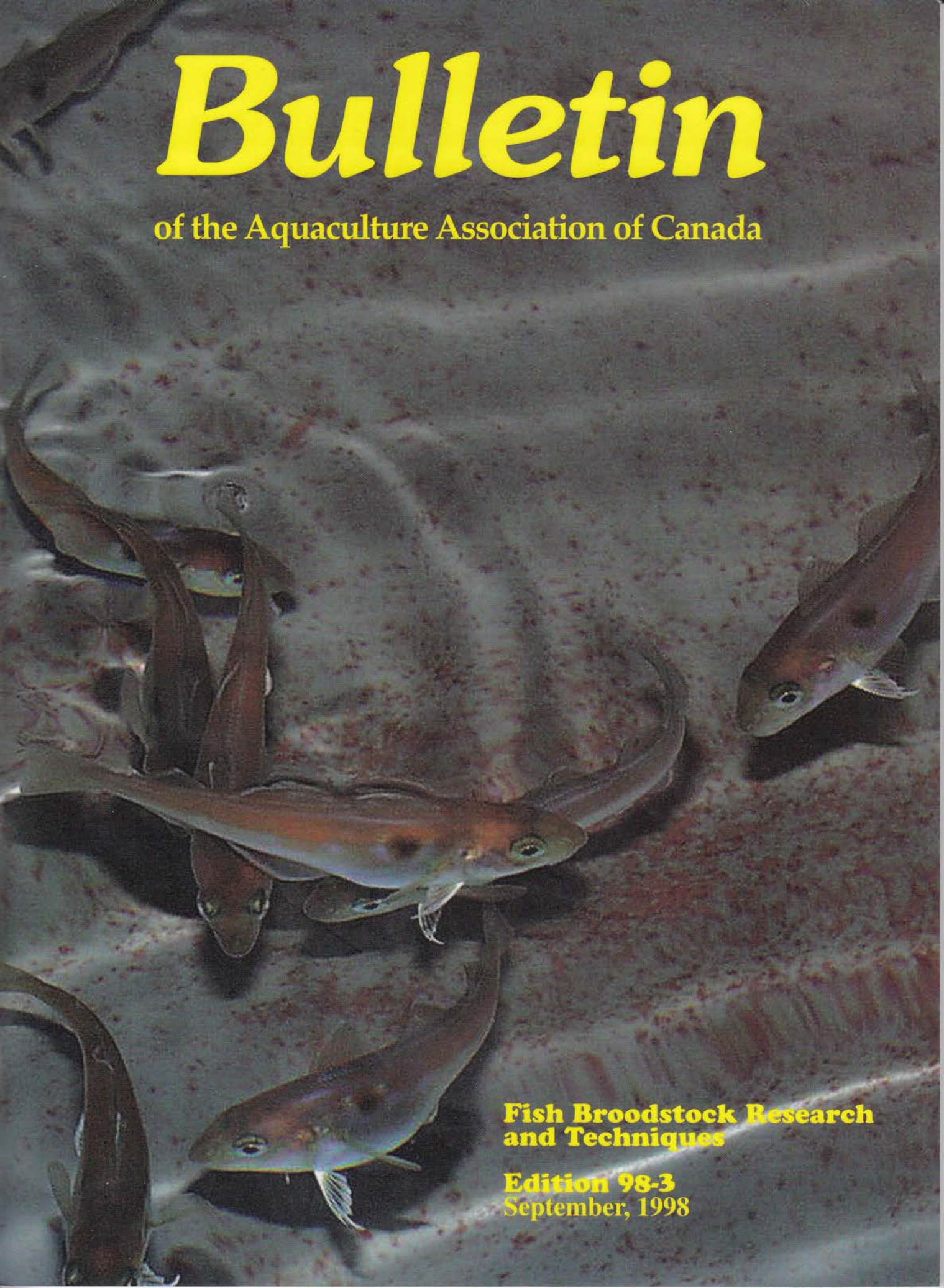

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of the Aquaculture Association of Canada

Fish Broodstock Research and Techniques

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Cover: 4-month-old haddock *(Melanogrammus aeglefinus)* hatched in the spring of 1998 by the Marine Fish Culture program at the Biological Station, Department of Fisheries and Oceans, St. Andrews *(DE Aiken photo).*

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Introduction

This issue provides highlights of the Fish Broodstock Research and Techniques Session held during Aquaculture Canada '98 – the 15th annual meeting of the Aquaculture Association of Canada — in St. John's, Newfoundland. Broodstock issues are important. At the root of any successful aquaculture enterprise is a successful broodstock program. This only seems natural, as broodstock problems, if they occur, will occur early in the process and manifest themselves in critical early life history stages, thereby putting constraints on juvenile production and facility operations.

It is therefore imperative that potential broodstock problems be identified and solved to ease facility operations and increase production. Like all aspects of finfish culture, broodstock research and management will continue to broaden as we enter the next millennium. The global aquaculture industry still firmly depends on salmon, particularly Atlantic salmon, though strong initiatives exist worldwide to develop other coldwater marine species for commercial culture. Hence, this Special Session was organized to provide state-of-the-art broodstock information on both salmonids and new candidate species.

There is no reason to isolate experts working on salmonids from those that research non-salmonids. We can all learn from each other. Salmonid aquaculture, with its 20- to 30-year history, has already confronted hurdles concerning selective breeding. In contrast, new candidate species such as halibut and haddock are still in their infancy and significant information concerning basic reproductive biology remains to be discovered and applied to improve culture success. This session was thus organized to represent all of these concerns and developments.

The session was well attended and was comprised of nine speakers of national and international background. Speakers emphasized not only their recent biological advances, but also new technology and available tools employed or developed. It is the suite of techniques available to the broodstock manager that will allow for efficient and improved culture operations. Thus, special emphasis was given to this facet of culture. Areas covered spanned genetics, selective breeding, egg quality, spermiation, cryopreservation, ultrasound, biomanipulation and breeding technology.

Some of the highlights of each of the presentations follow. Dr. Gerry Friars (St. Andrews, NB) reviewed his involvement in long-term selective breeding of Atlantic salmon for cage culture in the Bay of Fundy and discussed the associated production gains and methodology used. Dr. Larry Crim (Memorial University, St. John's, NF) presented information on the development of new species for aquaculture and the kinds of obstacles one may face in such an undertaking. Dr. Suzanne Brooks (University of Suffolk, UK) provided a detailed presentation on fish egg quality incorporating both biochemical and genetic characteristics. We learned about the handling and storage of fish semen from Dr. Krischen Rana (FAO, Rome) for successful egg fertilization and cyropreservation techniques. Mr. Vern Pepper (DFO, St. John's) reviewed comparative growth trials pertaining to Atlantic salmon strain selection for rearing in Newfoundland south shore waters. Dr. Jim Powell (Victoria, BC) presented his research findings on the use of hormonal implants to increase spermiation of Pacific salmon, both seasonally and in total quantity. Ms. Debbie Martin-Robichaud (DFO, St. Andrews) showed us new technology to determine sex and maturity condition of halibut and haddock using ultrasound. Dr. Mike Reith (National Research Council, Halifax, NS) spoke about the concepts of and early experiments in biomanipulation at the egg stage to gain control over offspring characteristics. And I showed how captive paired matings in haddock may be used to control for egg quality in production facilities, and revealed how the technique could be used to begin selective breeding programs for marine batch spawners.

Many of these speakers provided written contributions that appear in this issue and in their entirety form a well-rounded and timely contribution to this important area of fish culture. Funding for some of the speakers to travel to St. John's was kindly provided by the Atlantic Canada Opportunities Agency (ACOA), and assistance with session organization was generously given by Dr. Jay Parsons and Mr. Cyr Couturier.

It is hoped that those reading this contribution will find it valuable, and perhaps allow it to act as a stimulus for their own practices or research. For greater detail and updates on the science and ideas presented, readers are encouraged to contact the individual authors.

E.A. Trippel
Biological Station, St. Andrews

Transfer of Breeding Technology from Terrestrial Agriculture to Aquaculture

G. W. Friars

The gains made through controlled mating and selection in terrestrial species have accrued on the strength of resources in the form of natural variation. Early estimates of genetic parameters and selection advances indicate that similar gains in aquaculture are possible. The merging of conventional breeding practices with new technologies, at the molecular and cellular levels, holds great potential.

Introduction

Aquaculture, in this author's mind, is a component of agriculture. Breeding technology has had a long history in terrestrial agriculture and the transfer of this specialty to aquaculture represents tremendous opportunity and challenge. Nature contributed significant advantages to agriculture where, for instance, wheat was a cross of wild grasses. The plant breeders have been able to develop and improve this natural crossbred to its current state, where it is a food source for a large portion of the world's population. Similarly, the derivation of maize from wild progenitors has enhanced the development of modern agriculture.

The objective of this paper is to delineate some of the potential and point out some of the challenges ahead in applying terrestrial breeding practices to aquaculture.

Genetic Gains in Terrestrial Agriculture

The field of poultry improvement has contributed some of the best documented gains from controlled breeding. Data in chickens[10] revealed an average increase of approximately 26 eggs per 50 weeks, per hen housed, between 1950 and 1970 and similar gains were reported for the period 1970 to 1980.[10] The accompanying decline in the number of pounds of feed required to produce a dozen eggs attests to the improved efficiencies in commercial egg production.

Genetic gains in the efficiency of poultry meat production have paralleled those in egg production stocks. The data in Table 1, extended from an earlier publication,[2] reveal that experimentally selected lines showed appreciable gains over controls. Commercial strains, selected for six additional years, demonstrated even more striking gains. Decreases in the number of pounds of feed required to produce a pound of broiler were also realized. However, the increase in fat content represented a negative trend in terms of consumer preference, and probably also deterred improvements in feed conversion. Hence, the problem of selecting on the basis of a single trait is revealed and must be borne in mind in all breeding programs aimed at improved performance.

Potential for Genetic Gains in Aquaculture

Evidence of heritable variation in traits of economic importance has been observed in fish over the years. For example, heritability estimates for growth range from 0.17 ± 0.19 to 0.38 ± 0.22 in rainbow trout (Table 2).[9] The higher value was at two and one-half years of age, whereas the lower value was at four years of age. The feasibility of selecting for market size, on the basis of early growth, often has serious limitations. For instance, estimates of genetic correlations between growth of pre-smolt, in fresh water, and post-smolt, in sea water, became weaker as the period in sea water was extended.[5]

Age at sexual maturity is also heritable as observed in rainbow trout (Table 2).[9] Similarly, the estimate of heritability of the ability to reach market size after two winters, without becoming sexually mature, was 0.19 in Atlantic salmon. [11] Many other reports on heritabilities of traits of economic importance in aquacultural species can be found in the literature.

Importance of Pedigrees in a Breeding Program

Controlled mating systems are of paramount importance in breeding programs, in terms of managing in-

Table 1. Dressed carcass weight, abdominal fat as a percent of carcass weight, and feed conversion in 47-day-old male broilers.

Genetic Group	Selection	Dressed Carcus Weight (plucked and bled) (g)	Abdominal Fat (% of dressed carcass weight)	Feed Conversion* (kg feed/kg live body weight)
Control	No selection	717	1.2	2.01
Select 1	High 63-day and low 147-day weight until 1972	967	1.9	1.97
Select 2	High 63-day weight until 1972	1088	1.4	1.92
Commercial 1	Selected commercially until 1978	1688	2.4	1.88
Commercial 2	Selected commercially until 1978	1656	2.0	1.91

*Averaged over males and females; male weights only are shown here.

Table 2. Genetic parameters and their standard errors of traits. (9)

Traits	Age in Years	Number of Observations included	Additive Genetic Effects (h^2)
Weight	2.5	747	0.38 ± 0.22
	4.0	699	0.17 ± 0.19
Age at sexual maturity		641	0.21 ± 0.14

breeding levels. Families need to be identified in order to accomplish this feat. Family information also enhances selection progress. Where progeny from different families must be mixed in various growing operations such as seacage rearing, marks distinguishing families are required. Branding and fin clipping have been used in this connection. However, the identification of individuals within families requires further detailed marks. Operculum tags and passive integrated transponder tags have been used with certain technical limitations. The application of molecular markers may be feasible in the future as technologies become more automated and cost effective.

Pedigrees and family marking are also used to enhance selection. Family averages can be combined with information on individuals to sharpen the estimates of breeding values in prospective parents. Also, some traits such as those requiring slaughter for market quality, or mortality due to disease challenge, can only be assessed on the basis of family information as dead fish make poor breeders.

Multiple Objective Selection

Several traits need to be improved simultaneously in stocks developed for commercial production. In the Salmon Genetics Research Program,(1) for example, some traits considered were as follows:

- percent smolts per family at 18 months of age;
- market size of individuals and their siblings;
- percent non-grilse per family; and
- resistance to bacterial kidney disease based on challenged siblings.

In order to combine this information into a single score, estimates of genetic and phenotypic variances of each trait and covariances between traits were required. Also, relative economic weights must be assigned where, for instance, the relative value of a market salmon is five to ten times higher than that of a smolt. Matrix algebra was used to combine these sources of information.

Table 3. Realized gains from selection in Atlantic Salmon.[5]

Gene Pool	Weight (kg)				Length (cm)			S1[a] smolt (%)		
	Gen[b]	Con[c]	Sel[d]	SD[e]	Con	Sel	SD	Con	Sel	SD
Strain 1	1	4.3	4.6	0.32	69.8	72.0	0.35	44.6	53.6	0.40
Strain 1	2	3.5	4.4	0.83	66.6	70.6	0.80	87.8	89.8	0.25
Strain 2	1	3.7	3.9	0.47	66.5	69.3	0.31	68.7	75.9	0.40

[a] S1 fish reach the smolt stage after approximately 18 months in fresh water.

[b] Generation numbers.

[c] Controls were maintianed through the use of 40 to 50 single pair matings of fish chosen at random, without selection, with the avoidance of full sib matings.

[d] Select fish were progeny of parents selected on the basis of multiple traits.

[e] SD – standard deviation.

Table 4. Realized genetic gain for resistance to Marek's disease in chickens.[7]

Parental generation	
Mean Marek's disease incidence of all individuals	50.8 ± 1.2[a]
Mean Marek's disease incidence of selected females	34.5 ± 3.1
Mean Marek's disease incidence of selected males	25.3 ± 6.1
Reach=50.8-(34.5 + 25.3)/2=20.9 ± 3.9	
Offspring	
Mean Marek's disease incidence of control line	46.2 ± 1.4
Mean Marek's disease incidence of selected line	32.1 ± 1.4
Realized gain=46.2-32.1=14.1 ± 1.9	
Realized heritability of index=Realized gain/Reach=14.1/20.9=0.67 ± 0.30[b]	

[a] Standard errors are approximated

[b] variance of $h^2 \cong \frac{\text{variance gain}}{(\text{gain})^2} + \frac{\text{variance reach}}{(\text{reach})^2}$

Gains from Selection

Similar to the earlier reported gains from selection in terrestrial species, substantial advances have now been observed in aquacultural stocks. The realized gains in Atlantic salmon[5,6] (Table 3) resulted from the use of multiple objective selection, as described in the previous section. The gains depicted by standard deviations (SD) allow comparisons between traits. The results indicate that the increases in weight and length are cumulative and accrued from generations 1 to 2 of selection in strain 84JC, depicted as Strain 1. The fact that improvements can be accumulated over generations of selection allows for long-term benefits. The decrease of the SD for percent S_1 smolt is associated with the fact that improvement in management and environmental factors between generations contributed to appreciable increases in smoltification rate, and reflects the fact that standard deviations automatically decrease as percentages reach high levels. The results have been repeated in a single generation of selection in a second strain, 87JC.

