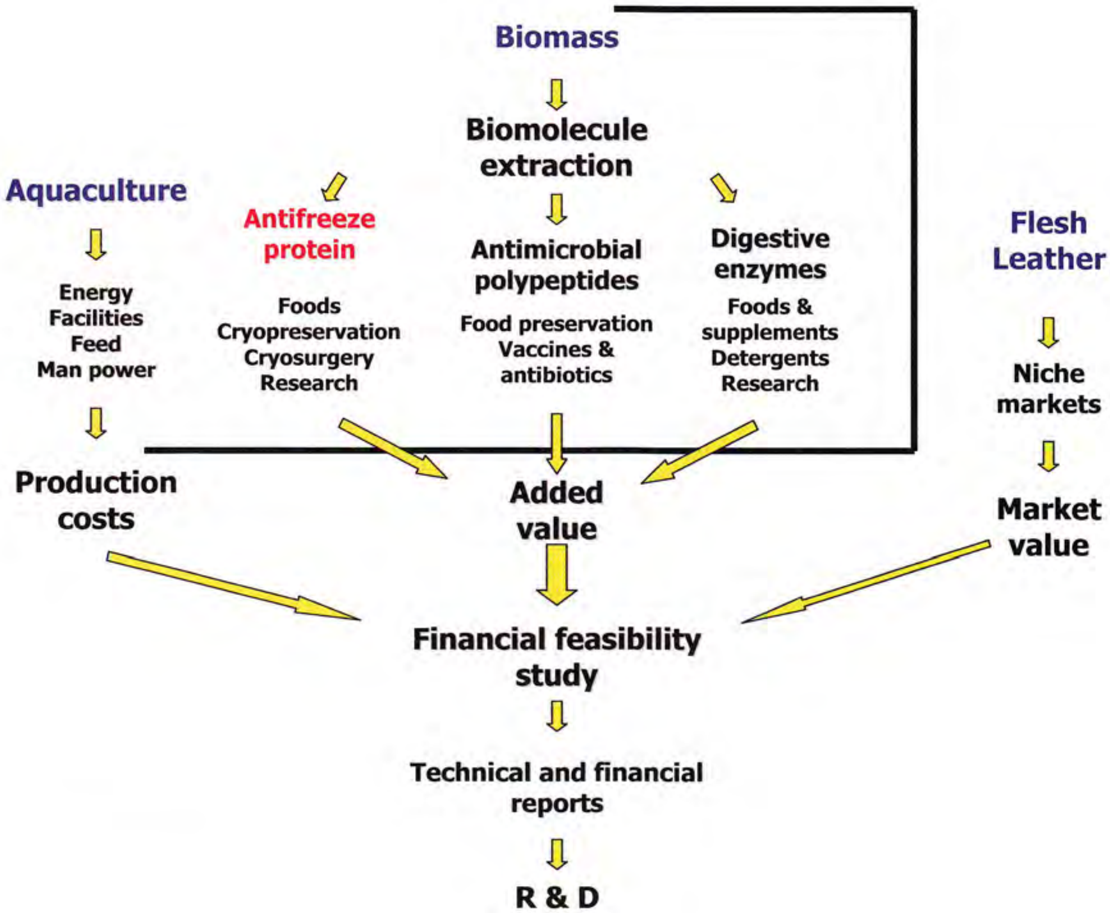
In the Province of Quebec (Canada), there is growing interest in marine fish farming, as the coastal environment offers an abundance of rearing sites. The provincial and federal governments are actively supporting the development of mariculture (scallops, mussels, finfish, etc.), but there is still no marine-based production of fish in Quebec. The spotted and Atlantic wolffish (*Anarhichas minor* and *A. lupus*, respectively) have been identified as species with high potential in Quebec's environmental conditions.[1] Wolffish are tolerant of the cold, resistant to disease and stress, are able to withstand low oxygen as well as low salinity conditions, and can be reared at very high densities. They hatch fully developed and readily feed on commercial formulations, so live prey is not required. Because of their sedentary habit, they convert most of their energy to growth and grow rapidly in captivity. Unlike marine fish species such as Atlantic cod (*Gadus morhua*) and Atlantic halibut (*Hippoglossus hippoglossus*), the culture of wolffish is similar to that of salmonids (fecundity, size of eggs and larvae, acceptance of formulated feeds, survival, rearing requirements), making technology transfer to the wolffish industry easily conceivable.

Wolffish are not targeted by commercial fisheries (by-catch) in Canada and the catches elsewhere (mainly Iceland) can be deficient during the year, providing opportunities to establish a niche market supplied by year-round aquaculture production. Popular in Europe, wolffish is a quality product that has never been strongly targeted in North America. Recently, wolffish was given the Gold Award for the best new product at the 2000 International Boston Seafood Show. It features long, thick, white-fleshed fillets, that are delicate in taste, firm in texture, boneless, parasite-free and rich in omega-3 fatty acids. Norway is currently the only producer of cultured wolffish and there are pre-commercial spotted wolffish farms in the region of Tromsø. Newfoundland and Quebec are both presently involved in R&D activities on spotted and Atlantic wolffish, in collaboration with Norway. Iceland and Chile are also interested in these novel mariculture species.

In the aquaculture sector, a company's profitability depends on production costs and market price, which is subject to regular fluctuations. Therefore, product diversification could stabilize and potentially increase returns. There are three biomolecules that could be extracted from wastes of the aquaculture industry: antifreeze proteins (AFP),[2] antimicrobial polypeptides (AMP),[3] and digestive enzymes (DE).[4] Given their numerous applications in diverse biotechnological fields and, consequently, their high market prices, these biomolecules could confer a substantial added value to the basic aquaculture product.

The originality of this approach to aquaculture di-

Integrated Approach for the Evaluation of the Potential for Commercialization of High-Valued Biomolecules within an Aquaculture Production Cycle



versification is in the integration of the possibilities for diversification early in the process of establishing a new industry. This conference paper is aimed at the potential of antifreeze proteins only.

Antifreeze Proteins (AFPs)

Several species of cold-water marine fishes produce antifreeze proteins (AFPs) to avoid freezing of their tissues. Until now, four AFP forms have been characterised.[5] These proteins are over 500x more effective at depressing the freezing point of tissue than other blood osmolytes. They can also modify or suppress growth of ice crystals, inhibit recrystallisation, and protect membranes against cold-induced damage. AFPs are expected to have many applications and are presently being actively studied. Possible uses for AFPs include incorporation into frozen foods (e.g., dairy products) to prevent recrystallisation of ice crystals already present in the product (to preserve the original texture), and as cryoprotectants in cell culture, and gamete and organ cryopreservation. Another potential biomedical application lies in cryosurgery. This special technique uses AFPs to kill cancerous cells by affecting their action on crystal growth.

The principal source of AFPs is plasma from marine fish species living in cold water. Such fish can have AFP concentrations ranging from 1 to 30 g/L of plasma. The average price of antifreeze proteins is \$500/mg. According to predictions from A/F Protein Canada,[5] demand for this resource will soon exceed production capacity, so a number of researchers are attempting to develop large-scale AFP production by using genetic methods such as recombinant DNA. However, reticence about genetically modified organisms (GMOs) and the limit imposed by the natural resource itself could provide a marketing niche for naturally-derived AFPs and other biomolecules from the aquaculture industry.

Atlantic wolffish synthesize type III AFP in their blood, the best of the three types tested in protecting bovine oocytes from cold-induced damage. AFP production by wolffish could allow the rearing of this species in sea cages or net-pens all year long in Quebec. In most fish that have been found to synthetize AFP, two abiotic factors influence the production of the biomolecule: water temperature and, principally, photoperiod. Atlantic cod (*Gadus morhua*) and the winter flounder (*Pseudo- pleuronectes americanus*) produce AFP seasonally, while the ocean pout (*Macrozoarces americanus*) produces AFP year-round, although production is higher during the winter than the summer. Little is known about the pattern of production of AFP in wolffish. To assess the potential of AFP production by a wolffish aquaculture operation aimed at the biomolecule market, production should be characterised throughout the year. Considering that intra-species AFP production variations exist, the difference between species and the effect of age and size of the fish on protein production should be evaluated.

Perspectives

With recent developments in marine biotechnology and identification of this development axis as a priority for eastern Quebec, the efficient use of by-products originating from fisheries and aquaculture activities should be assessed. However, before entrepreneurs can include extraction and purification of high-value biomolecules in their business plans, more information is needed on the potential for production of these biomolecules from aquaculture operations, access to markets, and the value of these biomolecules. Therefore, technical and biological feasability studies on biomolecule extraction, plus an integrated market analysis, should be initiated.

This study is part of a larger-scale project on the evaluation and feasibility of extracting high-valued biomolecules from residual biomass produced by a wolffish culture facility.

Objectives

- Characterize the physiological response of cold resistance in both Atlantic and spotted wolffish, and
- Determine the annual pattern of production of AFP.

Sub-objectives

- Evaluate the influence of fish age and seawater temperature on AFP production,
- Evaluate inter- and intraspecific differences in AFP production, and
- Measure the effect of AFP production on hepatic energy metabolism.

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Status of Wolffish Broodstock Studies in Quebec

Nathalie Le Francois and Jean-Denis Dutil

In 1999, research and development activities on the Atlantic and spotted wolffish (*Anarhichas lupus* and *A. minor*) were initiated in Quebec, Canada. There is interest in these species because of their potential use in diversifying the mariculture industry.

Broodstock wolffish collected by local fishermen in the spring and fall season form two captive wild populations that are being maintained at Centre Aquacole Marin in Grande-Rivière and at the Maurice Lamontagne Institute in Mont-Joli. The populations consist of 60 spotted wolffish and 20 Atlantic wolffish. At Centre Aquacole Marin, optimization of the rearing facilities has made it possible to provide the relatively low temperatures that the broodstock require during the summer months. A method to monitor eggs maturation in maturing wolffish females has been developed using echography technology. The spawning behavior of the female fish has been carefully observed and documented.

Routine veterinary evaluation of the health of the broodstock is being done and no outbreaks of disease have occurred. Cicaterization problems (scarring) have occurred in the fish in the weeks following capture, but in most cases it was adequately controlled.

Several wolffish released eggs in 2000 and 2001, but none of the eggs were fertilized. Poor egg quality is suspected to be responsible for the results. In 2002, the broodstocks can be considered to be stabilized and are being maintained in near optimal conditions, including exposure to seawater temperature that is less than 10°C throughout the year. Improvements in the environmental conditions in which the broodstock are being held are expected to have a positive effect on egg quality and make it possible to pursue upcoming R&D initiatives on the juvenile stages of the spotted wolffish. Cryopreservation techniques have been evaluated in order to stabilize the sperm supply during fertilization trials. A commercial cryopreservation product currently available in Europe has been identified as promising.

Incubation and on-growing of juveniles has been successfully realized using egg masses of Atlantic wolffish obtained from the wild. Currently, over five hundred 3+ juvenile Atlantic wolffish are being held in Quebec and a large proportion of this captive population are sexually mature. Fertilization trials on these fish will be initiated in the coming year.

At this stage, preparation of a major proposal aimed at the development of hormonal techniques to follow ovarian maturation of both species of wolffish is the subject of our efforts to secure funding for wolffish research.

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Jean-Denis Dutil *is a researcher at the Maurice Lamontagne Institute, Fisheries and Oceans Canada, Mont-Joli, Quebec.*

New Publications

Rural Aquaculture, P Edwards, DC Little, & H Demaine, eds., CABI Publishing, 368p., 2002. Hardcover, ISBN 0-85199-565-9, US$90.00. Addresses key issues in aquaculture and rural development, including case studies from several countries in southeast Asia. Topics range from production and technical issues to social aspects and R&D methodology (www.cabi- publishing.org/bookshop)

Nutrient Requirements and Feeding of Finfish for Aquaculture, CD Webster & CE Lim, eds. CABI Publishing, 2002. Hardcover, 448p., ISBN 0-85199-519-5, US$140.00. Book provides information on nutrient requirements, feed formulations and feeding practices of species cultured around the world. Chapters have detailed feeding information on specific species (www.cabi-publishing.org/bookshop).

Diseases and Disorders of Finfish in Cage Culture, PTK Woo, DW Bruno & LHS Lim, eds. CABI Publishing, 2002. Hardcover, 400p., ISBN 0-85199 -443-1, US$149.00. Book is an authoritative reference to infectious and noninfectious diseases of cold and warm water finfish in cage culture. Intended for aquaculturists, consultants, pathologists and researchers (www.cabi-publishing.org/bookshop).

Aquaculture and Fisheries Biotechnology, by RA Dunham, 2002, CABI Publishing. 380 p., hardcover, ISBN 0-85199-596-9, US$100.00. Book gives an overview of the rapidly advancing area of genetic improvement of fish for aquaculture and related fisheries. Topics covered include polyploidy, sex reversal and breeding, cloning and nuclear transplantation, biochemical genetics, gene mapping, isolation and cloning, transgenic fish, and commercial applications. (website www.cabi-publishing. org/bookshop)

Responsible Fisheries in the Marine Ecosystem, M Sinclair & G Valdimarsson, eds., 2002, CABI Publishing. 400p., hardcover, ISBN 0-85199-633-7, US$140.00. Book was developed from an international conference in Iceland in October 2001. Contents include a global overview of marine capture fisheries, legal protection for marine ecosystems, and incorporating ecosystem considerations in fisheries management. (www.cabi-publishing.org/bookshop).

Fish Hatchery Management, 2nd ed., GA Wedemeyer, ed., American Fisheries Society, 2002. 751p. Epanded from the first edition, it includes topics on hatchery operation, production, water issues, transportation, stocking, open systems, semi-controlled systems, broodstocks and spawning, nutrition and feeding, and fish health. Available from the American Fisheries Society (e-mail afspubs@ pbd.com).

Biological Indicators of Aquatic Ecosystem Health, SM Adams, ed., 2002, American Fisheries Society. 520 p. Book provides information for designing and applying bioindicators in the field to assess the health of aquatic organisms and ecosystems. For information e-mail afspubs@pbd.com.

Arctic Charr Aquaculture, by G Johnston, 2002, Iowa State University Press. 304 p., hardcover, ISBN 0-85238-272-3, US$92.99. Provides the practical and theoretical information to successfully culture Arctic charr. Gives a general overview of the subject, a practical guide for a producer operating an Arctic charr farm, and the business of commercially producing Arctic charr. Iowa State University Press (tel 515 292-0140, fax 515 292-3348, website isupress.com).

The Icelandic Fisheries: Evolution and Management of a Fishing Industry by Ragnar Arnason, 2001, Iowa State University Press. 192p., hardcover, ISBN 0-85238-210-3, US$84.95. This book gives an overview of the Icelandic economy, the development phases and position of the fishery. It also discusses the catching, processing, and marketing aspects of the industry and examines the success of the Icelandic fisheries management system. Iowa State University Press. (tel 515 292-0140, website isupress.com).

Conservation of Fresh Water Fishes: Options for the Future, by Maria Collares-Pereira, Ian G. Cowx, & Manuela Coelho, 2002, Iowa State University Press. 472 p., hardcover, ISBN 0-85238-286-3, US$144.99. Book provides a wide range of view points from specialists around the world on key issues of fish conservation such as environmental pressures, introduced species, and over-fishing. Iowa State University Press (tel 515 292-0140, fax 515 292-3348, website isupress.com).

Management of Wolffish Broodstock

Helge Tveiten

This article summarises results of studies on the management of wolffish broodstock covering several aspects of the reproductive cycle of male and female fish: temperature requirements, photoperiod manipulation of maturation and spawning, endocrine regulation, and hormonal stimulation of sperm production. Exposure of common wolffish (*Anarhichas lupus* L.) to high temperature during ovarian growth (12°C versus 4°C or 8°C) resulted in delayed ovulation, reduced egg production, and low egg survival. An increase in temperature from 4°C to 8°C or 12°C during the breeding season (time of final oocyte maturation and ovulation) delayed ovulation and reduced the survival of eggs to the eyed stage from 73% at 4°C, to 48% at 8°C, and 8% at 12°C. Sperm quality did not differ between the groups, irrespective of whether fish were exposed to the various temperature treatments during the summer months or during the breeding season in late autumn. Computer assisted sperm analysis (CASA) was used to examine the motility characteristics of spotted wolffish (*Anarhichas minor*) sperm. Sperm motility was greatest in solutions of 200 to 500 mOsm, decreased rapidly below 200 mOsm, and declined more slowly above 500 mOsm. Gonadotropin releasing hormone analogue (GnRHa) treatment had little effect on sperm quality in spotted wolffish. There was, however, a trend for fish given the highest dose of GnRHa to produce active sperm over a longer period of time than fish in the control group. More than two years of photoperiod manipulation were required to shift broodstock to a reproductive cycle that was 6 months out of phase with the normal breeding period. The effect of photoperiod on the timing of ovulation was reflected in a corresponding temporal change in plasma sex steroid hormones.

Introduction

This article summarises results of studies on the management of wolffish broodstock carried out at the University of Tromsø, Norway. It includes published data and preliminary results from recent experiments on several aspects of the reproductive cycle in both male and female fish: temperature requirements, photoperiod manipulation of maturation and spawning, endocrine regulation, and hormonal stimulation of sperm production.

Effects of Temperature on the Reproductive Biology of Common Wolffish

The effects of temperature on reproductive development in the common wolffish were studied in groups of male and female fish subjected to a range of temperature treatments during different stages of the reproductive cycle. Exposure to high temperature during ovarian growth (12°C versus 4°C and 8°C) had a negative effect on egg production (lower fecundity and smaller eggs) and egg survival (Fig. 1).[1] As a consequence, total reproductive output was reduced in fish exposed to high temperature. Elevated temperature also affected the time of final maturation: ovulation was delayed by about 4 and 5 weeks in groups of fish exposed to 8°C and 12°C compared to the fish in the 4°C group (Fig. 2). Temperature exposure caused temporal shifts in sex steroid profiles. In fish exposed to 8°C and 12°C, the peaks of both testosterone and oestradiol-17β were delayed by 4 weeks in comparison with those held at 4°C throughout the experiment (Fig. 2).[2] These differences corresponded to shifts in timing of ovulation. The results indicate that reproductive endocrine homeostasis in wolffish is influenced by temperature treatment during ovarian recrudescence, and that temperature experienced at this stage of the reproductive cycle also influences the timing of ovulation.

Effects of temperature on egg survival were more pronounced when fish were exposed to higher temperatures during final oocyte maturation and ovulation than during ovarian growth. An increase in tem-

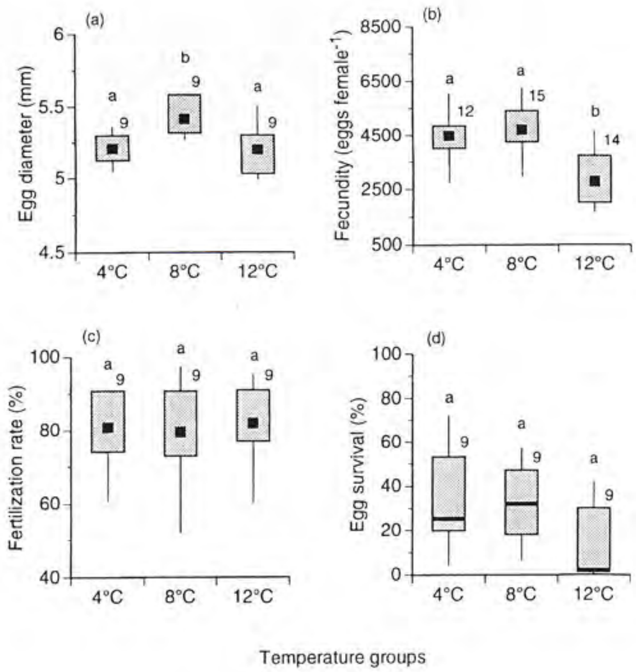

Figure 1. Egg diameter (a), absolute fecundity (b), fertilization rate (c), and egg survival to the eyed stage (d) of common wolffish exposed to different temperatures during vitellogenesis. Box-plots show average (a-c) or median (d), and the mid 50%, and 80% of the values. Values that are not significantly different ($P > 0.05$) share common superscripts. The number of fish in each group is indicated above the boxes. [from Journal of Fish Biology 55:809-819]

perature from 4°C to 8°C or 12°C during the breeding season delayed ovulation and reduced the survival of eggs to the eyed stage (from 73% at 4°C, to 48% at 8°C, and 8% at 12°C) (Figs. 3, 4)).[3] The high mortality of eggs from fish exposed to 12°C was associated with a high incidence of abnormal cell cleavage during the early stages of development. In eggs from fish exposed to 8°C, the increase in mortality occurred at the eyed stage and was independent of fertilization rate and early cell cleavage (Fig. 4). Although egg survival decreased with increasing temperature, survival was also related to the length of time that individual brood fish had been exposed to elevated temperatures prior to ovulation.

Temperature within the range used in our studies did not have any clear effect on the reproductive performance of male fish. Plasma concentrations of sex steroids associated with spermiation were low (< 2.0 ng/mL) and there was no indication that steroid concentrations were affected by temperature. The number of spermiating fish, sperm concentration, and sperm motility did not differ between the groups, irrespective of whether fish were exposed to the temperature treatments during the summer months or during the breeding season. We did not observe any effect of temperature on fertilization rates, indicating that temperature did not influence the ability of sperm to initiate cell cleavage.

Unusual Characteristics of Sperm of the Wolffish *Anarhichas minor*

Unlike the sperm of most teleosts, those of the wolffish are motile on stripping and can retain activity for at least 2 days. The sperm lose motility when exposed to sea water. Computer assisted sperm analysis (CASA) was used to examine the motility characteristics of wolffish sperm. Straight line velocity (VSL), beat cross frequency (BCF), and percent motility were the most sensitive indicators of movement. Sperm trajectories differed from those of other teleosts. Wolffish sperm display large side-to-side movements of the sperm head and an irregular trajectory. This may be an adaptation to swimming in the viscous gelatinous egg mass. Motility was unaffected by pH from 5.0 to 9.0, but was lower at pH 4.5. Sperm motility was greatest in solutions of 200 to 500 mOsm, but declined above 500 mOsm, and decreased rapidly below 200 mOsm. The unusual characteristics of wolffish sperm may relate to the fish's spawning strategy, which involves mixing of the sperm with eggs that are contained in a gelatinous mass.

Effects of Gonadotropin Releasing Hormone Analogue (GnRHa) on Sperm Quality of Spotted Wolffish

The effects of GnRHa treatment on sperm quality were examined by treating sexually mature male fish with 0 (control), 50 and 150 µg GnRHa/kg given intraperitonally in cholesterol pellets containing 5% coconut butter. Fish were sampled after 1, 2, 4 and 6 weeks. GnRHa treatment did not result in any significant change in sperm volume, activity, or density. In fish given the highest dose, however, there was a transient 54% increase in sperm volume after one week.