The genetic parameters for resistance to bacterial kidney disease (BKD) had to be guessed, but a recent heritability estimate of 0.38 for survival after a challenge with BKD[8] gives credibility to the procedure used. This result, together with the realized gain from selection (Table 4) for resistance to Marek's disease in chickens, indicates that genetic gains for resistance to disease in fish has possibilities, and needs to be considered in breeding programs.

Table 5. Diploid and triploid harvest size (kg) in Atlantic salmon.[12]

Location	Diploid	Triploid
Farm 1	4.13	4.51**
Farm 2	4.36	3.91*

*Significant at $P < 0.05$

** Significant at $P < 0.01$

Involvement of New Technologies in Aquacultural Breeding Programs

Progress in research at the cellular and molecular levels has exhibited giant strides in recent decades. One of these areas has been the use of triploidy to induce sterility in Atlantic salmon. This practice has potential in the reduction of disturbances of gene pools in natural stocks, due to escapees from aquaculture. Data indicate that the results are variable between farms in terms of the disadvantages in weight gain of triploids in contrast to sibling contemporaries (Table 5).[12] The reduction in triploid weights, compared to diploids in Farm 2, is typical of the down trends experienced where the fish are under stress. The replication in two farms, referred to here, is unique as information on multiple farms is difficult to obtain. However, this type of outcome has also been noted in fresh water.

The depression in gains due to triploidy appears to be more serious in some families than in others. This result infers the possibility that stocks could be developed to tolerate triploidy.

The possibilities of transgenics are being investigated in aquaculture with some phenomenal results. Atlantic salmon have been involved in this area of research.[4] Public concerns over the use of DNA from non-homologous sources have been circumvented,[3] where a gene construct from sockeye salmon was injected into coho salmon eggs. "On average, the transgenic salmon were more than 11-fold heavier than non-transgenic controls, with a range from no growth stimulation to one individual 37 times larger than controls." The combination of this technology with conventional breeding practices could have potential in the development of unique stocks. Similarly, other molecular techniques may have potential in breeding programs.

Dispersement of Genetically Improved Stocks

Improved poultry stocks, in the form of hatching eggs, and superior cattle genes, carried in frozen semen, are dispersed globally on a routine basis. Some stocks of Atlantic salmon, tilapia and other species of fish have been shipped between continents. The concern about disturbances with gene pools of wild progenitors and derangements of ecological systems seems to be more serious in aquaculture than is the case in terrestrial species. In terrestrial species, the pilfering of gene pools is often deterred through strain crossing, other biological techniques and legal agreements. The protection of genetic gains in aquacultural stocks is now drawing attention.

Generally speaking, the potential for genetic gains in aquaculture is very promising, but restrictions due to social, ecological and economic limitations are real.

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Optimizing Broodstock Management Technologies

L. W. Crim and C. E. Wilson

The development of new candidate fish species for cool-water aquaculture depends upon control of many factors, including a predictable source of offspring. While aquaculture projects may be started with founder stocks of juveniles or gametes collected from broodstock in the wild, a secure supply of offspring can only be assured by adapting and manipulating captive broodstock to perform well under culture conditions. Normally, the conditions for holding newly acquired broodstock closely emulate the field situation, with similar light and oxygen levels, thermal regimes, and feeding schedules. But for reasons of either convenience or practicality, broodstock usually will be acclimated to extraordinary and possibly stressful rearing conditions, which may negatively impact reproductive performance. While most fish species appear capable of undergoing gonadal development in captivity, few are able to spontaneously spawn viable gametes (e.g., Atlantic cod). Therefore, considerable research is aimed at developing gamete collection and fertilization protocols for captive female broodstock. For example, both Atlantic halibut and yellowtail flounder broodstock spawn eggs in batches requiring frequent (24 to 72 h) handling and stripping to collect freshly ovulated eggs from each ripe female. For internal fertilizers, such as ocean pout and wolffish, artificial egg insemination protocols must be modified, highlighting the need to develop broodstock management strategies for individual species. Efficient protocols for handling females will not only yield eggs of the highest possible quality, but will also maximize egg production in comparison to the annual gamete potential (fecundity). The definition of egg quality is based on various criteria, namely egg morphometric description, egg fertilization and hatch rates or, best of all, larval survival. Under ideal conditions, more than 50% of the juvenile offspring will survive (e.g., yellowtail flounder). Finally, control of broodstock reproduction has been effected using gonadotropic releasing hormone analogue (GnRH-A) to synchronize spawning in females and increase milt volume in males. This hormone technology in combination with environmental manipulation of the reproductive cycle may be a promising means of broadening the spawning season to increase the efficiency of aquaculture hatchery operations.

The development of new candidate finfish species from the northwest Atlantic for aquaculture depends upon the control of many biological and physical environmental factors. It is especially important to have a predictable source of offspring. While aquaculture projects may be quickly started with founder stocks of juveniles collected from the wild (strategy 1), or even from viable gametes produced by adult fish from the wild (strategy 2), predictable offspring supplies in the long-term can only be assured by adapting and manipulating captive broodstock to produce healthy young under culture conditions.

Strategy 1 for Rapid Start-up

Starting with wild-caught juveniles, the rapid start-up with a new species for aquaculture requires considerable knowledge and experience with culture practices (Table 1). For example, assuming access to sufficient numbers of juveniles in the wild, proper methods must be known for their capture (draggers, hook and line, and SCUBA divers), live transport (oxygen levels, temperatures, and anaesthetics) and holding the newly-caught juveniles once they reach their farm location (water flows, tank size, stocking density,

type of diet) to avoid highly stressful regimes that could result in unhealthy individuals or cause large-scale mortalities.

Over time, wild juveniles must be acclimated to artificial rearing conditions which may be judged by health records and growth of the young fish under these new conditions. In the beginning, wild fish often must be fed diets composed of fresh chopped fish or invertebrates (shrimp, sea urchin) to initiate the feeding response in captivity. While this may be necessary, such fresh diets are not ideal because of their expense, inconvenience, or incomplete nutrient supplies (energy levels, vitamins, trace minerals). Therefore, the newly acclimated fish must be weaned onto either home-made moist pellets or commercial diets which will serve to optimize growth.

Strategy 2 for Rapid Start-up

Another route to a relatively rapid aquaculture start-up with a new species is based upon collection of good quality gametes (eggs and sperm) from ripened wild broodstock (Table 1). While the high fecundity (annual gamete production) of many marine fish species potentially allows for collection of large numbers of eggs and sperm, egg overripening is frequently a problem, so eggs must be freshly collected after ovulation to retain high viability. If gametes in proper condition are collected from wild broodstock, then the ordinary practices of in vitro fertilization, egg incubation and hatching of larvae are normally followed to produce large numbers of yolk-sac larvae. Within a few weeks after hatch, enriched live-food production including algae, rotifers, and artemia must be made available for the first-feeding larvae to develop through metamorphosis and yield a supply of juveniles (Table 1).

Table 1. Steps involved in rapid aquaculture start-up beginning with juveniles from the wild or gametes produced by wild broodstock.

Wild Juveniles	Gametes from Wild Broodstock
Acclimation	In vitro egg fertilization
Feed and grow	Egg incubation
Market	Hatch of larvae
	First feeding
	Metamorphosis
	Weaning
	Feed and grow
	Market

Strategy 3 for Long-term Aquaculture

In both strategies 1 and 2, offspring sources dependent upon wild collection of juveniles or gametes from ripened broodstock will always be unpredictable and necessarily unstable. Therefore, there is the need to develop a domesticated broodstock which requires the acquisition and holding of broodstock fish and the development of management technologies necessary to produce stable offspring supplies (Table 2). Much like the collection of juveniles, wild broodstock must acclimated to artificial culture conditions that minimize stress and metabolic disturbances. Again, wild broodstock are often initially fed with chopped fish or shrimp before being switched onto commercial diets. Both the quantity and quality of the diet is important for reproductive performance. For example, broodstock nutrition is important before spawning to insure gonad development and spawning of high quality gametes. After spawning, reconditioning of broodstock is important to restore body resources which will impact fecundity and quality of gamete production in the coming year(s). In addition to the type of broodstock diet,[2] the plane of nutrition may have an important bearing upon the decision of broodstock to either remature annually or remain non-reproductive for one or more years.[3] Work on broodstock nutrition is needed to reduce the excessive variability in gamete quality frequently encountered in a domesticated broodstock (see below).

Table 2. Requirements for developing a domesticated broodstock for stable aquaculture of a new species.

Broodstock	Eggs and Sperm	Juveniles
Collection	Gamete supply	Juvenile supply
Feeding and acclimation	Egg fertilization	Feed and grow
Gonad development	Egg incubation	Market or mature
Spawning	Hatch of larvae	
Reconditioning	First feeding	
	Metamorphosis	
	Weaning	

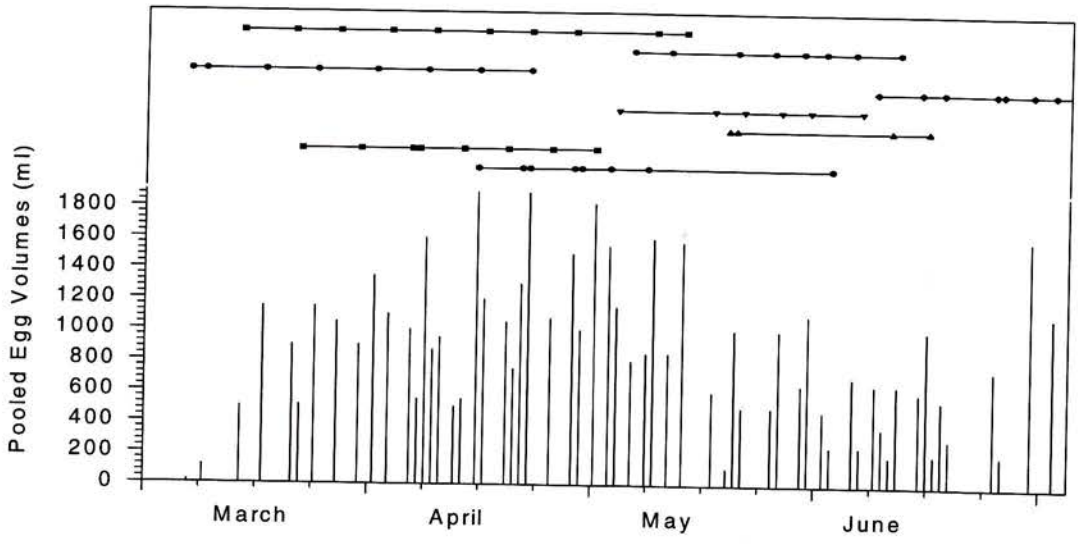

Figure 1. Daily pooled egg volumes (vertical bars) collected during the 1996 cod spawning season. Duration of ovulatory activity for 8 females is represented by horizontal lines. Dates for egg collections are indicated on lines by various solid symbols.

Table 3. Enhanced broodstock reproductive technologies.

Broodstock	Requirements	Technology
Spawning protocols	Spontaneously spawning vs Manual stripping	Artificial insemination
Gamete quality	Fresh eggs and sperm	Gamete storage
Control of the timing of reproduction	Induced spawning Synchronized spawning In-season and off-season breeding	Hormone treatment Environmental regulation

Spawning Protocols and Gamete Quality

Having met the basic requirements necessary to provide a stable supply of offspring for aquaculture production (above), much can be gained from specialized reproductive technologies to optimize the performance of domesticated broodstock (Table 3).

Under ideal circumstances, domesticated broodstock will spawn spontaneously in captivity, obviating the need for handling to determine the reproductive status of ripening fish and the stripping of gametes. For example, mixed populations of male and female Atlantic cod readily spawn under captive conditions yielding high quality fertilized eggs which float and are obtained simply by placing strainers over the tank outflow.[4] Beginning late in the winter, groups of mature cod held under these conditions repeatedly spawn for many weeks producing large volumes of fertilized eggs. There is good evidence from individual records that female cod are multiple or serial egg-batch spawners (Fig 1). For the approximate 4-month spawning season, females spawned an average of 7 times (egg volume >5 mL) with most females spawning at regular intervals averaging 6.3 ± 0.7 days. Under these circumstances, good quality eggs were collected based upon the average egg fertilization rate of 84 ± 3%.