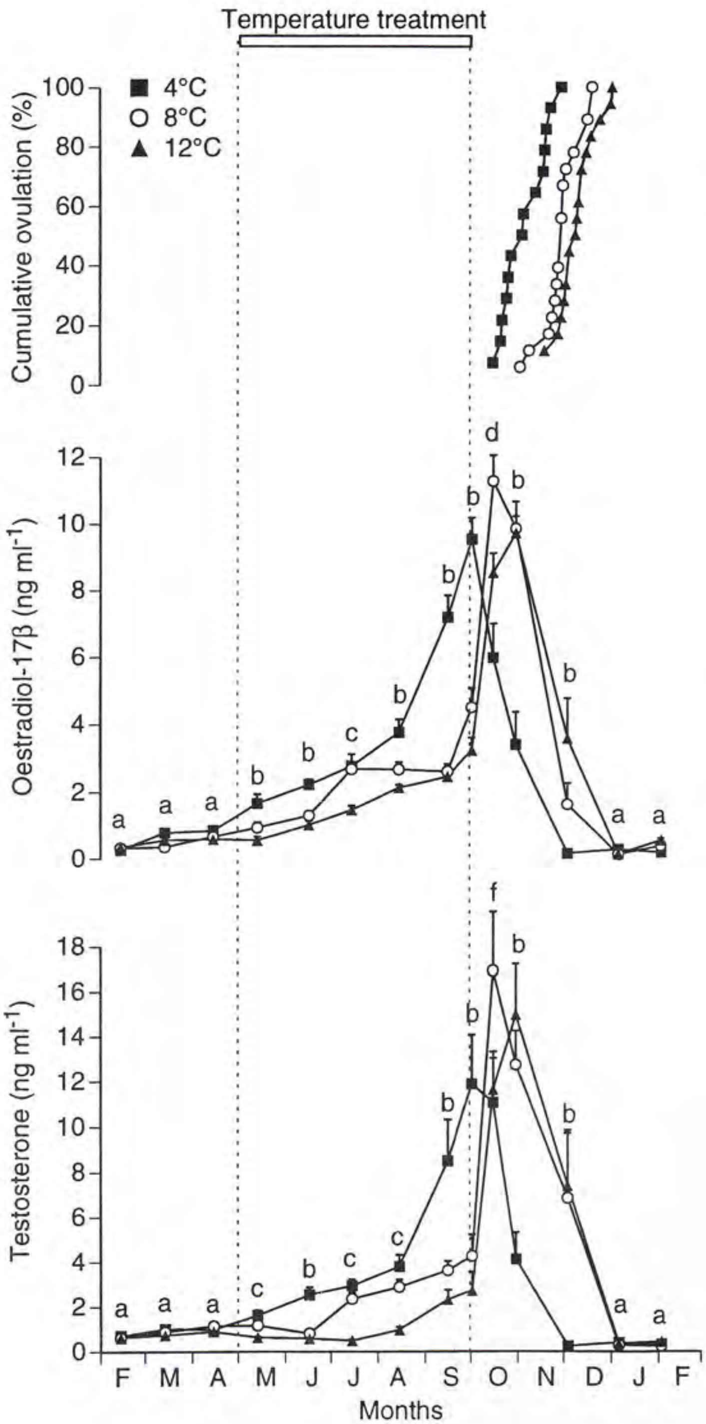

Figure 2. Timing of ovulation (upper panel), plasma oestradiol-17β concentrations (mean ± S.E.) (mid panel), and testosterone concentrations (lower panel) in female common wolffish exposed to different temperatures during ovarian recrudescence (a = no difference between groups; b = 4°C different from 8°C and 12oC; c = 4°C and 8°C different from 12°C; d = 8°C different from 4°C, but not from 12°C; f = 8°C different from 12°C, but not from 4°C. The number of maturing fish were 14, 18 and 18 in the 4°C, 8°C and 12°C groups, respectively. [from Journal of Fish Biology 59:374-385]

There was also a trend for fish given the highest dose of GnRHa to produce active sperm over a longer period of time than fish in the control group. Sperm density decreased gradually over time in fish from all treatment groups.

Year-round Supplies of Eggs and Fry: Using Photoperiod to Manipulate Maturation and Spawning

It is advantageous to be able to manipulate maturation and spawning time so that eggs and fry are produced year-round. Manipulation of maturation might also benefit the grow-out of wolffish if maturation could be prevented and the reduced flesh quality associated with maturation avoided. Our aim was to alter spawning time of the spotted wolffish in an attempt to establish spring and autumn spawning broodstocks (i.e., 6 months out of phase with the normal cycle). Under normal light and temperature cycles the broodfish usually breed during late autumn. One group of spotted wolffish, previously exposed to continuous light, was subjected to two seasonal light cycles compressed into 9 months, and thereafter to a simulated natural light regime. This gave a photoperiod regime that was 6 months out of phase with that experienced by fish held under natural photoperiod conditions. Both groups were held at ambient temperature (4°to 8°C).

During the first breeding period, after 3 to 4 months of photoperiod manipulation, only a few fish ovulated and there was only a small advancement in the timing of ovulation in fish exposed to the compressed light cycle. Towards the end of the second compressed light cycle, ovulation commenced in July in fish exposed to the compressed light cycles, and the breeding period was advanced by 3 to 4 months compared to that of fish exposed to the natural light cycle. The effect of photoperiod on the timing of ovulation was reflected in corresponding temporal changes in plasma sex steroid hormones. During the second reproductive cycle, under which the fish were exposed to a simulated natural light cycle 6 months out of phase with the natural light regime, ovulation started in April, 6 months earlier than in fish exposed to a natural light cycle.

Thus, over 2 years of photoperiod manipulation were required to establish broodstock showing 6-month shifts in the timing of the

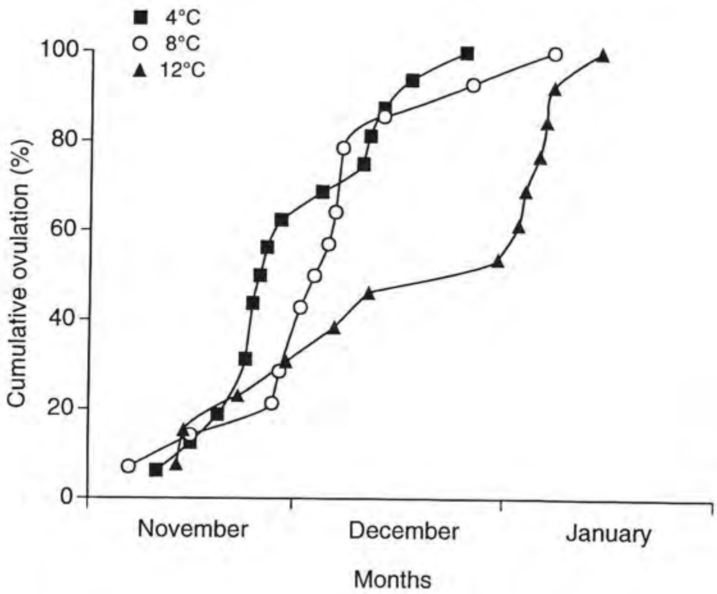

Figure 3. Influence of temperature experienced during the spawning season on the cumulative percentage of ovulated female common wolffish over time. [from Journal of Fish Biology 58:374-385]

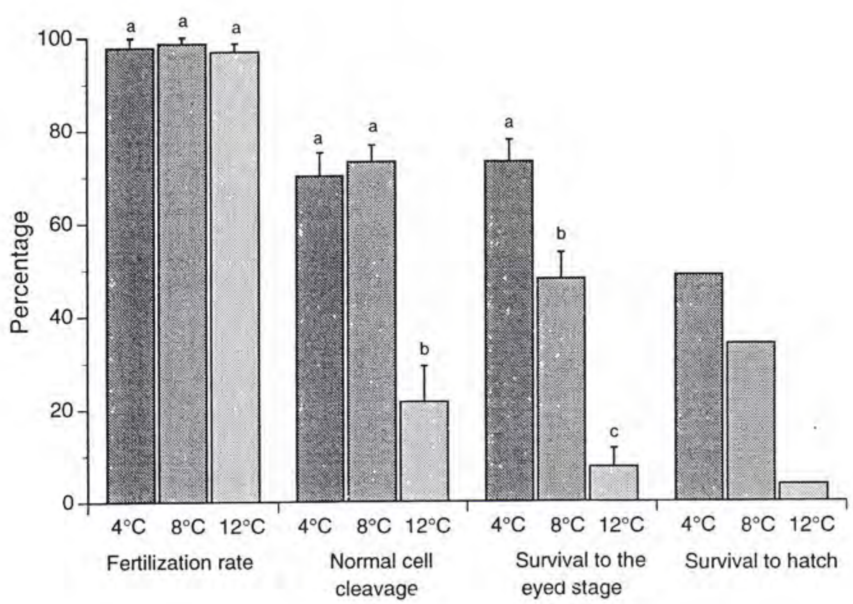

Figure 4. Fertilization rate (mean ± SE), and percentages of normally cleaved eggs, survival of eggs to the eyed stage and to hatch from female common wolffish exposed to different temperatures during the spawning season (n=10, 10 and 9 in the 4°C, 8°C and 12°C groups, respectively). Eggs were incubated at a temperature of 6°C. Values that are not significantly different (P > 0.05) are given common designation. [from Journal of Fish Biology 58:374-385]

breeding period. The effect of photoperiod treatment is expected to be influenced by the photoperiodic history of the fish, the direction of change of photoperiod, and the timing of photoperiodic change relative to the stage of the reproductive cycle. As such, it may be possible to establish broodstocks that spawn out-of-phase with each other using photoperiod manipulations of shorter duration, but this remains to be investigated.

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Factors Affecting Survival and Growth of Early Life Stages of Spotted Wolffish (*Anarhichas minor* Olafsen)

I.B. Falk-Petersen

Incubation temperature affects egg developmental rate, yolk consumption, incubation time, and survival, and final size and survival of spotted wolffish larvae. The highest survival during egg incubation, hatching, and start-feeding occurred in egg batches incubated at constant 6°C. The size of newly hatched larvae was positively correlated with initial egg size and the largest larvae hatched from eggs incubated at 4°C. Hatching may represent a critical phase: both premature and postmature hatching result in high mortality during early larval life. Disinfectants affect egg survival and the hatching process. Handling or transportation influence egg survival and the early egg stages are the most sensitive to such stress. Strong light is detrimental to eggs. The 21-24 mm well-developed larvae start feeding on formulated feed and *Artemia* soon after hatching. Mortality during start-feeding varies between batches and with temperature, and is related to the temperature during egg incubation and the disinfection procedure used on the eggs. The highest survival during start-feeding was registered at 8°C (96%) in groups originating from eggs incubated at 6°C. Initial daily growth rates were highest at both 12°C and 14°C. A newly-developed formulated feed increased survival and growth of larvae and early juveniles compared to those reared on various other commercial feeds and *Artemia* sp. The commercial feed Marin nutra (Skretting) also produced high growth rates and good survival during the start-feeding period.

Introduction

The spotted wolffish *Anarhichas minor* Olafsen with its rich and tasty fillets, popular skin, and rapid growth rates in captivity, is a promising candidate for cold-water aquaculture. A production line for spotted wolffish has been established based on several years of active research on the biology of the species at the Norwegian College of Fishery Science, University of Tromsø, in cooperation with R&D companies.[1-4] Wolffishes were introduced as potential new species for cultivation 15 years ago and biological studies on the common wolffish (*A. lupus*) were initiated. Growth rates of the spotted wolffish appear to be better than those of the common wolffish,[5] so the focus has now changed. In 1992/93, we collected adult spotted wolffish from the Barents Sea and established a broodstock at the Aquaculture Research Station, University of Tromsø. The first eggs hatched in 1994 and the offspring from this and later generations are now contributing to the broodstock population at Troms Steinbit A/S, the only egg and juvenile production plant established in our area.[1]

Successful production of high quality eggs and larvae depends upon favourable environmental conditions for the broodstock as well as during the long egg-incubation phase, the start-feeding phase and the early growth phases. Wolffish juveniles can be stocked at high densities in shallow raceways and they grow well under favourable conditions.[6-8] Under culture conditions the fish are apparently social, despite the fact they are probably territorial during most of the year in nature. Wolffish broodstock have been fed both salmon and marine fish feed, but the recent development of a more optimal formulated floating dry feed has given promising results.