In the absence of spontaneous (volitional) spawning, domesticated broodstock are in need of close

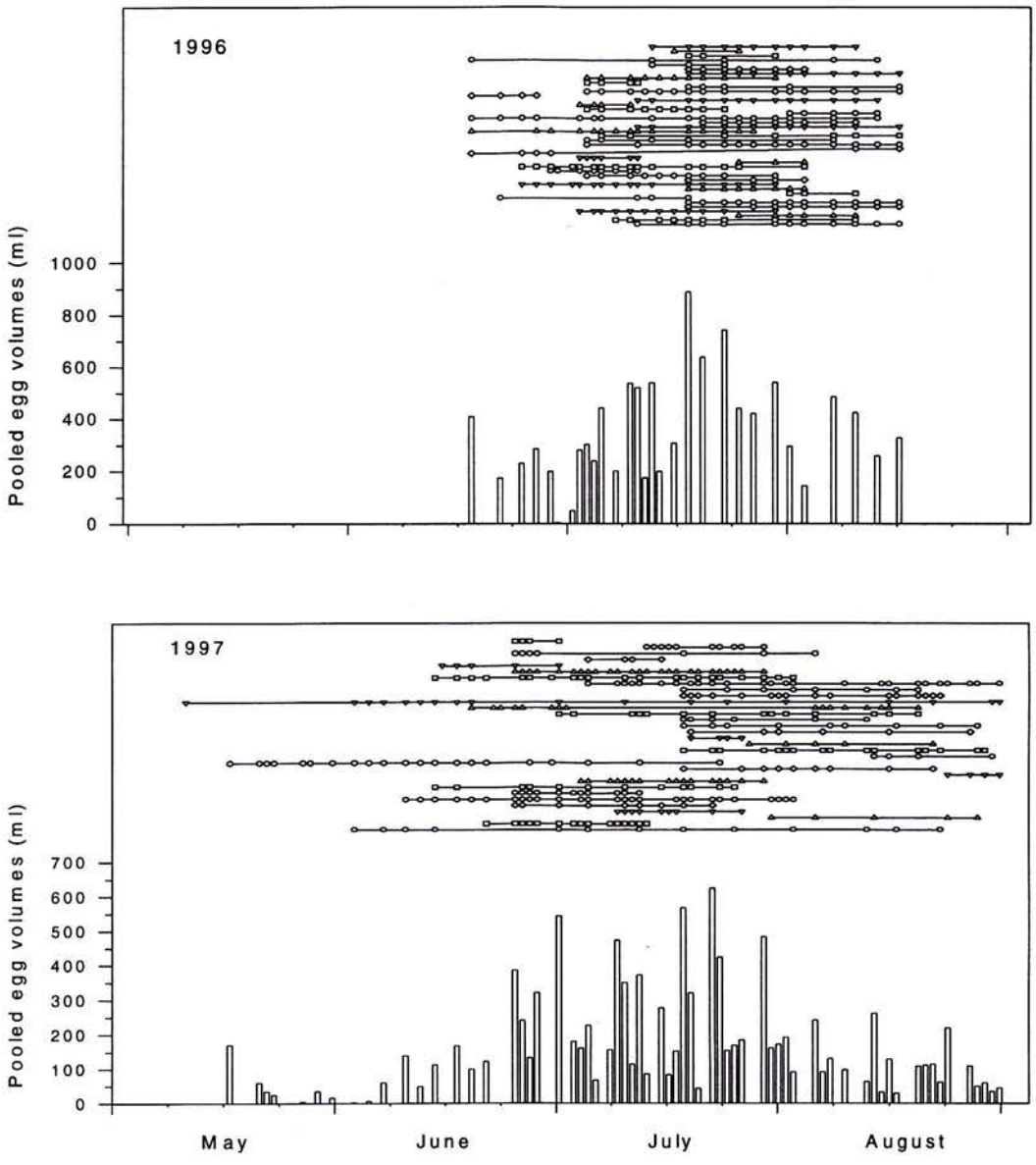

Figure 2. Daily pooled egg volumes (vertical bars) collected during the yellowtail flounder spawning season. Duration of ovulatory activity for 41 females in 1996 and 43 females in 1997 is represented by horizontal lines. Dates for egg collections are indicated on lines by the various open symbols.

monitoring to determine their reproductive condition and to strip fresh gametes because eggs quickly overripen after ovulation and begin to deteriorate. Handling of serial egg-batch spawners such as the yellowtail flounder is particularly difficult because they characterstically ovulate egg batches repeatedly at short intervals (1 to 2 days) and because egg quality is highly variable between individuals and between egg batches from the same female.[5] In our recent work with yellowtail broodstock, females were checked for spawning under two different conditions: 3 times/wk (Monday, Wednesday, and Friday) in 1996 and daily in 1997 (Fig. 2). The data show that yellowtail begin to spawn as early as May and spawning continues into August. Based on pooled egg volumes, July is the peak spawning period. Among the records for individual spawning performance (egg volume >5 mL), examples are seen of females spawning very briefly

Table 4. Yellowtail broodstock spawning performance and egg quality data for 1996 and 1997.

	1996	1997
Duration of spawning (days)	76	121
Number of spawning females	41	43
Mean egg batches per female	8	12
Mean egg batch volume (mL)	33.5 ± 1.4	25.8 ± 1.1
Mean egg viability rate* (%)	59 ± 3.5	60 ± 1
Mean egg viability rate (1st half)	75 ± 2.8	64 ± 2.9
Mean egg viability rate (2nd half)	46 ± 4.4	58.5 ± 1.2

*Egg viability rate is based upon the proportion of round, clear and floating eggs lacking a perivitelline space prior to fertilization.

for a period of just a couple of weeks. In contrast, for most females the duration of spawning lasted more than a month. In 1997, more than half the females spawned at least 10 times and the best 5 females were stripped of eggs on more than 20 occasions. Finally, by comparing egg viabilities in 1996 and 1997 (Table 4), it is seen that egg quality over the entire spawning season was the same in both years. In analysing these results more carefully, it was noted that mean water temperatures were lower during the first half of both spawning seasons (~7°C) compared with the second half (~11°C). After dividing the spawning season into two halves, it was shown that egg quality was most severely reduced during the second half of 1996 which suggests that the combination of less frequent checking of broodstock and increased water temperatures may be detrimental to egg quality.

Control of the Timing of Reproduction

One notable feature of yellowtail broodstock is that the females tend to spawn at different times throughout the summer. Therefore, the somewhat protracted spawning season appears to be due to both asynchrony of spawning and the serial egg-batch spawning characteristics of this species (Fig. 2). To try to improve this situation, Larsson et al.[6] used hormone treatment in 1995 to synchronize spawning of yellowtail females. This work was successful and demonstrated that, after ripe females received gonadotropin releasing hormone analogue (GnRH-A) treatment at the beginning of the spawning season in June, spawning was synchronized, egg-batch volumes were increased, the duration of the spawning season was shortened, and egg quality was maintained or improved.

Another application of this type of hormone technology has been used to determine to what degree the spawning season could be advanced in female yellowtail. When females were treated with GnRH-A in February, roughly 3 months prior to the normal spawning season, no advancement of spawning occurred in the majority of females.[1] By contrast, when females were treated with GnRH-A in April, they began spawning in May and spawning was completed by June, showing that the spawning season could be both advanced and synchronized using hormone treatment. Therefore, this work suggests that hormonal regulation of the timing of spawning may be feasible but will most likely depend upon how well the gonads are developed at the time of hormone treatment. This approach, if successful, has the potential to not only reduce the handling of broodstock fish but also increase hatchery output by broadening the spawning season to include more eggs in the "off-season". Perhaps the most effective approach to control reproduction in fish will depend upon use of a combination of environmental manipulation (photoperiod and temperature) and hormonal treatment of hatchery broodstock to maximize the broadening of the spawning season.

In conclusion, a domesticated broodstock is important to make fish farming practical. Broodstock husbandry and handling methods have an important influence on the production of good quality gametes. Collection of good quality gametes from serial egg-batch spawners can be difficult and requires special attention. Control and manipulation of broodstock reproduction can improve hatchery efficiency.

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Induced and Synchronized Spawning of Captive Broodstock Using Ovaplant and Ovaprim

J. F. F. Powell, J. Brackett, and J. A. Battaglia

In a large population of salmon, maturation occurs at different rates and fish spawn at different times. In some cases, spawning within a group of fish may occur over several months. In these fish, it would be a distinct advantage to synchronize and compress the spawning season. In other circumstances, it is desirable to have some fish in a population mature ahead of others. The ability to advance maturation provides greater flexibility in the hatchery and with seawater entry dates of smolts. As well, early gametes permit the out-crossing of different strains. With regard to alternate species, several obstacles to research and development could be overcome if fish spawned in a predictable fashion. Under ESC (Health Canada) approval we have developed a method to advance and synchronize maturation in captive broodstock. In controlled studies and trials conducted in British Columbia, New Brunswick and Chile, coho (*Oncorhynchus kisutch*), chinook (*O. tshawytscha*) and Atlantic salmon (*Salmo salar*), trout (*O. mykiss*) and sablefish (*Anoplopoma fimbria*) were induced to mature using peptide implants. In treated coho salmon, spawning dates were significantly ($P<0.05$) advanced, the spawning season was shortened, milt quality was increased and fry reached first feeding earlier. In several trials, this method has proven both effective and safe for humans and fish. The implications of this technology for producers are profound in the development of a broodstock management programme.

Introduction

Several methods are available to induce maturation in captive fishes, including injection of gonadotropin hormones (GTH), pituitary extracts containing GTH,[1] human chorionic GTH, and gonadotropin-releasing hormones (GnRH). All these methods either supply GTH, or GTH-like peptides, or elicit the liberation of native GTHs from the pituitary which then induce maturation.[2] Recently, hypothalamic hormones such as native GnRH or their analogues have gained favour among commercial fish producers. GnRH peptides are smaller, easier to prepare, and are more effective at inducing maturation than other peptides. Because they are naturally occurring peptides that use the endocrine pathways of the fish, they are more reliable and potent without causing harmful side effects. As well, analogues of both the mammalian and salmon GnRH (sGnRHa) forms are more potent and degrade slower than natural hormones.[3]

Methods for administration of GnRH peptides to fish include topical absorption,[4] injection of soluble GnRH in vehicle, intubation,[5] or a sustained release preparation.[6] Of these methods, three are commonly used: saline solutions of GnRH,[7] injection of commercial preparations that contain a salmon GnRH analogue such as Ovaprim[8] or sustained released pellets[6] (Ovaplant; Syndel Laboratories, Vancouver). Pure synthetic GnRH analogues have been on the commercial market for over a decade, but require field preparation and refrigeration to prevent degradation. Preparations such as Ovaprim or Ovaplant are preferred because of handling and storage considerations without loss of efficacy.

In a study to demonstrate the efficacy of induced maturation using liquid injectable sGnRHa or implants that contain sGnRHa, coho salmon (*Oncorhynchus kisutch*) were either injected with Ovaprim, implanted with Ovaplant, or administered both treatments. To determine species differences, rainbow trout (*O. mykiss*) were treated with Ovaplant to induce spawning. The objectives of this study were to

determine whether treatment to induce spawning causes an increase in prespawn mortality, advances spawning date, compresses the spawning season, or has any deleterious effect on the progeny. The study was conducted under controlled conditions and in a commercial production setting.

Methods and Materials

Fish

One month prior to normal spawning, 250 maturing male and female coho salmon (*O. kitsuch*) were transferred from seawater netcages to freshwater raceways at the Chiloe Aquaculture Research Center, University of Chile, Castro. Four earthen raceways (4 x 2 x 25 m) were divided in half by a screen placed midway along the length. Groups consisting of 25 males and 25 females were randomly selected and placed in each section of the divided raceways. Fish were acclimated to the holding conditions for two weeks at water temperatures of 8°C and at flow rates sufficient to provide saturated levels of dissolved oxygen. After two weeks, each group of fish was assigned a treatment in the following manner: groups closest to the inlet were designated controls and all other groups were randomly assigned treatments. Control fish were so designated to prevent possible effects of pheromones released from treated fish upstream. Group designations and treatments appear in Table 1. Average weight of the coho was 3.5 kg.

Trout were transferred from seawater netcages approximately 6 weeks prior to their normal spawning date. Fish were randomly placed in partitioned raceways to form two groups containing 32 fish each. Average weight of the trout was 5 kg.

Procedure for Ovaplant experiment

As detailed in Table 1, coho and trout were implanted with Ovaplant implants (Groups 1, 2, and 6) or placebo implants (Group 4) containing no peptide. Ovaplant implants each contained 150 µg of sGnRHa (sGnRH-D-Arg^6-Pro^9-Net) in inert, biodegradable vehicle. Final doses for fish averaged 43 µg/kg for coho and 30 µg/kg for trout. The pellet has a life expectancy of 21 days making the daily average dose of sGnRH analogue 2 µg/kg/day for coho and 1.4 µg/kg/day for trout.

Fish were crowded in one area of the raceway and removed by dip net to an anesthetic bath containing freshwater, salt, and 500 ppm benzocaine. Anesthetized fish were transferred to a scale and weighed. After weighing, fish were implanted, the affected area was swabbed with a topical disinfectant and the fish were placed in the raceway to recover.

Checking the fish

All fish were checked for maturation twice a week after implantation. Maturation was considered achieved when fish expressed gametes upon receiving gentle pressure to the abdomen. Once spawning was initiated, remaining fish were checked for ripeness daily.

Ovaprim injection

When the first coho expressed gametes, all fish in groups 1 and 3 were anesthetized and weighed. They received 0.5 mL/kg of Ovaprim delivered interperitonealy using a 22-gauge needle connected to a 3-mL syringe. Trout in Group 6 were treated similarly. After treatment, fish were returned to the raceway and checked for maturity every day thereafter.

Incubation protocol

Paired mating, the use of milt from one male to fertilize the eggs of one female, permits a more accurate evaluation of gamete viability. Egg incubation at the Chiloe Aquaculture Research Center was carried out using routine procedures. Fertilized eggs from paired matings were incubated separately to the hatching stage at which time they were pooled with cohorts from the same experimental group until the experiment terminated.

Males

A positive response to treatment was the release of

Table 1. Group and treatment designations for fish.

Group		Treatment	Number of Males	Number of Females
Coho	1	Ovaplant and Ovaprim	25	25
	2	Ovaplant	25	25
	3	Ovaprim	25	25
	4	Placebo Implant	25	25
	5	No Treatment	25	25
Trout	6	Ovaprim and Ovaplant	13	19
	7	No Treatment	15	17

Table 2. Parameters measured in spawning fish.

Parameter	Measurement
Males	
Motility	Time to cessation of movement (seconds)
Cell Count	Neubauer Bright line chamber (cells/mL)
Volume	Volumetric measure (mL)
Females	
Volume of eggs	Volumetric measure (L)
Egg size	Measure of subsample (mm)
Fertilization rate	Cell division 24h post-fertilization
Survival to eyed, hatch and first feeding	Survival of total eggs received per female (%)
Hatching	Time from treatment to hatch (days)
First Feeding	Time from treatment and weight (days)

Table 3. Comparison of spawning rate in coho and trout. Coho salmon were treated on 2 May and trout on 30 April. Shown are values for the number of days from treatment to initial spawning, the number of days required for 50% of the group to spawn, and number of days for the group to complete spawning. Duration of the spawning season is also shown.

Group	Time to Initial Spawning (days)	Time to 50% Spawning (days)	Time to End of Spawning (days)	Duration of Spawning Period (days)
Coho				
1	10	10	14	4
2	10	12	16	6
3	11	11	17	6
4	17	25	31	14
5	16	24	32	16
Trout				
6	14	15	31	17
7	31	47	56	24

milt after palpation of the abdomen. After each fish expressed milt, the adipose fin was clipped in order to determine the number of newly expressing males for each sampling period. Milt were activated with the addition of buffered 0.6% saline solution. Samples were repeated in triplicate (the parameters measured appear in Table 2).

Females

Spawning in females was defined as the free-flow of eggs from the genital papillae with slight pressure to the abdomen of the fish. Fish that released eggs were dispatched with a blow to the head, weighed, exsanguinated, and dried with towels. Eggs were stripped from the carcass and enumerated volumetrically. Numerical tracking of individual egg batches was by group number and spawning order. Parameters measured appear in Table 2.

Statistical analysis

Statistical analyses conducted on the data were one-way analysis of variance (ANOVA) using Tukeys and Kruskal-Wallis methods for parametric data and ranked Dunn's method for non-parametric data. Significance accepted at the $P<0.05$ level unless otherwise noted.