Broodstock individuals collected from the Barents Sea, and their offspring grown in the facility, have an apparently normal reproductive cycle in captivity. The spawning season extends from July until January, with spawning occurring primarily in October and November in fish kept at normal seasonal seawater temperature (varying from 3°to 10°C) and day length.[1] The spawning period can be extended with photoperiod manipulation of the reproductive cycle. Temperatures that are too high during the final phase of oogenesis may effect egg quality. In addition, high temperatures may induce outbreaks of atypical furunculosis (caused by *Aeromonas* sp.).

Temperature is the major factor influencing the developmental rate and survival of fish, and optimal environmental conditions are important for successful cultivation.[9] Yolk is generally utilized faster at higher temperatures. The efficiency with which yolk is transformed into body tissue and the effect of temperature on yolk utilization probably are important determinants of early survival. The present project has focused on the survival and developmental biology of eggs incubated at various temperatures, the hatching periods and survival of larvae hatching at different developmental stages,[4] the efficiency of various egg disinfectants and concentrations,[10] as well as experiments with various start feeds and start-feeding temperatures.[3,4] In addition the sensitivity of the eggs to mechanical disturbance (transport) at various developmental stages has been investigated and a few experiments have been carried out to study the effects of light intensity during egg incubation.

Material and Methods

Shortly after ovulation, female wolffish were stripped and the eggs were fertilized by careful mixing with sperm from one to three males. Fertilized eggs were left for 4 to 6 hours before being transferred to 20-L upstream incubation units, 2-L experimental units or larger raceway incubation systems. Eggs were carefully spread apart to prevent them from sticking together. The 5- to 6-mm eggs were incubated at various temperatures in darkness and routinely disinfected for 5 minutes with 150 ppm glutaraldehyde.[1,10] The fertilized eggs were transferred to incubation units in triplicate or duplicate groups. They were held at selected temperature regimes from 2° to 8°C, treated with various concentrations of disinfectants, and incubated at high or low light intensities. Mortality, yolk utilization, development rate, hatching success, larval size, first-feeding success, and growth of larvae were recorded. Dead and decaying eggs were noted and removed at frequent intervals.

Spotted wolffish larvae are 21-24 mm long and well developed when they start feeding soon after hatching. They accept formulated dry feed as well as *Artemia* (or combinations of the two). Larvae were transferred to shallow mini-raceways for start-feeding experiments at various temperature regimes using both commercial and "new" feeds.

Results

Incubation temperature

Good egg batches have shown ~ 100% fertilization rate and survival rates have been between 60 and 80% during the 800 to 960 degree-day (dd) incubation period.[1-4] The low fertilization rates in some egg batches presented a practical problem as the eggs degenerated and became infested with bacteria. Fertilization problems occurred in some cases when the egg quality was generally good.

The survival of eggs during the long incubation period was significantly different between groups at the three incubation temperatures and was best in batches incubated at constant 6°C [1-4] or at ambient temperatures which decreased from 6-8°C to 3°C during incubation. A constant temperature of 8°C also gave good results, while low temperature (constant 2°C) was disastrous, and results from 4°C have been variable. Most of the egg mortality occurs during the period from 150 to 300 degree-days. In some cases, there also has been high mortality during the hatching period; postmature hatching often results in dead embryos or low survival immediately after hatching.

Incubation time decreased with increasing temperature, but the number of degree-days required for completion of egg development increased with increasing temperature.[1-4] Time to the peak hatching period was ~ 940 dd (17 weeks) at 8°C, ~910 dd (22 weeks) at 6°C and ~850 dd (30 weeks) at 4°C. Morphological differences reflecting variations in differentiation were noted among larvae originating from the different temperature groups. Transfer of yolk to embryonic tissue appeared to be most efficient at 4°C.[4] Size of the visible yolk at peak hatching otherwise varied between egg batches and also with temperature. The largest larvae hatched from the eggs incubated at the lower temperatures.[4]

Some egg batches developed more synchronously than others. Within each batch, hatching took place over several weeks, but there was always a relatively sharp peak in hatching from which most of the viable offspring recruited. Premature hatching may be induced in eggs resting in microzones with low oxygen tension. Temperature elevations, short disruptions in the water supply, or mechanical disturbances also induced premature hatching. Lowering the temperature from 8°C to 6°C has in some cases inhibited hatching in batches with such tendencies. In addition to the size and weight differences caused by incubation temperature there is an increase in embryo length and particularly weight with time of hatching.[11]

Egg disinfectants

Disinfection of eggs with 150 ppm glutaraldehyde for 5 minutes the first day after fertilization and thereafter once or twice per month until ~600 dd increased survival until hatching.[1-4] Treatment with 300–1200 ppm glutaraldehyde twice per month increased survival during the egg phase, but inhibited hatching and resulted in 87-100 % mortality, compared to ~60 % mortality, but normal hatching, in untreated controls. Treatment with the iodophor buffodin (1.5-2%) also reduced mortality during incubation and did not inhibit the hatching process.[12]

The frequency of disinfection also affected survival of eggs. Disinfection twice a month appeared to improve survival until hatching at the temperatures tested.[11] There was a tendency towards higher numbers of prematurely hatched larvae in eggs disinfected twice a month compared to those treated once a month. The survival curves reflected a combination of effects caused by incubation temperature and disinfection frequency. Survival was lowest at the lowest incubation temperature (4°C).

Mechanical disturbances and light intensity

Mortality during the egg stage and at hatching was also caused by mechanical disturbances during the early incubation period. The eggs were most sensitive during early cleavage and gastrulation.[13] Exposure to constant high light intensities during incubation resulted in total mortality of the eggs within the first month and medium light intensities resulted in very low survival at hatching.

Feeding and temperature

Wolffish larvae can be fed formulated feed particles immediately after hatching. A newly developed formulated feed produced by H.K. Strand, Fiskeriforskning (feed based on fish liver residues, squid meal, immunostimulants, attractant, vitamins and binders (alginate/dextrin)) has increased survival and early growth of both common and spotted wolffish compared to some commercial feeds and *Artemia* sp.[6,7] The commercial feed Marin nutra (Skretting) has also resulted in high growth rates and good survival, and a newly tested feed from Danafeed has shown even more promising results.

Experiments on start-feeding of larvae held at various temperatures (6° to 16° C) showed that larval growth rates increased with temperature up to 14°C, but survival was lower at the higher temperatures. In one experiment, mean wet weight and mean survival of spotted wolffish larvae 0-63 days post-hatching were compared among groups fed dry feed (commercial marine fish feed from Skretting) at ambient seasonal temperature and constant temperatures (6°, 8°, 10° and 12°C). Growth was best at 12°C, but mortality was significantly higher at this temperature. In another experiment, mean wet weight and mean survival were compared during 0-63 days post-hatching, fed dry feed at variable temperature regimes (the first 30 days constant temperatures of 8°, 10°, 12°, 14° and 16°C were used, thereafter all groups were regulated to constant 8°C). Individual growth rates increased with increasing temperatures up to 14°C, but at 16°C growth was retarded. Survival was low in both the 16°C and 14°C groups, intermediate in the 12°C and 10°C groups, and best in the 8°C group.[7]

In general, yolk reserves are completely exhausted after ~3 weeks in feeding larvae. After 4 weeks most non-feeding individuals are dead. The number of unsuccessful feeders varied between batches and the reasons for the variations are obscure. Early survival has been between 80% and 96% in the best groups.[7]

Temperature history of eggs

Water temperature during egg incubation affected subsequent larval growth and survival. Studies on growth of spotted wolffish larvae hatched from eggs incubated at different temperatures (4°, 6° and 8°C) showed that early growth from hatching until 42 days post-hatching at 8°C was better in the groups originating from eggs incubated at the lower temperatures. Survival during first-feeding was highest among larvae originating from the 6°C group, and lowest among those from the 8°C group.[4]

Hatching time

Mortality during the 10 weeks of start-feeding was higher among prematurely and postmaturely hatched individuals.[10]

Histological studies

Wolffish larvae hatch at an advanced developmental stage with apparently functional organ and tissue anatomy and histology.[14-16] Histomorphological studies of developing larvae have been conducted in connection with various feeding experiments. Clear differences were noted in liver histology and subcutaneous and intramuscular fat accumulation in wolffish larvae fed various diets and rations.[6,17]

Cooperation Model

I would like to point to the successful cooperation model among R&D partners in Tromsø (Troms Steinbit Ltd., Akvaplan-niva, NFH/ University of Tromsø, Norwegian Institute of Fisheries and Aquaculture Ltd.), which efficiently has brought cultivation of spotted wolffish to its present stage. Contributions and expertise of the partners are:

- Reproductive biology, eggs and larvae (NFH, University of Tromsø, Troms Steinbit)
- Fish health (Norwegian Institute of Fisheries and Aquaculture and NFH, UiTø; Troms Steinbit)
- Fillet quality (NFH, UiTø; Troms Steinbit)
- Marketing (NFH,UiTø, Norwegian Institute of Fisheries and Aquaculture Ltd., Eksportutvalget for fisk, Akvaplan-niva, Troms Steinbit

There have been regular seminars for scientists, commercial partners and potential farmers, and a newsletter (in Norwegian) has been produced.

Conclusions

Good quality egg batches from spotted wolffish show high survival when incubated at constant 6°C or in seasonally decreasing temperatures. With a few exceptions, higher mortality rates and frequencies of abnormal larvae were noted in eggs incubated at constant 4° or 8° C. The results may reflect the temperature adaptation of the broodstock, as spotted wolffish in nature probably spawn at about 4°C to 6° C.

The number of degree-days required for the eggs to complete development and hatch decrease with decreasing incubation temperatures and larvae hatching from eggs incubated at the lower temperatures are the largest. Within individual egg batches, an increase in larval size and weight was also noted with hatching time.

Prematurely and postmaturely hatching larvae generally have low viability. Modifications of the start-feeding units so that larvae are handled more carefully could possibly increase the survival in the premature group, but would only be worthwhile in batches of eggs that have a large proportion of early hatching larvae because of accidental environmental disturbances in the hatchery.

Disinfection of eggs with 150 ppm glutaraldehyde for 5 minutes once or twice per month during the first 600 degree-days increases egg survival during incubation. Treatment with higher concentrations inhibited the hatching process and most eggs died. Mechanical disturbances during early cleavage and gastrulation affect egg survival until hatching.

The commercial start-feed Marin nutra (Skretting) has given satisfactory growth and survival rates of spotted wolffish larvae and juveniles. Results from start-feeding experiments with new formulated feeds, however, are even more promising. It is important to develop optimal start- and on-growing formulated feeds for "new" marine cold-water species.

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Biochemical Analysis of Egg Quality and First-Feeding Condition of the Common Wolffish, *Anarhichas lupus*

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This paper summarizes the research and development efforts of the Newfoundland Wolffish Working Group, which is supported by industry and regional development associations. Studies of the lipid and amino acid composition of common wolffish eggs (*Anarhichas lupus*), as well as the nutritional analysis of first-feeding larvae fed experimental and commercial diets, were conducted to help the industry develop criteria for selecting the most appropriate diets. In both eggs and first-feeding larvae, total and specific fatty acid and lipid class content (e.g. docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and their ratio, triacylglycerol (TAG), and phospholipid (PL)), as well as amino acids (e.g. taurine), were positively correlated with egg survival and juvenile survival and growth.

Introduction

Newfoundland's involvement in wolffish research began in the 1980s with studies of wild stocks, but the focus shifted to aquaculture in the 1990s. The Cape Freels Development Association, which operates the Wesleyville Hatchery, and Memorial University of Newfoundland recently collaborated on a research and development project on the relationship between egg quality and subsequent growth and survival of juvenile wolffish, *Anarhichas lupus*.

Memorial University is working with Atlantic Agrifoods Ltd., an industry partner from the west coast of Newfoundland, to develop wolffish farming. The western region of Newfoundland has potential sites for land-based marine culture facilities, access to high quality cold water, and the incentive to encourage new industry, as the area has been severely affected by the collapse of the wild fishery.

Research by Norwegian, Russian and Canadian scientists has resulted in the development of spawning and culture techniques for wolffish and has produced information on the effect of temperature on reproduction and egg development.[1-4] Our paper summarizes some of the results of biochemical analyses (fatty acid and amino acid profiles) performed to assess egg quality and condition of first-feeding wolffish.

Materials and Methods

Eggs and first-feeding wolffish

Egg masses were collected from the wild and incubated at 6°C in upwelling Heath tray recirculation systems used for salmonid egg incubation or at seasonal temperature in a Heath tray flow-through system. Eggs were sampled several times throughout the 7- to 9-month incubation phase and at hatch (each time three 5-egg samples were taken from each egg mass; over the 3-year experiment approximately 35 egg masses were sampled). Newly hatched wolffish were stocked at a density of 90 fish/L (initially ~0.1 g/fish) in 33 shallow-water white mini-tanks. Rearing parameters included: 1 L water volume, 70-90 mL/min water flow, $6° \pm 0.5°C$ water temperature, $33 \pm 1‰$ salinity, 200 to 270 lux light intensity at an 18L:6D photoperiod, and constant aeration. Feeding conditions consisted of simultaneously offering enriched *Artemia* (1000/L) and a dry diet in excess during the first 30 days, weaning at day 40, and providing only a dry diet from day 40 to 100. All diets were provided in 3 feedings per day. Replicate fish samples were taken at 20-day intervals from day 0 to 100, for determination of growth rates and analysis of lipid and amino acids.

Biochemical Analysis

Samples of eggs, diets, and fish tissues were analyzed for proximate composition, lipid profiles and amino acids. Fatty acids were determined using gas chromatography (GC) with a 30-m DB225 (J&W Scientific) column after 2:1 chloroform and methanol extraction, the addition of a C17:0 internal standard, and derivatization to fatty acid methyl esters with H_2SO_4. Lipid classes were determined using an internal standard of 3-hexadecanone and a Mark V Iatroscan. Amino acids were determined using an AEC internal standard, deproteinization, the buffer lithium method and a Beckman 121MB Amino Acid Analyzer, using a Benson D-X8, 0.25 Cation Xchange Resin and a single column. Total amino acid samples were hydrolysed with hydrochloric acid. The amounts of fatty acid and amino acid were calculated based on quantity per egg, the wet weight of fish tissue, and the dry weight of the diets.

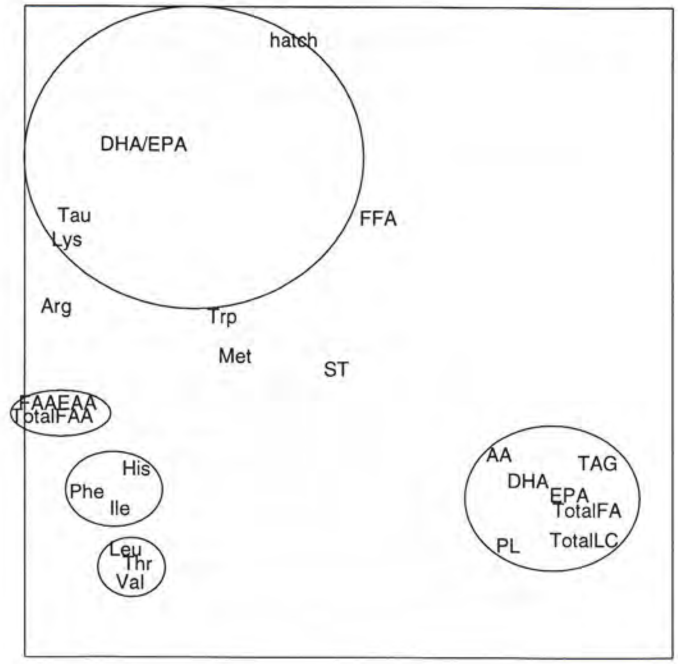

Figure 1. Principal components analysis of fatty acids, lipid classes and amino acids of common wolffish eggs (*Anarhichas lupus*). Abbreviations: fatty acids (DHA = docosahexaenoic acid, EPA = eicosapentaenoic acid, DHA/EPA = DHA/EPA ratio, AA = arachidonic acid, TotalFA = total fatty acid); lipid classes (PL = phospholipid, TAG = triacylglycerol, FFA = free fatty acids, ST = sterol, Total LC = total lipid class); free amino acids (TotalEAA = total essential amino acid, TotalFAA = Total free amino acid, Tau = taurine, Lys = lysine, Phe = phenylalanine, His = histodine, Ile = isoleucine, Leu = leucine, Thr = threonine, Val = valine, Arg = arginine, Trp = tryptophan, Met = methionine).

Results and Discussion

Egg quality

Temperature,[3] genetics and parental condition are probably the major factors contributing to variations in egg quality and survival rates of wolffish eggs. In this study, hatching rates ranged from 0 to 97%. In Figure 1, principal components analysis shows the relationships among various fatty acids, lipid classes, and amino acids. Particularly strong is the association amongst hatch, DHA/EPA ratio (docosahexaenoic acid/eicosa-pentaenoic acid) and taurine, with triacylglycerol and phospholipids also related to DHA and EPA content and being major components of the egg composition. Figure 2, adapted from Halfyard et al.,[5] shows the decrease in total and specific lipids (e.g. EPA, DHA, TAG) over the development period and the increase in major amino acids. Poorer eggs tended to show selective conservation or decrease of these components compared to better quality eggs. Taurine, a non-essential amino acid thought to have an osmoregulatory function, was a major free amino acid that was significantly lower in poor eggs than in higher quality eggs. Taurine levels are also relatively high in high-quality commercial first feeding diets.[5-7] Egg quality and hatching, as well as the safety of the researcher, were also compromised by the use of glutaraldehyde for disinfection.

First Feeding

Halfyard et al.[4] reviewed studies on first-feeding and juvenile wolffish that have investigated the effect of different live and commercial diets, varying stocking densities, and the differences between the two species. Studies on live food requirements,[8,9] and effect of light intensity,[8] feeding frequency, and highly unsaturated fatty acid content (HUFA)[10] have shown that wolffish have very high growth rates under optimal rearing conditions. Principal components analysis of lipid and amino acid profiles for first-feeding wolffish fed on various experimental and commercial

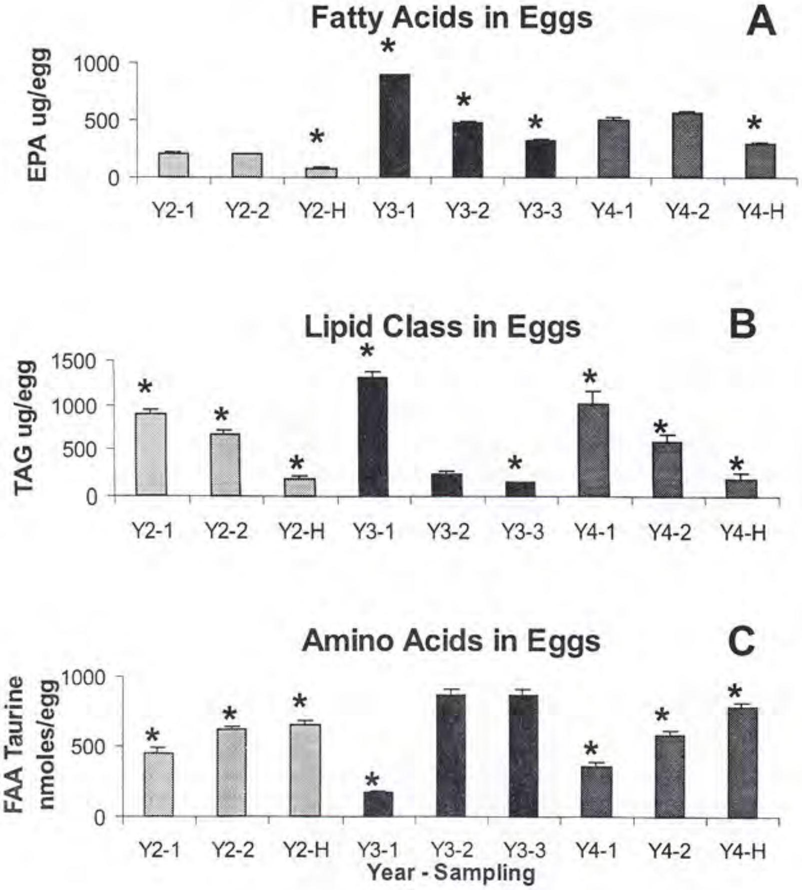

Figure 2. Eicosapentaenoic acid (EPA), triacylgylcerol (TAG) and free amino acid taurine in Year 2,3,4 in common wolffish eggs (*Anarhichas lupus*) sampled at times 1,2,3 and H (hatch) during the incubation phase. (from Halfyard et al. 2001)

dry diets suggests a strong association between survival, growth, DHA/EPA ratio and free amino acid content, particularly taurine. Figure 3, adapted from Halfyard et al.,[10] shows that diets with high HUFA content affected tissue deposition. Wolffish fed higher HUFA content also showed increased survival rates. Differences in egg quality and parental condition, as well as the benefits of co-feeding live food, were also reflected in survival, growth rates, and lipid and amino acid profiles of the post-hatch larvae. The commercial diets that resulted in the best larval performance had optimal HUFA content, phospholipids and higher levels of chemoattractants (e.g. alanine and glycine).

Conclusion and Recommendations

The eggs of the common wolffish used in this study were collected from the wild; therefore the biochemical analyses provided valuable baseline data for comparison with eggs from cultured fish. There is obviously a need for similar studies on spotted wolffish eggs spawned from domesticated broodstock. Sampling of spotted wolffish egg batches from Troms Steinbit A/S, Norway is currently underway. Linkages between Norway and Canada could facilitate research collaboration on the histological, biochemical and enzymatic aspects of egg quality.

Development and refinement of broodstock, first feeding, and grow-out diets is essential to reduce the length of the production cycle (~3 years are currently required for spotted wolffish to grow from 5 g to 3-5 kg). The lipid content of the diets will need to address the marketing issue of excessive fat deposition in the tissue, and also the effects of broodstock diets on egg production and egg quality. Studies on the requirements for vitamins, minerals, pigments and chemoattractants should also improve growth parameters. The fostering of close working relationships between Norwegian and Canadian industry and research partners, as well as commercial feed manufacturing companies should stimulate the development of wolffish farming.

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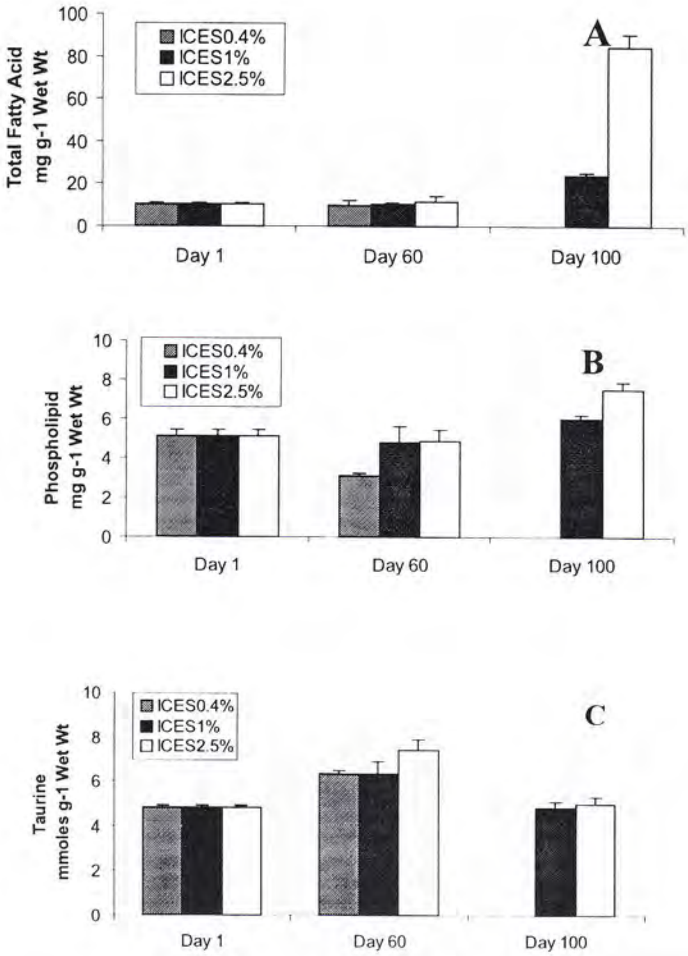

Figure 3. Total fatty acid, phospholipid and taurine content of the tissue of first feeding common wolffish *(Anarhichas lupus)* fed experimental ICES diets containing 0.4, 1 and 2.5% highly unsaturated fatty acids (HUFA) over a 100 day period. There is no data for ICES 0.4% at day 100 since survival was 0% (from Halfyard et al. 2001).