Table 4. Milt characterisitics of coho salmon and trout. Volume of milt is for single attempts at time of spawning. Sperm density or count is expressed as millions of cells per milliliter. Motility of sperm is expressed in duration of activity after activating a small sample of milt. Data are expressed as mean value and standard error of the mean.

Group	Volume (mL)	Sperm Density (x 10^6/mL)	Motility (duration in sec)
Coho			
1	34.8 ± 3.4	45.4 ± 4.6	1929.8 ± 23.4
2	44.4 ± 3.8	41.8 ± 6.1	1742.7 ± 36.4
3	41.1 ± 2.3	49.1 ± 3.3	1905.0 ± 28
4	47.2 ± 3.4	43.2 ± 4.1	1778.5 ± 178.5
5	49.8 ± 3	50.4 ± 0.5	1834.0 ± 172.3
Trout			
6	37.9 ± 10.5	46.25 ± 3.1	15107 ± 181.9
7	27.9 ± 13.2	48.7 ± 1.4	1824.2 ± 106.8

Results

Prespawn mortality

After 48 hours post-treatment, there were no mortalities in any group and no differences in overall mortality between treatments.

Time to spawning

Coho. Treatment with either Ovaplant, Ovaprim, or with preparations in combination significantly ($P < 0.05$) advanced spawning. Further, the spawning season was significantly compressed using experimental treatments (Table 3). The remaining two fish in the group spawned on the following days to extend the spawning season in this group to four days. In group 2 (Ovaplant), all fish spawned within 6 days of the start of spawning on 12 May. Group 3 (Ovaprim) completed spawning in 6 days after beginning spawning two days post-treatment. Placebo control fish began to spawn 8 days after the treated groups and continued spawning for 14 more days. Untreated fish began to spawn 6 days after the other fish were treated and continued to spawn for 16 more days. There was no difference in spawning time for the control groups.

Trout. Treatment with Ovaplant and Ovaprim significantly advanced the spawning date in trout. Treated trout began to spawn two weeks post-treatment and the spawning season lasted 17 days with 89% of fish spawning in 10 days. Control fish began to spawn as the last treated fish was spawned, 31 days post-treatment. The spawning season for control trout spanned 25 days. The time to 50% spawning for both populations differed by a month (Table 3).

Characteristics of milt

Coho males in group 1 and 3 had lower volumes of milt than control fish, but had higher sperm counts (Table 4). In trout, treated males had a higher ($P<0.053$) milt volume than control fish. There were no other differences seen in milt.

Eggs

There were no differences between treatment for the following parameters: volume of eggs, size of eggs, survival to eyed stage, survival to hatch, and survival to first feeding. Experimental groups reached first feeding significantly faster than control groups. When considered in relation to time from spawning to first feeding, the treatment further increased this effect. The duration of first feeding also differed with respect to treatment (Table 5).

Discussion

Fish treated with either Ovaplant and/or Ovaprim spawned in advance of control fish. Eggs and larvae from treated groups had similar survival and growth as those from control stocks. This demonstrates there is no latent effect of treatment on gamete viability. As such, this represents a significant advantage to freshwater culture operations. The reduced residency of spawning fish in freshwater decreases the potential of

Table 5. Days to first feeding from treatment and from spawning, and duration of first feeding for coho salmon fry from the start of spawning.

Group	Time from Spawning to First Feeding (days)	Time from Treatment to First Feeding (days)	Duration of First Feeding (days)
1	65	75	5
2	66	73	8
3	67	83	10
4	71	96	19
5	72	106	23

disease transmission to the current and subsequent generations by removing a pool of potential pathogens and decreases the need for therapeutant use. Advanced spawning also permits hatcheries to take advantage of warmer water temperatures which in turn promotes better growth and feed conversion in offspring.

The treatments also compressed the spawning season. That is, the duration of the spawning season within the treated groups was less than in the control groups. In the case of trout, spawning was complete by the time the control fish had begun to spawn. This was also the case in coho where the spawning season for treated groups averaged five days compared to 15 days for control fish. A compressed spawning season provides considerable economic advantage as it decreases the amount of time crews must attend adult fish.

Batch spawning of fish also permits batch ponding and first feeding of fry as exemplified by the current study. This results in more uniform growth of fish within a population by decreasing the duration of the first feeding phase. Uniformity in the size of fish is desired because it reduces the need for husbandry practices such as grading and mixing sizes of feeds. It is commonly thought that smolts of a uniform size also perform better in seawater than smolts of varied sizes.

This study has shown that induced maturation and coordinated spawning is a valuable tool for fish culturists. Firstly, the study indicates that greater efficiencies in hatchery operations are possible with regard to operational and capital costs. These savings are realized both immediately with adult fish and later with juvenile production. Secondly, there are implications for fish health as the freshwater residency period of adult fish is reduced; hence the use of therapeutants and the potential for horizontal transmission of disease is reduced. Thirdly, the genetic potential of broodstock can be maximized. By controlling the date of spawning, a greater number of viable gametes can be introduced into the breeding programme. As well, these techniques permit the out crossing of strains and the maximization of single-paired matings. In sum, advanced maturation helps maximize options in broodstock management.

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Sex Determination of Flatfish and Gadids Using Ultrasonography

D. J. Martin-Robichaud, M. A. Rommens and L. Vallee

Ultrasound is a non-invasive method of determining the sex of sexually monomorphic fish. A study was conducted to determine the feasibility of using ultrasound images to sex juvenile and mature halibut, and mature winter flounder and haddock. Ultrasonography has applications in broodstock management for the determination of the sex ratios of groups of volitional, serial spawners such as haddock, and the sex of immature halibut being reared as future spawning stock. The degree of ovarian maturation in haddock may also be assessed using ultrasound. Our study used an Access 10 Ultrasound with a variable frequency scanhead. Determinations were made by comparing ultrasound images with those from fish of known sex. Immature ovaries were relatively easy to identify, but in most cases testes were only distinguishable in mature fish. The ultrasound techniques that we used on flatfish and haddock are described.

Introduction

The ability to determine sex and assess stage of maturity of finfish is essential for applications in both fisheries management and aquaculture. For fish species that are sexually monomorphic, a variety of biochemical techniques has been developed to determine the sex of live specimens, including the immunochemical identification of female specific proteins in the plasma or mucous, and radioimmunoassay of reproductive hormones in the plasma. These procedures, however, are costly, time-consuming, and can not be easily adapted for industry use. Invasive techniques such as urogenital catheterization and ovarian biopsy have also been used to determine sex. A rapid, non-invasive technique to determine gender and gonad condition would be useful and ultrasound, which has been used to determine the sex of mature cod after gonadal development commenced in the fall,[1] has potential.

Ultrasonography has other potential uses as well. Optimum sex ratios are particularly critical for serial, volitional spawners such as haddock (*Melanogrammus aeglefinus)* and cod (*Gadus morhua*). Being able to determine the sex of mature fish prior to their allocation to tanks for spawning could increase the production of viable eggs. Knowing the sex of juvenile fish selected from specific genetic lines as future broodstock would allow retention of specific quantities of each gender. Maximizing the number of female broodstock would reduce the cost and space requirements of rearing fish to maturity, particularly in large species such as halibut (*Hippoglossus hippoglossus)* and sturgeon (*Acipenser* sp.). Many culture operations for these species still rely on wild-caught broodstock and the rapid assessment of sex of newly captured fish would also be useful.

The purpose of this study was to evaluate the feasibility of using ultrasonography to determine the sex of juvenile and mature halibut, and of mature haddock and flounders throughout the year. Emphasis was placed on describing the methodology and the characteristics of the images obtained with ultrasound to aid in the conduct, evaluation, and interpretation of ultrasound images by others.

Materials and Methods

An ATL Ultramark 4 Plus ultrasound with an Access mechanical multi-frequency sector scanhead (5.0, 7.5, and 10 MHz) was used. Ultrasound is acoustical energy produced by a transducer at megahertz (MHz) frequencies. The energy is absorbed or reflected back

to the transducer as it passes through the tissues. Higher frequency acoustic signals result in greater resolution but less depth penetration. The amount of signal reflected back to the transducer is dependent on tissue density. Dense tissues reflect more sound waves and appear white whereas less-dense, more fluid-filled organs and tissues are similar to water, reflect less, and result in a darker image. Image measurements were taken with system measurement controls and a trackball which permitted area, perimeter, and length determinations.

Initial ultrasound scans were conducted on 10 wild-caught, mature winter flounder (*Pseudopleuronectes americanus*) and yellowtail flounder (*Limanda ferruginea*) and 6 wild-caught juvenile halibut (52 to 67 cm FL). Fish were anaesthesized with metomidate hydrochloride (3 mg/L) in 44 L of water. The ultrasound scanhead was suspended in the water about 1 to 3 cm above the fish. The transducer frequency was 5 MHz and penetration depth was set at 75 to 90 mm. Cross-sections of the gonads were scanned directly posterior to the gut region. After scanning, the fish were dissected to confirm the assessments determined by ultrasound. These initial images were used as a reference to determine the sex of 4-year-old cultured juvenile halibut (n=31, 54.8 to 71.2 cm and 2.3 to 5.2 kg).

Mature Atlantic halibut (females 96 to 120 cm, 13.3 to 28.4 kg; males 82 to 105 cm and 6.1 to 19 kg) of known sex were scanned with ultrasound after spawning in July 1997 and during the subsequent spawning period in May 1998. Halibut were placed on a neoprene-covered table 6 to 12 cm underwater. The scanhead was suspended in the water approximately 1 to 7 cm above the surface of the fish. The transducer frequency was 5 MHz and penetration depth was set at 110 to 120 mm to produce an overall image of gonad morphology. To increase the resolution and to obtain magnified images of individual oocytes in spawning fish, the transducer frequency was set at 7.5 MHz and 40 mm depth. As with the flounders, the gonads of halibut were scanned in a cross-sectional plane directly posterior to the gut region.

Mature haddock (average 62.4 cm and 2.1 kg) were

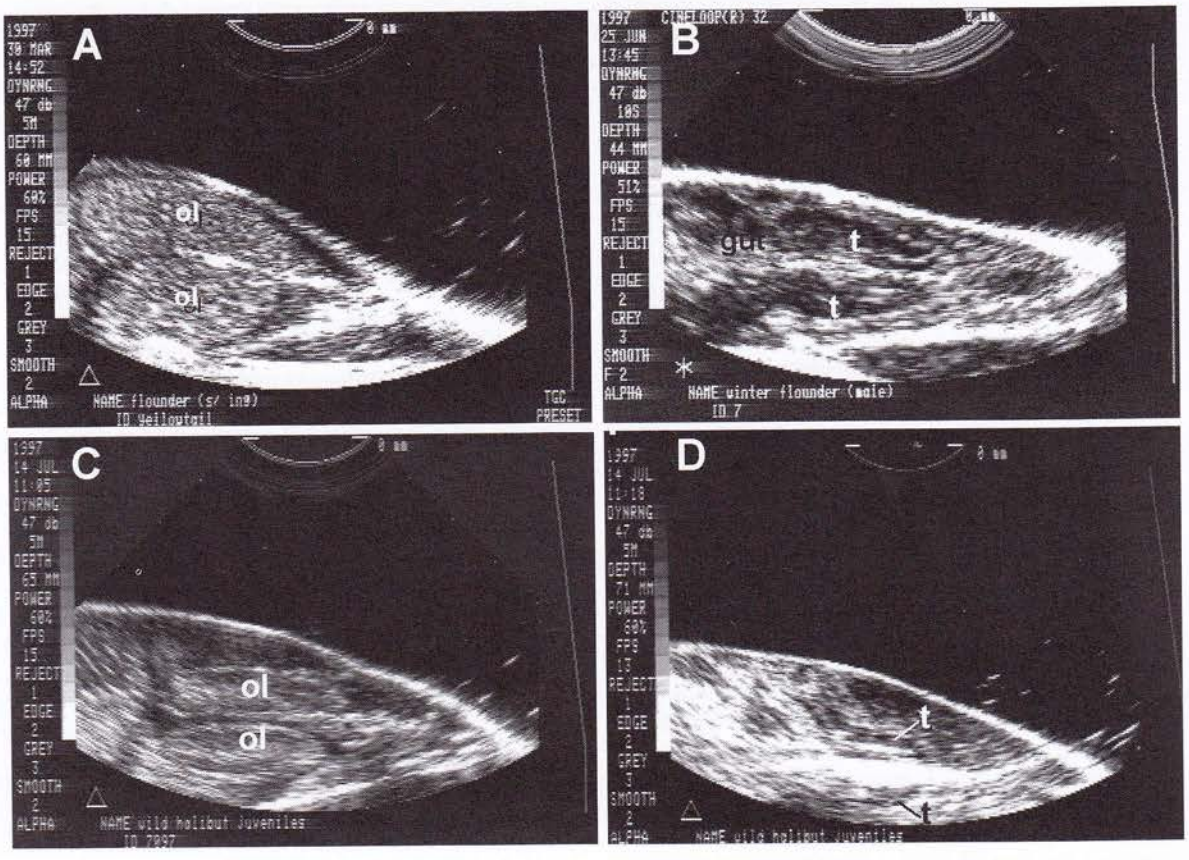

Figure 1. A) Gravid yellowtail flounder, B) mature male winter flounder, C) immature female halibut (53.6 cm FL), and D) immature male halibut (52.0 cm FL) (ol–ovarian lobe, t–testis).

scanned with ultrasound every 4 to 6 weeks from January 20, 1997, to April 23, 1998. Anaesthesized haddock were positioned and held stationary with the ventral surface up. The scanhead was suspended 1 to 4 cm in the water over the ventral surface of the fish and positioned directly anterior to the urogenital pore to obtain a cross-sectional image of the body cavity. Sex was confirmed by urogenital catheterization.

Results

Flatfish were positioned on one side, so the image showed one gonad above the other separated by the median mesentery and vertebral spinal rays. The ovary of yellowtail flounder appeared as two whitish-gray granular lobes, one lying over the other when the scanhead was positioned directly posterior to the gut (Fig.1A). The testes of mature flatfish were dark, fluid-filled, bi-lobed structures (Fig. 1B).

The sex of immature halibut was easily determined from the presence or absence of ovarian lobes behind the gut. When the scanhead was moved slowly towards the posterior end of the fish, the ovarian lobes appeared after the gut structures were visible (the ovary lies in a groove posterior to the gut cavity). The critical area is small and can easily be missed if the scanhead is passed over the fish too quickly.

The ovary of immature halibut appeared as two gray, granular lobes, one lying over the other (Fig 1C). The testes of juvenile halibut were more difficult to identify due to their small size. However, with experience, the testes were identified by accurately positioning the scanhead posterior to the gut cavity and viewing the characteristic bi-lobed dark structure (Fig.1D).