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Susceptibility of Spotted Wolffish to Infectious Diseases and Use of Immunoprophylaxis

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The spotted wolffish has turned out to be a perfect species for fish farming in cold waters. It grows rapidly and only minor disease or health problems have been discovered. Laboratory tests attempting to infect the spotted wolffish with the bacteria and viruses that are common problems in fish farming, show that the species is robust and strong. Atypical furunculosis is the only bacterial disease registered from wolffish farms so far. The disease is not a significant problem at present, and vaccines are available if needed. Similar to findings in all fish, parasites are present in the spotted wolffish. *Tricodina* and *Costia* have been found on the skin and gill surfaces of juvenile wolffish, but are easily treated with formalin. *Pleistophora* and *Gyrodactylus* occur in adult fish, but at very low incidence.

Introduction

Along with the expanding fish farming industry, problems with diseases have emerged with economically disastrous consequences for fish farmers. In Norway, substantial efforts have been made to improve fish health, especially in Atlantic salmon, and effective vaccines against several bacterial diseases are now available. This has reduced the use of antibiotics to a minimum level in the Norwegian salmon industry. In recent years, marine species such as cod, halibut and turbot are being raised in aquaculture in Norway, and disease problems have appeared in these species as well. The experience gained from managing disease outbreaks in Atlantic salmon and other marine species can be used to prepare for potential disease problems in wolffish and for protecting wolffish against the pathogens.

Wolffish Diseases

Atypical furunculosis has been diagnosed in both wild and farmed spotted wolffish. It is caused by atypical strains of *Aeromonas salmonicida,* a heterogeneous group of bacteria. Both salmonids and marine fish species are susceptible to infection and healthy wild fish can be "carriers" of the bacteria. Outbreaks of the disease in wild-caught wolffish broodstock have occurred after exposure to various kinds of "stress" (e.g., high temperature, handling, transport).

In the laboratory, we have exposed wolffish to various bacteria and viruses known to be pathogenic to other fish species and these challenge experiments have given us information about the diseases that may appear in large-scale production. *Vibrio anguillarum* is a common bacterium in the marine environment and infects salmonid and marine species (fish and shellfish). There are several variants of the strain, usually with low host specificity, but some have species preferences. Injections of high doses of *V. anguillarum, V. salmonicida* (coldwater vibriosis) and *V. viscosus* (winter ulcer), caused high mortality. When the doses were lower, only *V. anguillarum* caused mortality, indicating this bacterium is a potential pathogen in wolffish.

IPN (infectious pancreatic necrosis) is a serious problem in the salmon industry, and the virus is also pathogenic to marine fish. Apparently healthy fish can be carriers of the virus. VNN (viral nervous necrosis) is caused by a nada virus diagnosed in several marine species worldwide. The juvenile stages are particularly vulnerable to this infection.

We exposed wolffish of different ages to these viruses. Bath challenge of 0.3-g fish with IPN-virus isolated from halibut clearly resulted in mortality. Fish were exposed to virus added in the water and were then transferred to a raceway. A group of healthy fish was placed "downstream" from the challenged fish, physically separated but exposed to the water from the infected fish. Mortality was registered first in the "directly" infected group and some days later in the fish infected through waterborne challenge. A similar experiment was performed with VNN-virus as the infecting agent. Bath-infected fish started to die first, then the "downstream" group infected through waterborne challenge. The wolffish were more resistant to IPN than VNN. Injecting 10-g fish with IPN-virus did not result in mortality, but injection of VNN-virus did.

From these laboratory experiments we have an idea of the pathogens that might be a problem in farming of spotted wolffish. But what are the actual health problems in wolffish farms today? This last year we have established a network between the fish farmers, veterinary services and research institutes in order to identify health problems, discuss the issues, gain experience from each other, and try to solve problems.

Every second month, 10 apparently healthy wolffish are sampled and examined to evaluate the general health status of the fish in the farm. If mortality or any clinical signs of disease appear, sampling and examination of the fish is also performed and reported.

From our survey so far, no "major" health problems have been identified and the mortality of fish in the farms has been very low. The most pronounced pathological or clinical symptoms are associated with the gills, similar to findings in all farmed fish. Microscopic examination of the gills shows enlarged cells, increased cell numbers, and increased secretion of mucus. This is probably caused by external irritants such as poor water quality and particles in the water or parasites and bacteria which adhere to the mucus secretions (or there can be a combination of problems).

Parasites are found in all fish species and in farmed as well as in wild populations. In our survey, the most common parasites were associated with the gills and skin, the ciliated *Trichodina* and the flagellated *Costia*. In juvenile fish, these external parasites are usually treated with formalin; adult fish are more resistant to the parasites. Another parasite found is the microsporidium *Pleistophora* which infects the muscle cells of the fish. This intracellular parasite has been detected in thin sections of muscle tissue from some fish. It may develop into large tumor-like structures and reduce the quality of the fish product. So far, infections have not been visible by eye in the wolffish, and we don't know if the fish can fight this infection and prevent further development of infection, or if it is a matter of time before it becomes a problem with economic consequences.

Immunoprophylaxis

As mentioned before, outbreaks of atypical furunculosis have been reported in wild and farmed wolffish. Since this is the only bacterial disease reported so far, we have tried to design an effective vaccine. First, we tested the vaccine used against typical furunculosis that is already on the market and used in the salmon industry. When challenged with an atypical strain of *A. salmonicida* isolated from wolffish, 100% mortality occurred in both the unvaccinated and vaccinated fish, demonstrating that the vaccine does not induce any protection in wolffish. A similar experiment on halibut in Iceland using the commercial vaccine induced protection in halibut challenged with an atypical *Aeromonas* isolated from halibut. Since vaccination is based on the principle of recognition, the most probable explanation is that the bacterial isolate from wolffish is different from the typical *Aeromonas* used in the vaccine, and also different from the halibut isolate. When we used a vaccine based on a bacterial strain isolated from wolffish, high protection was achieved when we challenged the fish with the homologous strain. Vaccination is therefore an effective strategy to protect wolffish against diseases, but the composition of bacterial strains in the vaccine is crucial.

During the years we have collected isolates of atypical *A. salmonicida* from different fish species around the world and used different phenotypic and genotypic methods to characterize and compare them. Using AFL (amplified fragment length polymorphism), we found that all the wolffish isolates group together while strains from other fish species (halibut, turbot) are more diverse. Based on this characterization we have designed several vaccines with single isolates, or mixtures of isolates, of which the latter give best protection.

At what size or age can the fish be vaccinated? When do the fish have a functional immune system to respond to vaccination? We have used the presence of immunoglobulin (IG) producing cells as a criterion of a specific immune system and in adult fish we find these IG producing cells in various lymphoid tissues: head kidney, spleen, gut, thymus, skin and gills. The *in situ* hybridization technique was used to detect mRNA transcripts of the IG molecule. At the time of hatching, no IG-producing cells were found in tissues. The first positive cells appeared in the kidney 1 week post hatching. Next, cells appeared in the spleen and later in the gut and thymus. At a fish size of 10 cm, IG producing cells were still not found in skin which is an important physical and immunological barrier to the surroundings. To study the presence of systemic immune responses in the juveniles, we vaccinated (injection) two size groups of fish, 50 mm and 100 mm. At a size of 50 mm, it is practically feasible to inject the juveniles and IG producing cells are still not present in the primary lymphoid tissues of all fish. After challenge all the unvaccinated fish died within 4-6 days while the vaccinated fish in both size groups showed a high survival rate that was similar in the two groups. Wolffish obviously have a competent immune system at the size of 50 mm and can be protected against diseases through vaccination strategies.

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Tolerance, Growth and Haloplasticity of Atlantic wolffish (*Anarhichas lupus*) Exposed to Low and Intermediate Salinities

Nathalie Le François,
Simon Lamarre and Pierre Blier

Tolerance, growth and adaptability of Atlantic wolffish exposed to reduced salinities was investigated over a 19-week period. The fish were directly introduced to experimental salinities of 7, 14, 21 and 28‰, and held at a constant temperature of 10°C. No mortalities were recorded over the experimental period. Adjustments in the level of activity of Na^+K^+ATPase and metabolic enzymes in relation to the external salinity followed the general U-shaped model. The growth trajectories of the fish in the 14 and 28‰ salinity groups were significantly different, with the fish held at 14‰ displaying enhanced growth compared to those at 28‰ salinity. No detrimental effect on growth was observed in fish exposed to 7‰ salinity.

Introduction

Two members of the Anarhichadidae, the Atlantic wolffish (*Anarhichas lupus*) and the spotted wolffish (*A. minor*), are marine fish species for which biological and technical efforts aimed at the development of a sustainable mariculture industry are being applied in the northern hemisphere (Norway and Canada).[1] Despite the inferior growth performance and greater susceptibility to stress in captivity of the Atlantic wolffish, compared to the spotted wolffish, the species is of interest for mariculture research in Quebec because 1) the fish display good growth performance; 2) the species is *a priori* more tolerant of the low salinities and higher water temperatures that characterise most estuarine coastal areas; 3) fertilized egg masses are readily available from diving operations; 4) the species could present interesting opportunities for aquaculture because of their ability to synthesize antifreeze proteins and possibly other biomolecules that have high commercial value (e.g., digestive enzymes, antimicrobial polypeptides); and 5) there is high potential for transfer of knowledge gained from the culture of Atlantic wolffish to spotted wolffish culture, particularly in regard to reproductive physiology, nutrition and rearing technology.

The Atlantic wolffish is a coastal species that should be tolerant of a wider range of environmental temperatures and salinities than the spotted wolffish, which is found in the open waters of the Gulf of St. Lawrence. To our knowledge, however, no studies have been conducted on the physiological tolerance of the Atlantic wolffish to low salinity, the effect of salinity on branchial Na^+K^+ATPase activity and associated energy metabolism, and the potential impacts of low salinity on survival and growth. Most studies on the osmoregulatory capacity of fish have been conducted on salmonids following transfer from freshwater to saltwater; few have examined the metabolic adjustments that occur when fish experience a change in salinity. Knowledge of the hypo-osmoregulatory capacities of marine fish is limited. In general, marine fish do not tolerate prolonged exposition to freshwater, but several species are tolerant of low salinity (e.g., *Sparus sarba*, *Gadus morhua*, *Hippoglossoides platessoides*, *Pomacanthus imperator*, *Mylio macrocephalus*, *Scophtalmus maximus*, *Dicentrarchus labrax*, *Crysophrys major*). This is of interest from a mariculture perspective since the total area of estuarine environment in the world is estimated at 392 million hectares.

In hypo-osmotic environments, fish are in hyper-osmoregulatory mode trying to maintain their internal osmolality at values higher than the ambient environment. In hyper-saline waters, fish are in hypo-osmoregulatory mode to maintain their internal osmolality at levels lower than the external environment. These adaptation mechanisms are essential to the maintenance of internal homeostasis and vital body functions and require substantial amounts of energy. In teleosts, branchial metabolism relies on glucose and lactate as metabolic fuels. In salmonids, the level of several enzymes involved in branchial intermediary metabolism display pronounced elevation in activity when fish are exposed to increases in salinity and during smoltification. Recent studies on brook charr revealed a clear elevation of Na^+K^+ATPase activity following prolonged exposure of the fish to seawater, but there was no proportional elevation in activity levels of key enzymes of energy metabolism (citrate synthase (CS), cytochrome C oxidase (CCO), lactate dehydrogenase (LDH), and pyruvate kinase (PK)).

Few studies have described branchial enzyme activity in marine fish in relation to salinity.[2] Significant modifications have been observed in the level of activity of some branchial metabolic enzymes but they did not closely mirror the changes observed in Na^+K^+ATPase activity. In general, reduction or elevation of salinity can change the amount of energy available for growth by exerting pressure on the metabolic expenditure linked to the iono-osmoregulation activities. The accepted hypothesis states that the energetic cost for homeostasis is reduced in iso-osmotic environments and that these energy savings are translated into growth enhancement. In the present study, we 1) evaluated the tolerance and growth of Atlantic wolffish at different life stages (0^+ and 1^+) following exposure to low and intermediate salinities; 2) characterised the adaptative response at the branchial tissue level (Na^+K^+ATPase and energy metabolism) and the iono-osmotic adjustments (% water, osmolality, ionic composition) at regular intervals following transfer to and prolonged exposure to low and intermediate salinities (0, 7, 14, 21 and 28‰); and 3) evaluated the short term adaptation responses (ionic composition, osmolality, water content).

Material and Methods

Wild fertilized egg masses of Atlantic wolffish were collected by a team of divers in Conception Bay, Newfoundland, and transported to the Centre Aquacole Marin aquaculture facilities (Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec, Grande-Rivière, Québec). The egg masses were fragmented into small pieces and incubated in modified Heath-tray incubators at 4°-5°C until hatching in March 2000. The fish were transferred into experimental low-level raceways for the first-feeding and on-growing phases.

A group of approximately 6 000 juvenile fish were fed formulated feed (Nutramarine, Skreeting), coupled with enriched *Artemia* in the first week of life, until their mean mass reached 28.01 ± 5.93 g. The fish were then randomly assigned to four experimental salinities (7, 14, 21 or 28 ‰, n=100 per replicate per experimental salinity) for 19 weeks in order to characterise tolerance, growth, Na^+K^+ATPase activity and haloplasticity of the branchial metabolic enzymes at low and intermediate salinities. Condition factor (CF) and hepatosomatic index (HSI) were calculated. The water water, lipid and protein content of the white muscle were also evaluated. Growth performance (length and weight) at the group level was estimated. Branchial Na^+K^+ATPase activity, cytochrome C oxidase (CCO), citrate synthase (CS), pyruvate kinase (PK), and lactate dehydrogenase (LDH) activities were measured and expressed in units per mg of protein per hour. Plasma osmolality (mOsm/Kg) was measured using a single-chamber micro-osmometer.

Results

Osmolality

Osmolality values of the group held at 7‰ salinity were significantly lower than all other experimental groups but the osmotic disequilibrium was nonlethal. Compared to the control group (28‰ salinity), fish at the other experimental salinities (14 and 21‰) reached similar values rapidly (after two weeks).

Growth performance

After 19 weeks, the groups held at intermediate salinities displayed slight growth improvements in weight and length. The calculation of the growth in weight and length trajectories indicated there was a significant difference with groups of wolffish held at 14‰ salinity growing more rapidly than those at 28‰. In general, growth performance, in terms of weight or length, displayed a response that was the inverse of that seen in the enzymatic adjustments; the highest growth occurred in fish held at about the iso-osmotic salinity.[14]

Na^+K^+ATPase Activity and Metabolic Enzyme Activity

After 14 and 19 weeks at the different salinities, we observed adjustments in the level of activity of Na^+K^+ATPase activity. The expected U-shaped response curve was revealed. Fish at intermediate salinities displayed significantly lower Na^+K^+ATPase activity than the group held at 28‰. At the 14^{th} and 19^{th} week following exposure to the experimental salinities, adjustments in PK and LDH activity levels were observed in the wolffish, with lower values at intermediate salinities compared to fish at 7‰ and 28‰ salinity. The results were consistent with the U-shaped model. Interestingly, $Na+K^+$ATPase and LDH activity are 22% and 16% lower, respectively, in the groups held at 14‰ compared to 28‰ salinity. After 19 weeks, the level of activity of Na^+K^+ATPase and LDH of the fish held at 14‰ salinity (near iso-osmotic value) are respectively 21% and 27.4% lower than at 28‰. A strong positive relationship between NA^+K^+ATPase and LDH activity was found. This indicates that from week 14 the NA^+K^+ATPase activity level adjustments in relation to salinity in Atlantic wolffish, a typical marine species, is associated with adjustments in LDH activity.

Discussion

The slight growth enhancement at intermediate salinities could be linked to: 1) greater time allocated for digestion (the proposed mechanism is better feed conversion at intermediate salinities linked to the reduced drinking behaviour at those salinities); 2) reduction in swimming activity,[3] or 3) enzymatic adjustments at the gill level that imply a reduction of energetic expense in favour of somatic growth at near iso-osmotic salinities.

Atlantic wolffish have high hypo-osmotic capacities and slight growth improvements are achieved at intermediate salinities, which likely expands the potential sites for rearing the species in the estuarine coastal habitat. The same observations have been reported for the spotted wolffish[4] in experiments conducted at 12, 17, 25 and 34‰ salinity.

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Canadian Freshwater Aquaculture: A Development Perspective

M. Gauthier, H. Mercille, M. Parent, J.-F. Thifault and A. Dumas

Canadian finfish production has grown rapidly during the last twenty years. Salmonids produced in the Atlantic and Pacific regions depend on the availability of both freshwater and seawater. Freshwater aquaculture occurs mainly in Ontario, Quebec and Saskatchewan. Table markets are much more important than stocking and recreational markets, except in Quebec. Most experts agree that the freshwater sector will continue to grow during the next decades. The issues that need to be addressed by the aquaculture industry (sustainable development, profitability, regulations, etc.) are discussed in this paper. Public perception of aquaculture is an important factor limiting development.

Introduction

The Canadian aquaculture industry increased its finfish production from approximately 3,249 metric tons (mt) in 1986 to 67,435 mt in 1998.(1) Consequently, one could say that this young sector has an interesting potential for development and that its role in the economy is of growing importance, particularly in rural areas.(2,3) That is likely to continue, particularly with the end of the moratorium in April 2002 on new aquaculture sites in British Columbia.

Many Canadian researchers, fish farmers and stakeholders have an international reputation and contribute to some extent to the worldwide improvement of fish feed and aquaculture technologies within a context of global competitiveness and sustainable development.

These positive aspects will certainly continue to favour aquaculture production in Canada. However, what benefits are there going to be for the freshwater sector? This paper deals with this question and discusses the challenges facing the industry in each region. In order to draw some development perspectives, it is imperative to review the history of Canadian freshwater aquaculture and its current status.

A Brief History of Canadian Aquaculture

The first fish reared in Canada were brook trout (*Salvelinus fontinalis*) and Atlantic salmon (*Salmo salar)*. Production began in government hatcheries in the 1850s in the Province of Québec and in the 1870s in the Province of Ontario. By 1876, aquaculture was relatively well established and more than 14 million eggs from various species of salmonids (*Salmo* sp., *Oncorhynchus* sp., *Salvelinus* sp., *Coregonus clupeaformis*)(4) were being raised and used mainly for restocking and recreational fishing markets. At that time, the Fisheries Commissioner wrote:

> *"Fish culture is a science, which leads us progressively and constantly toward the solution to a very important problem of our times by making our rivers as productive as our land resources through careful management, thereby making available greater resources to the growing world population"* — translated from Samuel Wilmot, 1876

His words are still surprisingly relevant to the Canadian freshwater aquaculture industry.

A Portrait of Current Canadian Freshwater Aquaculture Production

It is not uncommon to observe data on aquaculture production being separated between freshwater and saltwater environments. However, this dichotomy cannot be established so clearly for the Canadian finfish aquaculture industry. Most of the important aquaculture species such as Atlantic salmon and steelhead trout (*Oncorhynchus mykiss)* spend their early life stages in freshwater (usually between 12 to 18 months) while grow-out occurs in seawater. If we look at the statistics on aquaculture production in Canada without considering this feature of the industry, one could conclude that this young industry has evolved mainly in saltwater and that the freshwater environment is of relatively little importance. In fact, the aquaculture industry in Canada is very dependent on its freshwater resources. Indeed, salmon and steelhead represent approximately 86% of the total

Canadian aquaculture value and 92% of the finfish production in 1998.[1] Actually, the growth in fish farming over the years has been mainly due to anadromous species (Fig. 1). The freshwater sector's annual expenditures of $93 million and 1,300 full-time jobs (1999 statistics) underline its importance to the aquaculture industry.[5]

Finfish aquaculture in Canada occurs mainly in the Atlantic and Pacific regions. Salmon production in British Columbia and New Brunswick reached approximately 42,200 and 14,232 mt in 1998, respectively. At that time, total finfish production in Canada was 67,435 mt, corresponding to a value of $394,859 million.[1] In 2000, Nova Scotia produced 9,214 mt of finfish, mainly steelhead and Atlantic salmon.[6] Among other species raised in Canada, it is worth mentioning rainbow trout, or steelhead trout (anadromous rainbow trout), and brook trout. Species such as Arctic charr (*Salvelinus alpinus*), walleye (*Stizostedion vitreum*) and tilapia (*Tilapia* sp.) are produced commercially, but on a smaller scale or only in certain provinces. Much of the data on these species are not available because there are so few producers. In 2001, Rogers and Davidson[7] reported Canadian production of 960 mt for Arctic charr.

Ontario is the most important producer of strictly freshwater species, with an annual production of about 4,000 mt in 1999.[8] That year, total Canadian production reached 6,623 mt for these species. Far behind Ontario are Quebec and Saskatchewan with freshwater finfish production in 1999 of approximately 2,200 mt and 875 mt, respectively.[1,9] The aquaculture industry in Alberta is of growing importance with a production of 814 mt of freshwater fish in 2001.[10]

Most of the fish produced in the provinces mentioned above are for human consumption. According to a recent study, only 20% of the fish are used for stocking (in lakes and ponds) or recreational fishing.[5] The situation differs in Quebec where about 50% of the production from the private sector is sold for stocking or recreational fishing.[11] Over the brief history of Canadian aquaculture, fish farming has switched from raising fish for stocking and recreational fishing to producing fish for the table market. The latter appears to offer greater opportunities for the industry. However, the thin profit margins and increasing competition between provinces, as well as from other countries, are clouds on the horizon.

The selling of fish for stocking and recreational markets is controlled by regulations on the introduction, transport, and distribution of fish species. Consequently, the markets are local, relatively small, and limited to certain species. Conversely, they appear to suffer less from competition and generally have a larger profit margin.