In prespawning female halibut, the large ovarian lobes were positioned one over the other with a characteristic oval shape and granular appearance (Fig. 2A). In spawning females, a higher frequency (7.5

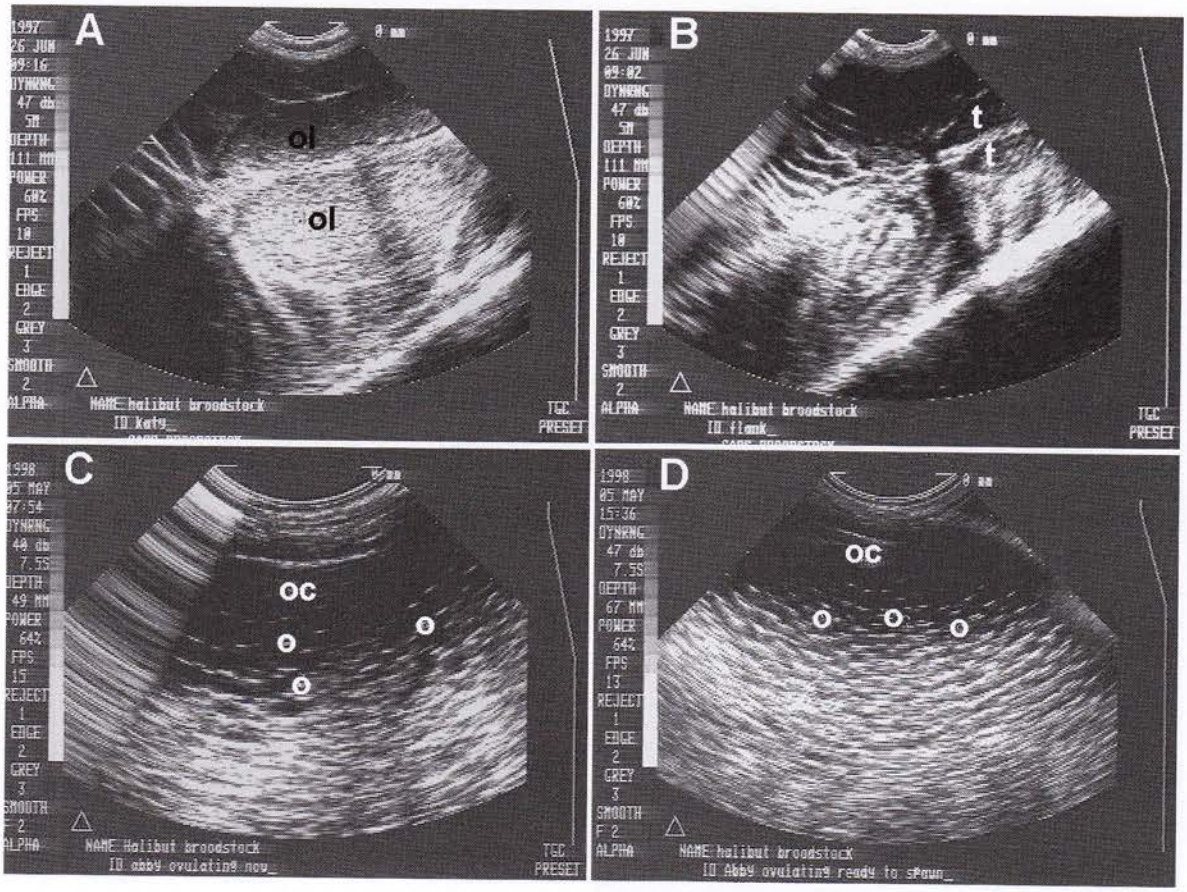

Figure 2. A) Mature female halibut a few months after spawning, B) mature male halibut, and C) ovary of female halibut with some individual oocytes released into the fluid-filled ovarian cavity 7.5 h before egg release, and D) ovary of the same female with the ovarian cavity filled with hydrated oocytes (oc–ovarian cavity, t–testis, ol–ovarian tube).

MHz) resulted in an image which clearly showed highly reflective, white individual oocytes surrounded by unreflecting, dark areas of ovarian fluid. During ovulation, the ovarian cavity was filled with hydrating oocytes (Fig. 2C, D). As with the juveniles, the testes were more difficult to detect and were identified as dark, fluid-filled bi-lobed structures (Fig. 2B). Often the vas deferens was detected as a small, white structure in the middle of each lobe.

The ovaries of mature haddock females scanned over the posterior ventral surface were seen as two round dense granular lobes adjacent to one another and positioned over the ventral surface of the highly reflective swimbladder wall (Fig. 3A). The ovarian lobes were surrounded by gut tissue, primarily liver. The testes of haddock were close to the swimbladder wall and were identified by their dark, fluid-filled lobes (Fig. 3B). The vas deferens were frequently visible, especially during the spawning season. During spawning, the ovarian cavity containing ovarian fluid was sometimes detected as a slightly darker area within the ovary (Fig. 3C). The ovaries in post-spawning haddock were difficult to identify due to the "empty" ovarian cavity and lack of dense ovarian tissue.

Discussion

Ultrasound has the potential to be a valuable tool for broodstock management and for monitoring gonadal development during reproductive studies. However, the successful interpretation of ultrasound images and correct use of ultrasound equipment requires experience. Martin et al.[2] provide a good description of the principles of ultrasonography and its application to fish. Understanding the internal morphology of fish, especially flatfish, in relation to image orientation is critical for the accurate interpretation of images. We have emphasized the preferred scanning location to acquire good gonadal images and for ease of interpretation. Haddock gonads, like those of Atlantic cod,

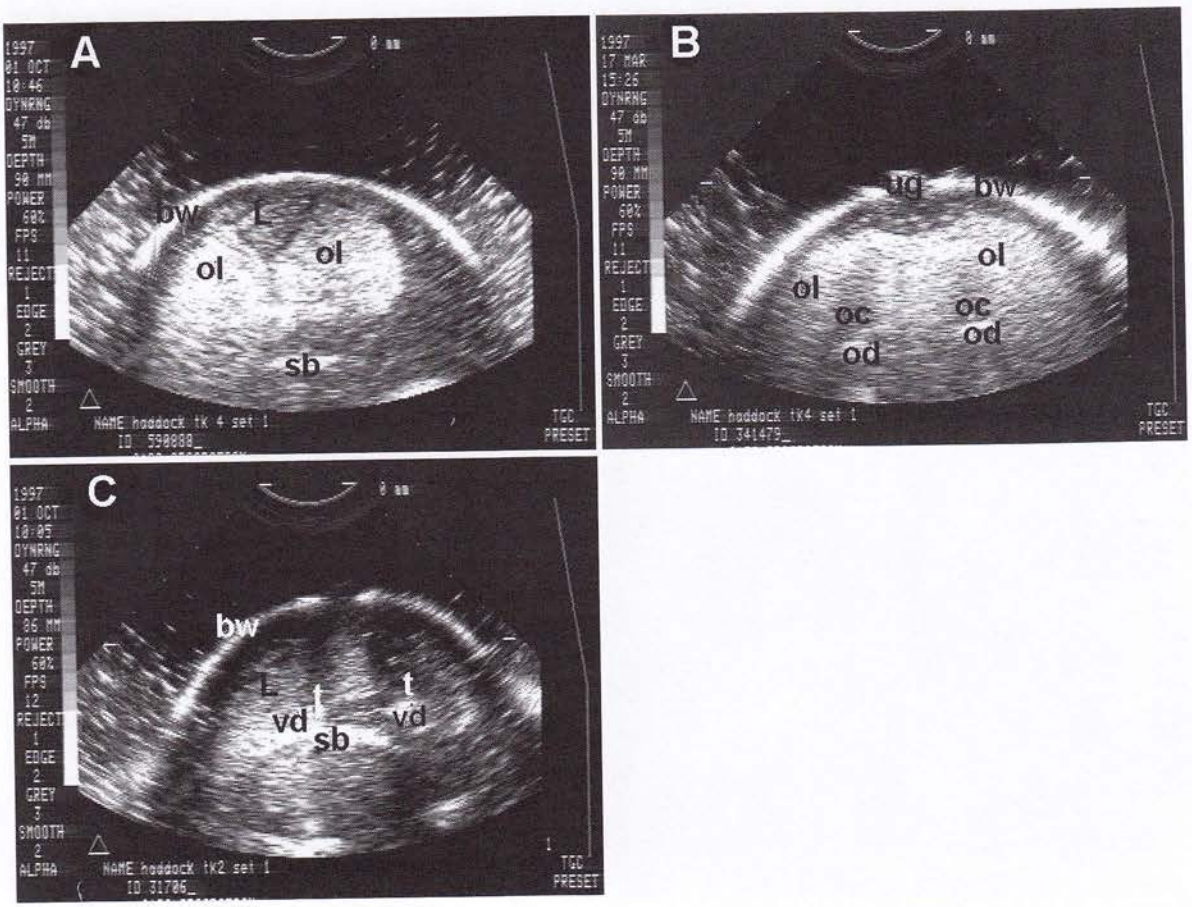

Figure 3. A) Vitellogenic ovary of mature haddock, B) ovary of haddock during spawning, and C) mature male haddock (bw–body wall, sb–swimbladder, ol–ovarian tissue, t–testis, vd–vas deferens, od–oviduct, oc–ovarian cavity, l–liver, ug-urogenital pore).

develop caudally and anteriorly.[1] Therefore, the maximum diameter of the ovary is at the posterior end of the body cavity where it can be observed by scanning the area directly anterior to the urogenital pore.

Testes of all the species examined were more difficult to identify than ovaries. Compared to the white, granular ovarian tissue of a mature ovary, the testes appeared darker due to their higher water content. Closer to spawning and during spermiation, the testes were lighter and more granular due to the presence of spermatozoa.[4]

The gonads of juvenile fish were small and could easily be missed if the transducer was passed quickly over the critical area. With a medium frequency transducer (5 MHz) it was possible to determine the sex of juvenile halibut accurately. The ovaries of juvenile halibut (>50 cm) were readily observed directly posterior to the gut. The size of the gonads observed in relation to the size of the fish was often used as a criterion for determining the sex of immature fish. For example, immature female salmon are identified by the presence of ovaries, while the lack of ovaries indicates the fish is an immature male.[3,4] Similarily, the sex of male juvenile halibut was frequently assumed from the absence of distinct ovarian lobes and the results were confirmed by dissection of six wild juvenile halibut.

For halibut eggs to be of good quality, it is critical to obtain eggs within 6 h of ovulation to prevent overripening of the oocytes. With experience it may be possible to evaluate the stage of oocyte development using ultrasonography. At 7.5 MHz, individual oocytes could easily be seen in the ovarian cavity and the distribution and density of ovulated eggs in the ovarian cavity may indicate the timing of ovulation. The presence of significant quantities of ovarian fluid surrounding large, hydrated oocytes indicated that ovulation had commenced.[5]

These results suggest that ultrasonography is a suitable technique for the determination of the sex of juvenile halibut and mature flatfish and haddock. The applications for ultrasound technology in broodstock management are diverse. Selecting juvenile fish based on sex would lead to the use of optimum sex ratios in adult broodstock holding facilities. Many culture operations still rely on wild-caught broodstock and rapid assessment of the sex of newly captured fish would be useful. The ability to determine the sex of gadids such as cod and haddock which are serial, volitional spawners permits the stocking of desired sex ratios into spawning tanks. Using ultrasound on mature halibut underwater to quickly evaluate ovarian development would be less stressful and invasive than the present practice of applying pressure to the ovary to extrude eggs for evaluation. Also, using ultrasound to determine the timing of halibut ovulation would be a valuable technique for ensuring high quality eggs.

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Performance of a Newfoundland Atlantic Salmon Strain for Aquaculture

V. A. Pepper, C. Collier, and T. Nicholls

The salmonid aquaculture industry of Bay d'Espoir on Newfoundland's south coast adopted the Saint John River strain of Atlantic salmon as its main production line in 1988. Because of concern in the Department of Fisheries and Oceans over importation of non-local salmon strains into Newfoundland, and industry interest in developing superior performance traits in its production salmon, industry and government collaborated on performance trials of Grand Codroy River (GCR) strain Atlantic salmon from Newfoundland's west coast. Saint John River (SJR) strain salmon, originally from the Bay of Fundy, were used as the control group for the comparative performance evaluations. Eggs of wild Atlantic salmon were collected from the Grand Codroy salmon stock in 1989. The two strains were evaluated on the basis of growth, survival, and food conversion ratio over two generations under commercial aquaculture conditions. First generation GCR stock performed poorly compared to the SJR strain. In the absence of sufficient hatchery space to conduct a rigorous breeding program, a mass selection approach was applied to the first generation brood stock. Second generation GCR salmon out-performed the SJR strain, indicating further experiments should be conducted to determine the potential of this strain for the Newfoundland salmonid aquaculture industry. Initial steps towards a breeding program for the industry are discussed.

Introduction

Currently, the only commercial Atlantic salmon farms in Newfoundland are in the Bay d'Espoir area on the south coast (Fig. 1). Although the Newfoundland salmon farming industry did experiment with local Newfoundland Atlantic salmon stocks (Grey River, Exploits River, and Conne River) from 1985 through 1988, these wild, predominantly-grilse stocks proved unsatisfactory. Since 1989, Newfoundland salmon farmers have been working with the Saint John River strain, currently the performance standard for the aquaculture industry in Atlantic Canada. To date, most Newfoundland experience with the Saint John River strain of Atlantic salmon has been satisfactory with respect to growth, feeding efficiency, grilsification, and product quality, though susceptibility to vibrio (*Vibrio salmonicida*) and atypical furunculosis (*Aeromonas salmonicida nova*)[1] has been an economic burden.

The salmon farming industry is interested in any salmon strain that will improve net financial benefits to their industry. To this end, the salmon growers are participating with the federal government in evaluating a local-origin strain relative to the present industry-standard strain. The Department of Fisheries and Oceans is anxious to support development of a Newfoundland strain of salmon for farming in the province due to concerns about potential genetic interactions between wild and aquaculture salmon.[2-4] The aquaculture industry is more interested in developing alternative strains as a potential strategy to improve economic performance. From an industry perspective, the greatest benefit from development of a local salmon stock would be from a breeding program aimed at minimizing grilsing and developing fish with superior growth, survival, and food conversion.