The performance of the aquaculture industry in Canada largely depends on environmental conditions. Huge amounts of high quality freshwater are available, but cold temperatures have a detrimental effect

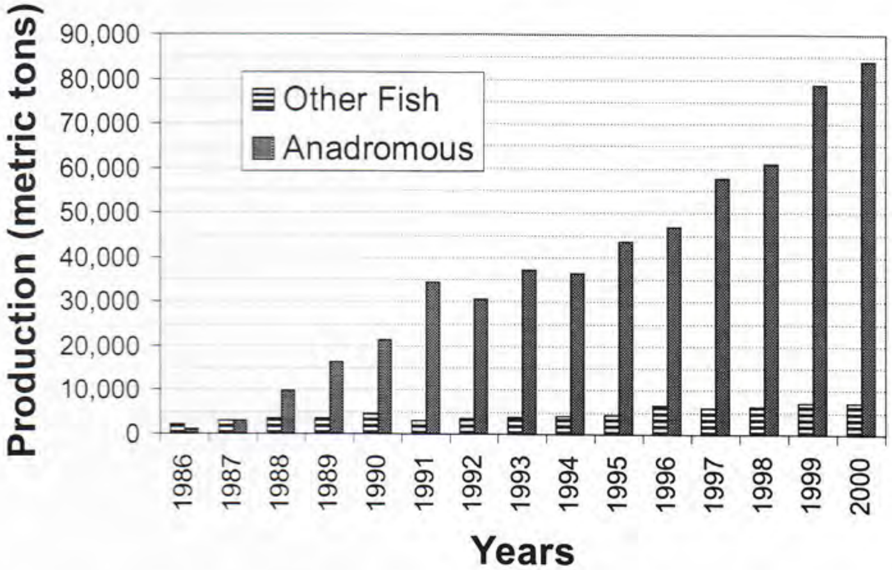

Figure 1. Fish production in Canada between 1986 and 2000 (Source: DFO 2002. Data for 1999 and 2000 are preliminary.

on the growth rates of cultured fish. For this reason, aquaculture is limited mainly to the southern parts of the country.

Fish feed is another factor influencing the performance of farmed fish. Fish meal, which is the main protein source in salmonid diets, currently comes from other countries and contributes to the release of phosphorus. Its increasing cost, combined with the decrease in the price obtained for salmon, have considerably reduced profit margins.

A final factor to consider is the legislative and regulatory environment in Canada, which can sometimes discourage the industry due to the delays that are imposed on the industry and the numerous agencies that are involved.[12]

In Quebec, environmental restrictions limit the establishment of new fish farms and have already brought about the closing of at least two existing farms. In Ontario, new regulatory initiatives related to First Nations land claims, fish health, and nutrient discharges have brought uncertainties to the aquaculture business.[8] Environmental agencies and organizations are continuing to closely monitor fish farming in British Columbia and New Brunswick.

The public often has a negative perception of fish farming. This, more than all previously mentioned constraints, can prevent aquaculture from increasing its activity. However, major opposition to the industry is generally not encountered until the aquaculture activity in an area reaches a certain level of production.

Development Perspectives

Canadian freshwater aquaculture faces two kinds of challenges: those that are common to all provinces and those that are regionally specific.

Mutual challenges include concerns about the environment, profitability, and regulations.[12,13] In freshwater, concentrations of phosphorus and suspended solids in fish farm effluents need to be addressed.[14-16] Environmental impacts related to these parameters can be reduced by improving digestibility of feed, nutrient utilization, and wastewater management.[17] Efforts to address these issues are already underway through research and development projects funded by AquaNet (Network of Centres of Excellence for Aquaculture in Canada). The Inter-Provincial Collaborative Initiative for Sustainable Freshwater Aquaculture is an initiative aimed specifically at developing low-phosphorus diets as well as improving water treatment technologies. It is a truism that the success of such networks depends on funding and true collaboration between governmental agencies, private corporations, and researchers.

One way to increase profitability in aquaculture is to reduce dependence on fish meal. More economical sources of proteins need to be found. Many research and development projects are being implemented to address this issue, not only in Canada, but in all major countries producing carnivorous species.[18,19] If plant proteins become a more important ingredient in fish feed, the Canadian prairies could become a major supplier.

According to OCAD,[12] there is willingness in the federal government to establish a regulatory framework that is more appropriate for the aquaculture industry and will help foster development.

There are relatively few new sites for salmonid farming in Canada because of climate, environmental, and regulatory restrictions. This is particularly true in the Atlantic region.

Freshwater aquaculture will probably never become as important in Canada as it is in Norway or the United States, but most experts agree that it will continue to grow during the next decades.[8,19] The enhancement of freshwater aquaculture relies particularly on the expertise, knowledge and ingenuity of people involved in this industry. Joint R&D projects aimed at solving previously mentioned problems should therefore be encouraged. It has already been demonstrated that strong institutional and governmental support for R&D play an important role in aquaculture development and it is critical that commitment to the industry continue. Development of value-added products and commercialization of new species such as walleye and Arctic charr, which have been traditionally supplied by wild stock, offer interesting potential for development, but still need further research.[7,21]

Only the specific challenges for the provinces most heavily involved in freshwater aquaculture are considered here. The ending of the moratorium on new aquaculture sites in British Columbia will probably contribute to the expansion of aquaculture production in the Pacific region. This will increase the demand for high quality freshwater. The fish farming industry in Ontario is strategically well positioned to undergo significant expansion in the future: the climate is suitable for salmonid aquaculture, land-based and nearshore operations are viable, and the scientific community has developed an international reputation while remaining close to the needs of the industry and governmental agencies.[8] In Ontario, however, the uncertainties that were previously mentioned will have to be addressed before further growth will occur. The situation that prevails in Quebec differs from that in Ontario. Care should be taken in Quebec to ensure the fate of aquaculture is not similar to that in Sweden, where a promising industry has been almost extinguished, mainly because of environmental restrictions.[21] In other provinces, some governmental agencies also support the sustainable development of aquaculture by financing multi-sector research and development projects which address relevant issues.[22,23]

The aquaculture industry has to demonstrate its commitment to apply and to promote sustainable practices in order to improve the public perception of aquaculture, while remaining competitive. Otherwise, significant decreases in production could happen and most salmonids sold in Canada would have to be imported.

Concluding Remarks

The Canadian freshwater aquaculture industry depends largely on the development of salmon farming in the Pacific and Atlantic regions. One can say that this industry will continue to grow significantly in these regions. In more central provinces, such as Ontario, Quebec and Saskatchewan, restocking and recreational fishing markets should remain at least stable, while the table market will fluctuate according to the industry's ability to remain competitive. Further expansion of the fish farming industry depends on the will and capacity of producers, government agencies and stakeholders to manage and to innovate within a continually changing context. Finally, it becomes imperative to reassure the Canadian public concerning the sustainability of fish farming by demonstrating a progressive approach.

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Broodstock Genetics:
A warning to the wise

Tim Jackson

In establishing a new species for aquaculture, there are areas of major concern to the aquaculturist: larval rearing, diet, environmental requirements, health, etc. One area that has often been overlooked is the genetic make-up of broodstock and the development of the strain. Many fish farmers will tell you that this is important, but not an initial priority. In our recent findings (Jackson et al., in press), my colleagues and I have shown that broodstock genetics cannot be overlooked without potentially disasterous consequences.

Genetic variability represents improvement potential when undertaking strain development. It also reduces the risk of inbreeding, which has been shown to have a negative impact on the fitness of aquatic species. We undertook a collaborative study to determine the result of the first round of selection on the pedigree and genetic variation in the broodstock used in the Atlantic halibut industry in the Maritime Provinces. The partners were the Department of Fisheries and Oceans St. Andrews Biological Station, the National Research Council's Institute for Marine Biosciences, Maritime Mariculture, Inc., and R&R Finfish Development, Inc.

In 1996, 27 of the Biological Station's 52 wild

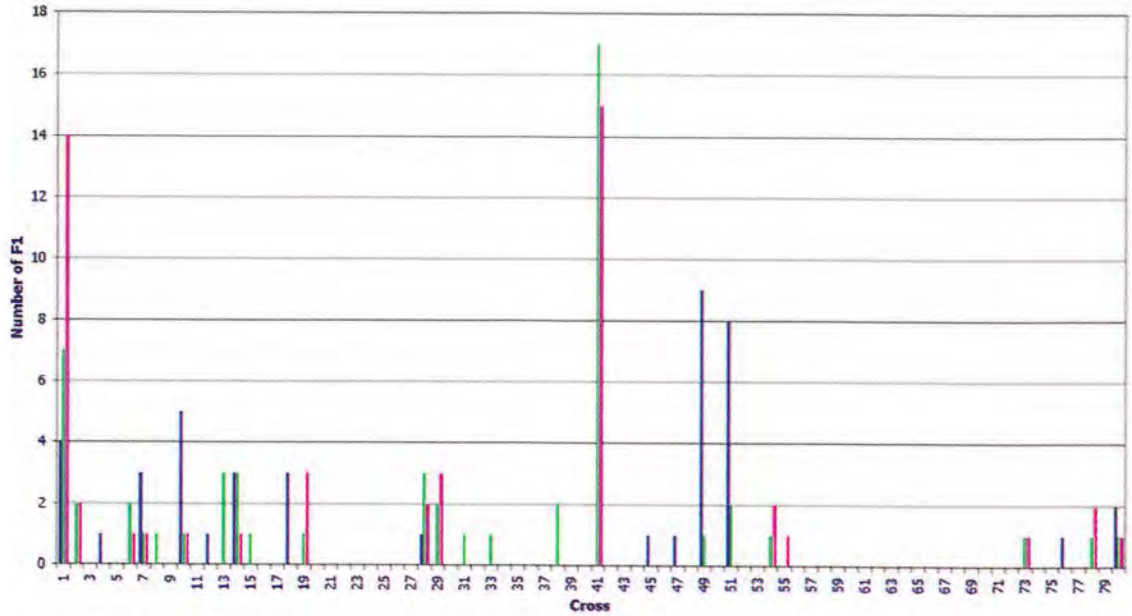

Figure 1. Number of F1 Atlantic halibut retained as broodstock from crosses performed in 1996. Different colours represent fish retained at three sites. Note some crosses are not represented while others have large numbers of full-siblings represented in the broodstock population.

broodstock (13 males, 14 females) were spawned to produce the 1996 F1 generation. One hundred and forty-five of those fish were retained as the first domestic broodstock and distributed across the two industrial hatcheries and the Biological Station hatchery. At the Institute for Marine Biosciences, McGowan and Reith (1999) developed five highly variable genetic markers for Atlantic halibut. Using these, we generated DNA fingerprints from fin-clipped tissues of all the wild broodstock and retained 1996 F1 fish. From this data we determined the parentage of 98% of the F1 fish and whether any families (brothers and sisters) existed in the three candidate broodstock groups of F1s. Of the 80 crosses performed in 1996, only 29 families were represented in the F1 stock and, as can be seen in Figure 1, there are some families (full brothers and sisters) that are troublingly large.

DNA fingerprinting data can also be used to generate indices of genetic variation in a group of individuals. One index, allelic diversity, showed us that the spawned parents represented 91% of the available wild genetic variation; however, only 74% of the available wild variation is found in the F1 fish. That represents a 26% loss in one generation. The loss in diversity and uneven family representation can be combined in the statistic "effective population size" or *Ne*. The *Ne* of the 27 wild broodstock is 27 (all are unrelated); however, the *Ne* of the combined 145 1996 F1 fish is only 12.5. As *Ne* is considered a reflection of genetically unique breeders in a group, a reduction in a single generation to 12.5 cannot be ignored.

In the case of the regional halibut producers, they are now returning to their wild broodstock and assessing fish from other spawnings (1998 F1s and others) to supplement the broodstock retained from 1996. Also, the fish in this study were internally tagged and sexed when fin clipped, so family data can now be applied for the safe breeding of the 1996 F1 (avoiding breeding full and/or half brothers and sisters). Although similar work has been conducted and is ongoing in the regional haddock broodstock program, this type of information is not generally collected or recognized as being important by the aquaculture industry at large. Many hatchery managers in the industry may be unknowingly inbreeding or severely reducing genetic variation in their broodstock. We hope that studies like these demonstrate the critical role DNA technology plays in the management of broodstock to our regional aquaculture industries.

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