Methods

Aquaculture cages and nets in support of these experiments were moored in Roti Bay (Fig. 1). Grand Codroy strain Atlantic salmon smolts (GCR) and a control group of Saint John River (SJR) strain smolts

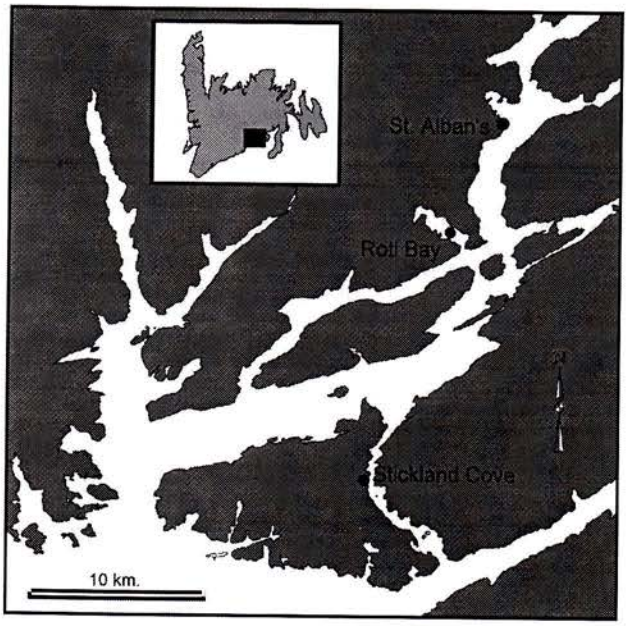

Figure 1. Location of study sites.

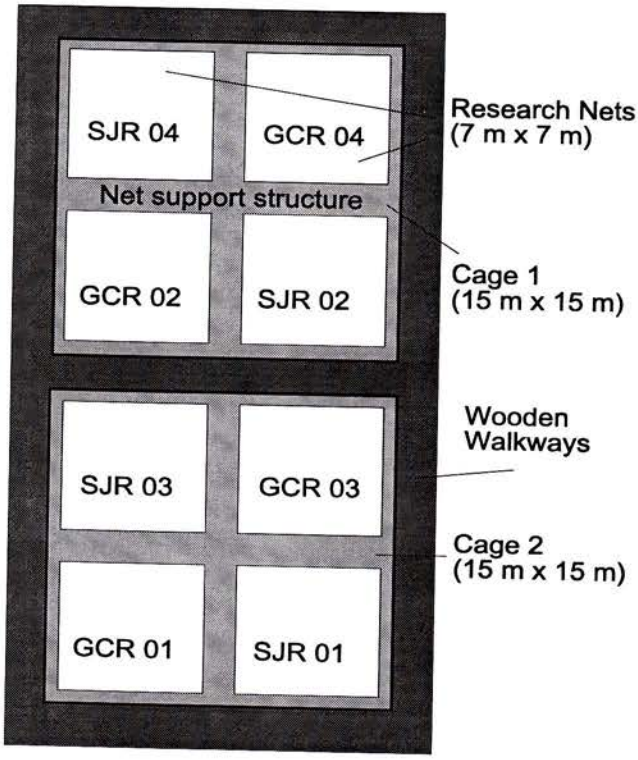

Figure 2. Layout of experimental cages.

from the Bay d'Espoir salmon hatchery were transferred to the estuarine cages during the interval of June 5 to 10, 1995. Cages were set up as shown in Figure 2. The four SJR nets received 5500 smolts each. There were more GCR smolts than anticipated, so the GCR nets received somewhat higher numbers (GCR-1, 6867; GCR-2, 6758; GCR-3, 5456; GCR-4, 6532). Salmon were fed to satiation three times daily at 0700, 1300, and 1800 h with Moore-Clark commercial dry diet. A veterinarian visited the site regularly to monitor fish health. The water column in proximity to the cages was monitored for temperature, salinity, and oxygen concentration throughout the experiment.

In mid-November 1995, the fish from the four cages of each strain were combined into large overwintering nets (15 m x 15 m x 7 m deep). The final specimen sampling for 1995 took place on November 21, after which the nets were drawn up for winter operation to give a water depth within the enclosure of 3.0 to 3.7 m. After the winter of 1995/96, the salmon were placed in circular cages (70 m diameter, two cages per strain) and then towed (May 1996) to an area of higher salinity at Strickland Cove (Fig. 1) where they were monitored through to sorting for market in October.

Smolts of the two strains were sampled (live weight, fork length, fin condition) at the Bay d'Espoir hatchery prior to transfer to the estuarine cages. Sampling of post-smolts in the estuarine cages was conducted monthly whenever possible but was interrupted occasionally due to medication procedures implemented by the veterinarian. At the end of the on-growing cycle in 1996, all salmon in the experiment were graded into three categories, grilse (maturing), small (non-maturing but < 2.7 kg) and large (non-maturing and ≥ 2.7 kg). Each of these groups was sampled at the time of grading (n=35).

The main performance indicators for this experiment were growth, survival, and food conversion ratio (FCR). Data collected during the experiment were examined for overall patterns of strain performance. Parameters were developed on the basis of the following:

Mortality

$$Z = \frac{-(\ln N_t - N_o)}{t}$$

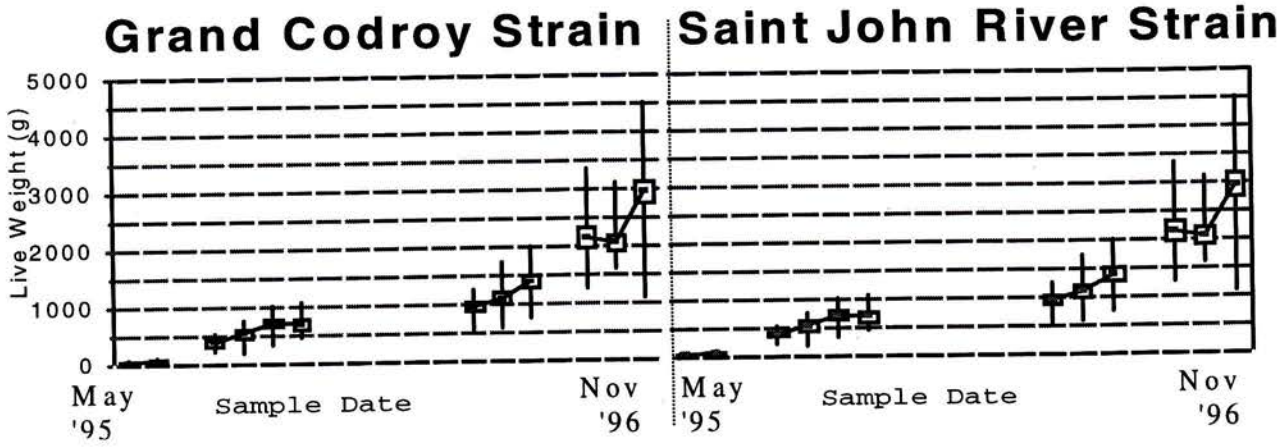

Figure 3. Progression in mean weight.

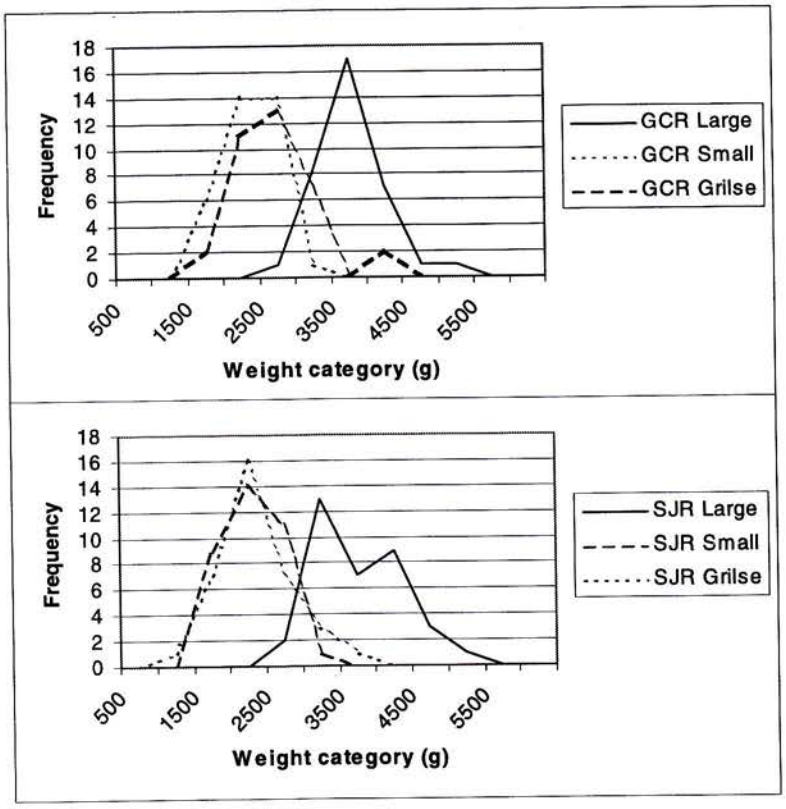

Figure 4. Distribution of final weights

where,

Z is the instantaneous rate of mortality of the group (i.e., the change in numbers in the specified time period t);

N_o is the number of individuals in the population at the beginning of the specified time interval;

N_t is the number of individuals in the population at the end of the specified time interval;

t is the time interval (in this case, days); and,

ln is the natural logarithm.

Growth,

$$G = \frac{(\ln W_t - \ln W_o)}{t}$$

where,

G is the instantaneous rate of growth (i.e., the change in biomass in the specified time period t);

W_t is the mean weight of individuals in the population at the end of the specified time interval;

W_o is the mean weight of individuals in the population at the beginning of the specified time interval.

t and ln are as above.

With the two quantities of G and Z, the instantaneous rate of change in biomass was calculated as follows:

$$R = G - Z$$

For the present experiment, FCR was calculated for the on-growing interval as the sum of the food provided to the fish on a daily basis relative to the increase in biomass of fish during the same interval. This is formalized as:

$$\overline{FCR_t} = \frac{(\sum_{t_o}^{t} FoodTaken)}{\Delta Biomass_t}$$

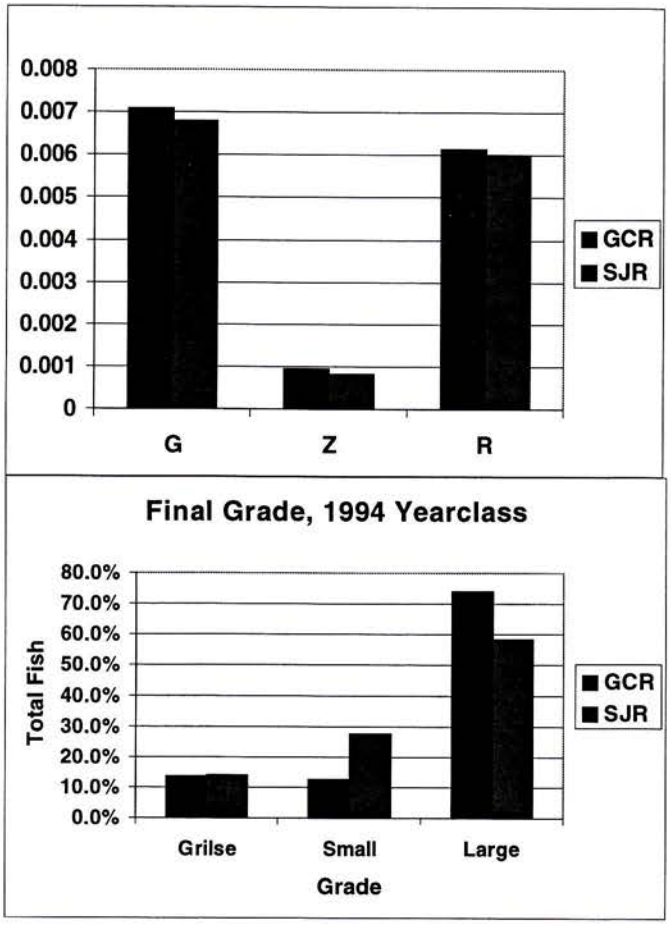

Figure 5. Relative performance.

Results

Distribution of salmon weight for the final samples of the 1995 experiments is illustrated in Figure 4. Relative performance between the two strains is illustrated graphically in Figure 5. Of considerable interest is the fact that, while both strains had an approximate 14% grilsing rate, the SJR grilse were 96% male while the GCR grilse were only 78% male.

Monitoring of the water column in proximity to the cages revealed highly variable conditions of temperature, salinity, and oxygen that fluctuated both temporally and with water depth. Summer temperatures ranged from 7 to 15°C and oxygen concentration from 7.9 to 11.9 ppm. Salinity was highly variable and ranged from a low of 6.2 ppt to 31.5 ppt in as little as 24 hours.

Growth of salmon in the two groups progressed in parallel fashion (Fig. 3) throughout the experiment. A maximum average summer density of 30 kg/m^3 was reached in one of the cages at the end of the experiment. Maximum densities in all other nets were well below this level. The box and whisker plots of Figure 3 illustrate both the range of the observed weights ("whiskers") and the 95% confidence

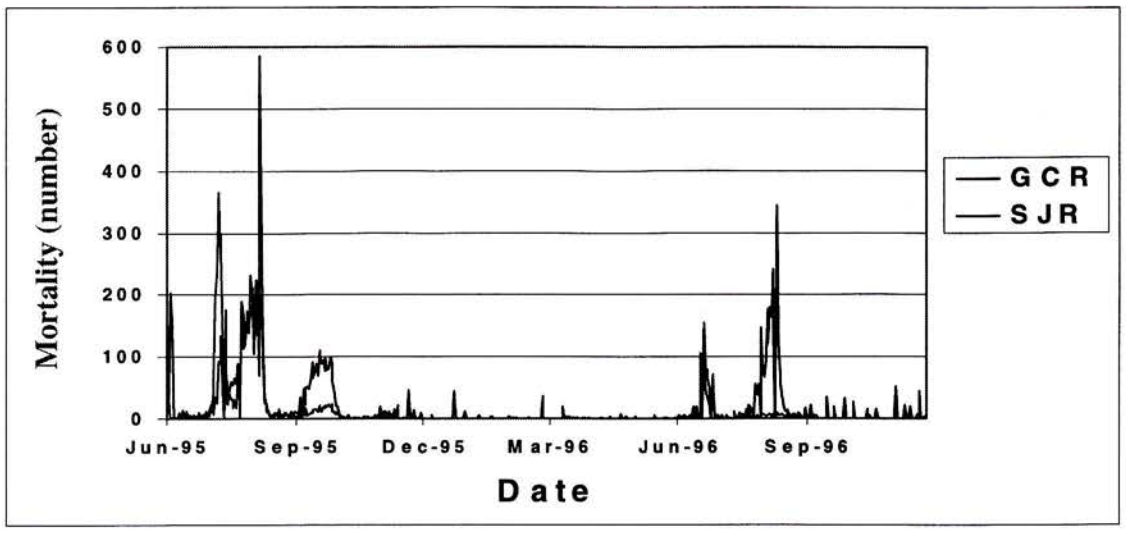

Figure 6. Daily mortality pattern.

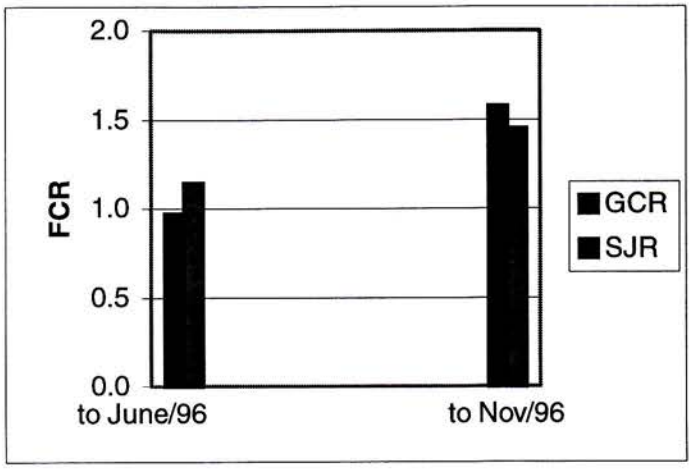

Figure 7. Food conversion values.

interval for the mean weights (boxes). The lines connecting the sampling series trace the mean weight values between dates.

Mortality of both strains was extreme during the interval following transfer to estuarine rearing (Fig. 6). Initial mortality was confirmed to be due to vibrio. The secondary mortality peak in 1995 was traced to atypical furunculosis. Thereafter, mortality during the first year in the estuarine cages was minimal. Performance of both strains was greatly compromised during July of 1996 when losses due to atypical furunculosis escalated. The GCR strain losses during this interval were much greater than those incurred by the SJR strain.

FCR for the interval of the entire experiment was high (i.e., poor) for both strains. However, FCR during the interval from the first sampling in the estuarine cages to the interval immediately preceding the mortality peak of 1996, was considerably better (Fig. 7). This mortality peak late in the ongrowing cycle removed a significant amount of the biomass that had been produced to that time.

Discussion

Experimental evaluation of performance of the "unselected" Grand Codroy stock (i.e., progeny from wild stock) started in 1989. The F_1 aquaculture generation from the wild foundation population, in comparison with fourth-generation Saint John River offspring, revealed some performance differences in the

estuarine cages. Most significant was an inferior FCR for the F_1 Grand Codroy stock, lower mortality during a disease outbreak, and a higher incidence of grilsification.

The best performing fish of the first generation Grand Codroy salmon were set aside as brood stock for stripping in 1993. Most performance indicators until the time of stripping were inferior for this first-generation Grand Codroy stock. However, it was expected that the best performers of the first generation GCR salmon, if subjected to a structured breeding program, might yet serve industry's interests.

Hatchery performance of second generation Grand Codroy salmon parr resulted in smolts of similar size and fin condition to the SJR strain smolts. Sampling at the Bay d'Espoir hatchery prior to transfer to the estuarine cages confirmed that the average weight for smolts of the two strains of S_1 smolts was similar immediately prior to transfer to the estuarine cages.

Growth performance of the two strains (Fig. 3) proceeded in a parallel fashion. The final samples, taken at the time the two strains were graded prior to market (Fig. 5), together with the FCR calculations (Fig. 7), provide a reasonable overview of the experiment. On the basis of Figures 6 and 7, it is apparent that mortality due to disease is the main challenge for Atlantic salmon operations, irrespective of strain origin. While there was some indication during the first generation evaluation that GCR salmon might have greater resistance to local salmonid pathogens, this was not apparent among the second generation GCR salmon. This is a surprise considering literature that indicates resistance to furunculosis is a heritable trait.[5]

The Newfoundland Salmonid Growers Association (NSGA) has responded to the fish health challenge with a comprehensive vaccination program that seems to be showing some promise. Rapid fluctuations in environmental conditions, especially salinity, are likely to be a source of considerable stress for Bay d'Espoir salmonids and may complicate the antibody response process. Although some improvement to strain performance could be achieved by the use of all-female salmon, thereby potentially eliminating the stress of maturation and improving production by >10%, the FCR values of Figure 7 suggest such a gain would be enhanced greatly by minimizing mortality among the oldest (i.e., pre-market) fish.

Experiments to date with these two salmon strains have resulted in considerable interest within the industry in continuing the development of these two strains. Genetic profiles are being determined to ensure that both strains have significant breeding potential. Although the Newfoundland industry already has been successful in generating an all-female line of SJR salmon, it now has undertaken to develop a similar line of GCR salmon as one of its production strategies. In concert with these initiatives, the Newfoundland Salmon Growers Association is constructing a research hatchery in support of a long-term breeding program that may encompass both strains and inter-strain crosses.

We thank the employees of SCB Fisheries Limited for help in the day-to-day activities associated with this project, especially Roy Abbot, Betty House, Brian Hull, Gary Kendell, and Corey Taylor. We also thank Sharon Kenny of DFO for her help with sampling and data processing for this research initiative and Dr. Leighanne Hawkins, Department of Fisheries and Aquaculture, for dealing with fish health issues as they arose.

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Controlled Breeding Technology for Haddock *(Melanogrammus aeglefinus)* in Mated Pairs

E.A. Trippel, C.M. Doherty,
J. Wade and P.R. Harmon

Haddock (*Melanogrammus aeglefinus*) were able to spawn in mated pairs when isolated in 3.5-m^3 tanks. Spawning duration of the six females monitored averaged 45 days, during which individuals spawned an average of eight egg batches. Batch-specific fecundity ranged from 10 000 to 270 000 and total fecundity from 480 000 to 930 000 eggs (female body size range: 52 to 59 cm FL and 2.1 to 3.1 kg). Egg diameter generally declined between successive batches, yet it remained relatively stable in some females for the first three to four batches before declining (first to last batch declines commonly ranged from 1.55 to 1.40 mm). Egg fertilization rates were variable among pairs and batches. Spermatocrit of haddock mainly ranged mainly from 7 to 37, whereas spermatocrit of Atlantic cod (*Gadus morhua*) was higher and ranged mainly from 50 to 85. Low spermatocrit, combined with low per capita milt production, suggest male haddock are less fertile than cod. Future research will address both maternal and paternal contributions to the production of viable offspring. This information is critical to the development of broodstock selection programs for marine batch-spawning fishes.

Introduction

Atlantic salmon (*Salmo salar*) form the cornerstone of coldwater finfish aquaculture worldwide. The species is ideally suited for sea-pen conditions and has provided substantial monetary gains for coastal communities involved in its commercial culture. Contributing to the success of salmon aquaculture has been the establishment of long-term selective breeding programs. Breeding of high performance strains of Atlantic salmon has resulted in gains in growth of 0.34 kg in a single generation.[3]

A key component to the success of these breeding programs has been the ease with which adult salmon can be handled during stripping of gametes with only mild stress being incurred by the parents. Eggs within the ovary ripen synchronously and salmon spawn just one batch of eggs per year. These two facets have greatly simplified the process of collecting eggs from parent broodstock for the development of pedigrees to be used in selection programs.

The need to develop selective breeding programs for non-salmonid marine fish will arise over the next several years, given the high probability that some species will attain commercialization and the inevitable demand to reduce production costs to remain competitive. In eastern Canada, haddock (*Melanogrammus aeglefinus*) are currently considered to be a prime candidate for coldwater marine aquaculture.[12] Haddock is a "round fish" and preliminary trials have revealed they grow well in sea pens used for salmon.

Table 1. Initial fork length and body weight of six adult pairs of haddock for which reproductive characteristics were monitored.

Tank	Male		Female	
	Length (cm)	Weight (kg)	Length (cm)	Weight (kg)
1	56	2.2	55	2.6
2	55	2.1	59	2.4
3	48	1.6	55	2.7
4	65	3.5	59	3.1
5	53	1.8	53	2.1
6	48	1.3	52	2.2

Table 2. Duration in days between successive egg batches of haddock.

Female Number	Batch interval (days)									Average interval (days)	Spawning duration (days)
	1-2	2-3	3-4	4-5	5-6	6-7	7-8	9-10	10-11		
1	19	13	8	10	9	9	4	–	–	7.0	72
2	4	6	6	6	4	2	6	–	–	4.9	34
3	5	4	7	9	6	9	–	–	–	6.7	40
4	4	3	17	5	–	–	–	–	–	7.3	29
5	8	9	6	5	3	15	3	–	–	7.1	50
6	10	5	6	4	6	4	2	4	3	4.9	44
									Mean	6.3	45

Hence, the commercial infrastructure for salmon appears to be usable for haddock, at least in the juvenile and adult outgrowing phases. However, breeding technology, as will be shown in this paper, is not directly transferable from salmon to haddock.

The main reason for the lack of technology transfer between the two species is that the reproductive physiology of haddock differs substantially from that of salmon. Haddock, particularly breeding females, are very sensitve, and cannot tolerate frequent handling. Haddock breed by spawning multiple batches of small pelagic eggs over a rather lengthy spawning period, as opposed to salmon which spawn a single batch of relatively large eggs. To date, the procedure followed for obtaining haddock eggs for experimental grow-out has been to gather them from large tanks (15 to 36 m^3) in which groups of adult haddock mate and spawn. Marine research facilities at which this approach is employed include the Department of Fisheries and Oceans (Biological Station) and Huntsman Marine Science Centre, St. Andrews, NB, Aquarium and Marine Science Centre, Shippagan, NB, National Research Council, Sandy Cove, NS, and University of Maine at Orono. Fertilized eggs are obtained from small-mesh surface collectors located in tanks. Although 2 to 3 liters of eggs can be obtained in this way over a 24-h period, there are some shortcomings to this approach. Specifically, the parentage of offspring cannot be determined when 50 to 75 fish are breeding together and up to five different females are generating eggs in a single day. Even if haddock could be easily handled, manual stripping of eggs from a serial spawner could result in low egg viability due to the difficulty in predicting the brief period when an egg batch has ripened and is ready to be released. Consequently, communal broodstock rearing practices for egg production purposes preclude controlled matings. Moreover, the approach makes it impossible to identify *individual* maternal effects on the size and quality of eggs and their fertilization success (i.e., maternal age, length, condition factor, batch effects). Thus, the communal broodstock approach is restrictive from both genotypic and phenotypic perspectives.

An alternative breeding practice is to isolate adult pairs by placing a female and male in a separate smaller tank during the spawning period. This would permit close monitoring of egg production from each pair. If viable eggs can be collected, a technique that has been successfully developed for cod[6,10] could be

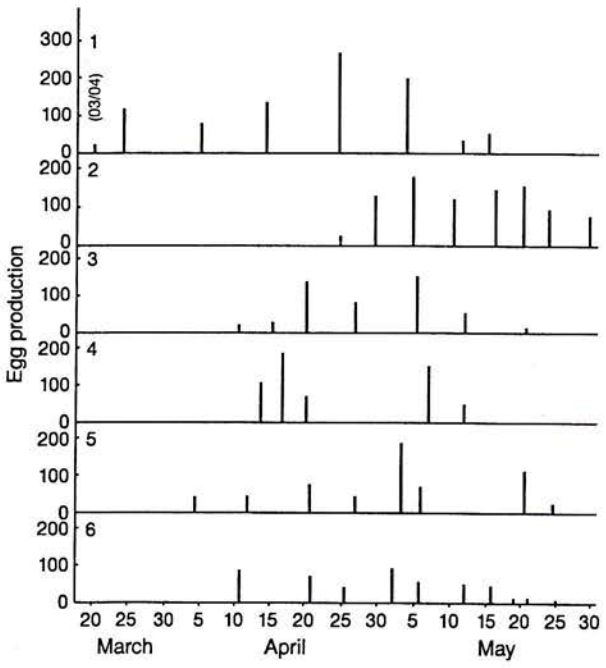

Fig. 1. Egg production (in thousands) by batch for six female haddock.

Table 3. Batch-specific fertilization rates of six adult pairs of haddock.

Pair Number	Batch number										Seasonal composite (%)
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	8	64	0	14	13	–	–	21.0
2	1	4	68	4	97	0	96	90	–	–	48.7
3	75	76	94	60	44	22	1	–	–	–	60.0
4	96	49	99	86	71	–	–	–	–	–	71.3
5	86	0	0	0	0	5	0	0	–	–	6.0
6	0	11	0	1	55	0	85	96	94	98	24.1
											Mean = 38.5

adapted to haddock.

Haddock exhibit wide growth variation within and among wild stocks.[1] For example, in the Bay of Fundy and western Scotian Shelf area, 4-year-olds range in size from 40 to 60 cm and 1.0 to 3.4 kg, yet broodstock collections do not discriminate on these variables (i.e., both slow and fast growing haddock are collectively held in communal spawning tanks). The large growth differential and the diversity of stocks that exist in the northwest Atlantic[4] suggest there may be considerable potential for haddock selective breeding. If egg size affects larval size, and changes in egg size occur between successive batches, then a comparison of offpsring performance between two females needs to account for egg size and batch number used (e.g., early, mid or late batches during a fish's spawning season). Thus, the merits of establishing a controlled mating system for haddock appear obvious, and could form the precursor for a broodstock program that would evolve as the industry develops.

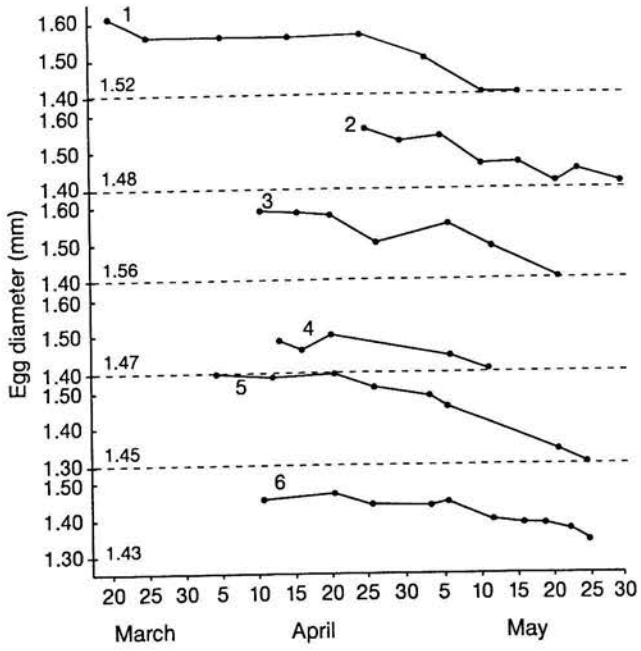

Fig. 2. Seasonal changes in egg diameter of six female haddock. Seasonal composite values are indicated for each female.

Objectives

When Atlantic salmon aquaculture began in North America about 20 years ago, scientists had already amassed an extensive amount of literature on all life history stages of this esteemed sportfish. In contrast, we know comparatively little about the basic life history traits of haddock, a traditionally important commercial species.[5,13] This paper begins to reveal some of the reproductive characteristics of haddock which need to be considered in its culture and selection. Some of these reproductive aspects have been recently studied for cod, a closely related species.[9-11] Various comparisons between haddock and cod, particularly in milt production, will be made.

In the present study, we:

1) determined whether haddock spawn in circular 3.5 m^3 tanks;
2) estimated batch fecundity, spawning duration, and time interval between batches;
3) assessed seasonal changes in egg diameter;
4) assessed fertilization rates; and
5) compared seasonal changes in spematocrit and milt production of haddock and Atlantic cod.

Mated Pair Methodology

This work is in its preliminary phases and here we report on the reproductive perform-

Table 4. Batch-specific fertilization rates of six adult pairs of Atlantic cod.

Pair number	Batch number											Seasonal composite (%)
	1	2	3	4	5	6	7	8	9	10	11	
1	99	74	100	88	98	90	99	99	81	100	99	93
2	0	98	98	58	98	99	–	–	–	–	–	75
3	98	99	93	14	–	–	–	–	–	–	–	76
4	81	85	97	98	59	–	–	–	–	–	–	84
5	0	10	0	71	85	–	–	–	–	–	–	33
6	0	68	99	96	90	6	57	–	–	–	–	59
												Mean = 70

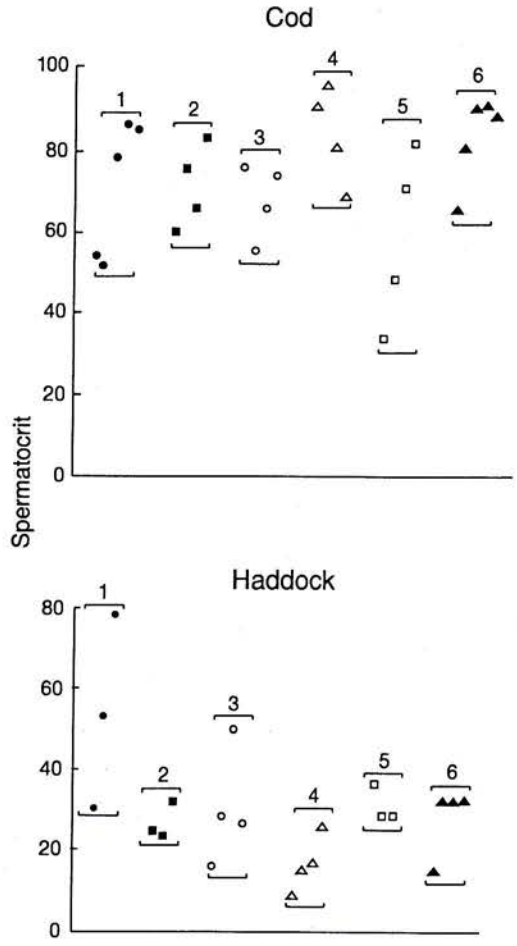

Fig. 3. Seasonal changes in spermatocrit of six male cod and six male haddock. Each point within a male "grouping" represents a sampling date. Initial and terminal sampling dates varied among males, with individual cod sampled once every 2 to 4 weeks within a period from January to May and haddock from April to June.

ance of six spawning pairs of haddock. The haddock were collected near Grand Manan on the western side of the Bay of Fundy (Northwest Atlantic Fisheries Organization Division 4X). They were maintained in large circular tanks (15 to 36 m^3) and fed a diet of mackeral (*Scomber scombrus*), northern shortfin squid (*Ilex illecebrosus*), and Aesop shrimp (*Pandalus montagui*). Fish were maintained at the Biological Station and the Huntsman Marine Science Centre in St. Andrews for 1 to 2 years prior to use. Then, each pair of haddock was placed into a separate spawning tank (diameter 2 m, depth 1.5 m, volume 3.5 m^3) at the Biological Station. Two pairs were initially placed in the tanks on March 1 and the other four pairs were distributed among four other tanks on April 1, 1997. Formation of adult pairs was simplified by using ultrasound to determine the sex of the brood fish.[7] Males ranged in length from 48 to 65 cm (1.3 to 3.5 kg) and females from 52 to 59 cm (2.1 to 3.1 kg) (Table 1).

Egg collectors were checked daily. The volume of eggs spawned in each batch was determined and, by use of egg diameter, equated to fecundity.[6] Milt from males was sampled once every 2 to 4 weeks (anaeasthetized males were hand stripped). Spermatocrit (% semen occupied by packed sperm cells) and volume of milt collected during the initial 15 seconds of stripping were determined. Water temperature in the tanks ranged from 3 to 4°C from January 1 to March 31, 4 to 5°C from April 1 to 30, and 5 to 7° from May 1 to 31.

Fertilization success and milt parameters were also evaluated for six spawning pairs of Atlantic cod. Male cod ranged in size from 75 to 86 cm (3.6 to 7.2 kg) and females from 68 to 86 cm (3.7 to 7.5 kg). Cod were placed in tanks between December 17, 1996, and January 24, 1997.

Table 5. Monthly averages of milt volume and spermatocrit of haddock and cod.

	Jan	Feb	Mar	Apr	May	Jun
Mean volume of milt (mL/sec)						
Cod	3.64	3.30	8.32	3.36	3.77	–
Haddock	–	–	–	0.34	0.58	0
Spermatocrit						
Cod	59.86	62.00	77.60	62.80	67.10	–
Haddock	–	–	–	22.15	36.70	0
Volume of milt (mL/sec/kg) end weight						
Cod	0.29	0.43	0.32	0.19	0.16	–
Haddock	–	–	–	0.18	0.31	0

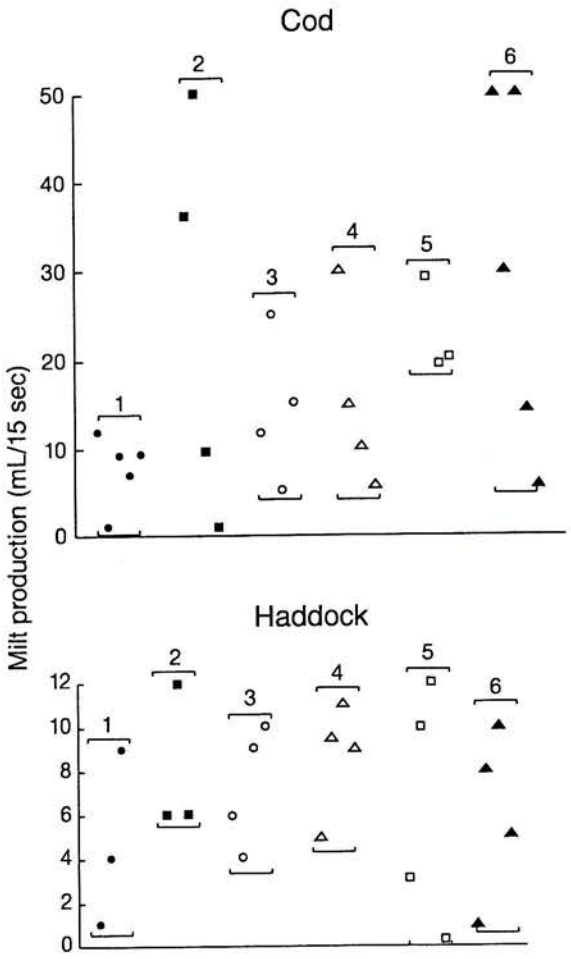

Fig. 4. Seasonal changes in milt production of six male cod and six male haddock. Initial and terminal sampling dates varied among males, with individual cod sampled once every 2 to 4 weeks within a period from January to May and haddock from April to June.

Spawning Habits and Egg Production

Haddock spawning occurred mainly during April and May, with one female commencing spawning in March (Fig. 1). Spawning duration averaged 45 days, and ranged from 29 to 72 days (Table 2). Although the average was 8 batches, one female spawned 11 batches. The number of eggs per batch ranged from 10 000 to 270 000 eggs. Total fecundity per female ranged from 480 000 to 930 000. The number of eggs produced per batch generally exhibited a dome-shaped pattern, with the smallest egg batches occurring late in the spawning period (Fig. 1). The number of days between egg batches (inter-batch interval) varied among females and averaged 6.9 days (Table 2). Female 1 averaged 10.2 days between egg batches, whereas females 2 and 6 averaged 4.9 days.

Egg diameter generally declined between successive batches, yet remained relatively stable in some females before finally declining (Fig. 2). The size of eggs produced by female 6 declined from 1.45 to 1.33 mm between initial and terminal batches. Other declines commonly ranged from 1.55 to 1.40 mm (Fig. 2). For the six females, the seasonal composite egg diameters ranged from 1.43 to 1.56 mm (batch averages weighted by batch fecundity).

Fertilization rates were highly variable among mated haddock pairs (11 of 46 batches (24%) exhibited > 80% fertilization) (Table 3). The seasonal composite values ranged from 6 to 71%, with the average being 38.5%. In contrast, composite fertilization rates in cod were higher, ranging from 33 to 93%, with an overall average of 70% (25 of 38 batches (66%) exhibited > 80% fertilization) (Table 4).

Milt Production

To understand why fertilization rates were generally poorer for haddock than cod, we examined seasonal changes in spermatocrit and milt volume of both gadids. Atlantic cod spermaocrit ranged mainly from 50 to 85 and within individual males tended to increase as spawning progressed (Fig. 3). Haddock spermatocrits were much lower, ranging from 15 to 40, and also exhibited a thickening trend over the season.

Volume of milt produced was much greater for cod, commonly ranging from 5 to 30 mL/15 seconds, with values as high as 50 (Fig. 3). Within a male, milt volume tended to increase at first and then declined as the season progressed (i.e., a dome-shaped pattern). Haddock milt production also followed a dome-shaped pattern, but milt flow rates were less than that of cod, and commonly ranged from 4 to 12 mL/15 seconds (Fig. 4). Some of the disparity in milt flow rates between the two species was due to body size (cod used were larger than the haddock). Thus, values were divided by body weight and expressed as mL/s/kg for each month of sampling. For cod, milt could be stripped from January to May, with February being the month of peak milt flow in which the average was 0.43 mL/s/kg (Table 5). Mean values for cod in the other months ranged from 0.16 to 0.32 mL/s/kg, with late season (April and May) values being the lowest. For haddock, the average milt flow rate for April was 0.18 and for May was 0.31 mL/s/kg (males did not produce milt in June). Average monthly spermatocrits ranged from 59 to 77 for cod and 22 to 37 for haddock. Note that spermatocrit is a good predictor of sperm density in cod[9] and likely for haddock as we. Consequently, haddock had lower sperm flows and thinner sperm than cod. These observations of sperm cell production/s/kg suggest male haddock may be ~20 to30% as effective as male cod, which may partly account for the lower egg fertilization rates observed for haddock.

Recommendations

Our research findings suggest that paired mating of haddock may be a fruitful avenue for generating viable eggs from specific families or pedigrees. It is noteworthy that fertilized eggs could be collected from each pair. The observed fertilization rates for haddock, however, indicate that further improvements may be necessary to produce a consistent supply of fertilized eggs. Experiments using GnRH implants began in 1998 in an attempt to stimulate haddock milt production and thereby improve fertilization success.[2,9] Moreover, a greater number of haddock needs to be tested using paired matings. Apparent differences in survival of broodstock held in paired versus communal conditions should also be evaluated. The type of breeding technology we are developing could provide a valuable tool to evaluate the performance of offspring originating from different parents within a stock or between stocks. For example, progeny produced from fast-growing haddock could be reared separately from those originating from slow-growing haddock. Whether isolated pairs will be able to supply sufficient quantities of eggs for commercial production is uncertain (i.e., breeders existing in large numbers of small tanks). However, the progeny developed from these pedigrees could be reared and eventually form large broodstock "pools" which could be kept in larger tanks or even sea pens from which gametes of high performance haddock could be collected and cultured.

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Calendar

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Aquatech '99 will be integrated with BioAtlantech 1999 and focus on the development of genomics and nutraceuticals for agriculture, aquaculture and forestry.

Information: Dr. Tillmann Benfey, University of New Brunswick (e-mail benfey@unb.ca). Registration information: telephone 506 444-2444, fax 506 444 5662, e-mail jgartley@fundy.net.

• **Aquaculture Europe '99**, 7 – 10 August 1999, Trondheim, Norway. Topics: larviculture, with focus on quality of offspring, fish health, genetics, interactions of farms with the environment, marketing of aquaculture products, and harvesting and market quality. Information: EAS Secretariat (tel +32 59 32 38 59, fax +32 59 32 10 05, e-mail eas@unicall.be, http://www. easonline.org).

• **US Trout Farmers Association Meeting and Trade Show,** 18 – 20 August 1999, Sheraton Inner Harbor Hotel, Baltimore. Program will highlight environmental concerns, such as effluents, and a workshop/short course will be held on fish production. Information: Pat Bethany tel 304 728-2189, fax 304 728-2196, e-mail ustfa@intrepid.net.

• **ICES Symposium on the Environmental Effects of Mariculture,** 13 – 16 September 1999, St. Andrews, NB. Forum to share research results and enhance international cooperation and collaborative research on 1) the environmental effects of bivalve and fish farming in the coastal zone, and 2) the influence of local environmental factors on mariculture productivity. Information: Dr. D. Wildish, DFO, Biological Station, St. Andrews, NB EOG 2X0 (tel 506 529-5894, fax 506 529-5862, e-mail wildishd@mar.dfo-mpo.gc.ca).

• **Aquaculture Canada '99,** 26 – 29 October 1999, Victoria Convention Centre, Victoria, BC. Annual meeting of the Aquaculture Association of Canada. Information: Linda Townsend (tel 250 741-8708, fax 250 755-8749, e-mail faep@mala.bc.ca).

• **52nd Annual Meeting of the Gulf and Caribbean Fisheries Institute,** 1 – 5 November 1999. Holiday Inn Beachside, Key West, Florida. Abstract deadline: 31 July 1999. Topics include: recent advances in caribbean aquaculture, management of marine parks and reserves, and impacts of anthropogenic activities on marine and freshwater fisheries. Information: LeRoy Creswell, GCFI, c/o Harbor Branch Oceanographic Institution, Inc., 5600 US 1 North, Fort Pierce, FL 34946 (e-mail creswell@hboi.edu).

• **Fish Rights '99 Conference, Use of Property Rights in Fisheries Management,** 11 – 19 November 1999, Freemantle, Western Australia. Conference will explore the strategic, political, and operational issues of different forms of rights-based fisheries management worldwide. Information: Secretariat Office, Petrie International, PO Box 568, Kalamunda, Western Australia 6076, Australia (tel (61) 08 9257 2088, fax (61) 08 9257 2099, e-mail petrcon@iinet.net.au).

• **Marketing & Shipping Live Aquatic Products '99,** 14 – 17 November 1999. DoubleTree Hotel, SeaTac Airport, Seattle. Agenda: improved handling technologies, resource management, regulatory concerns, unwanted introductions of non-indigenous species, economics, and animal welfare issues. Information: JB Peters, 5815 NE Baker Hill Road (fax 360 394-3760, e-mail JohnBPeters@compuserve.com).

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