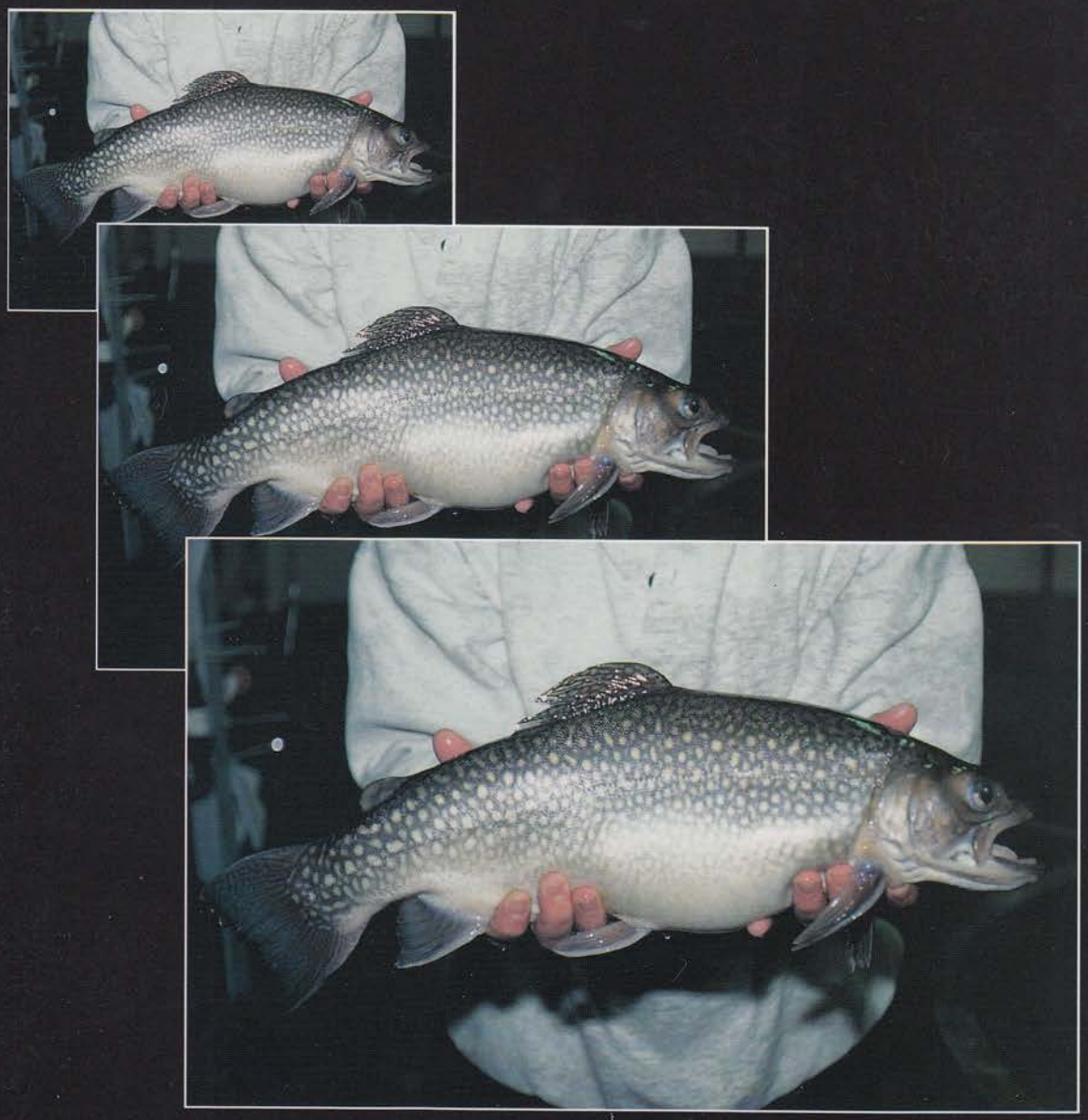
Bulletin

of the Aquaculture Association of Canada

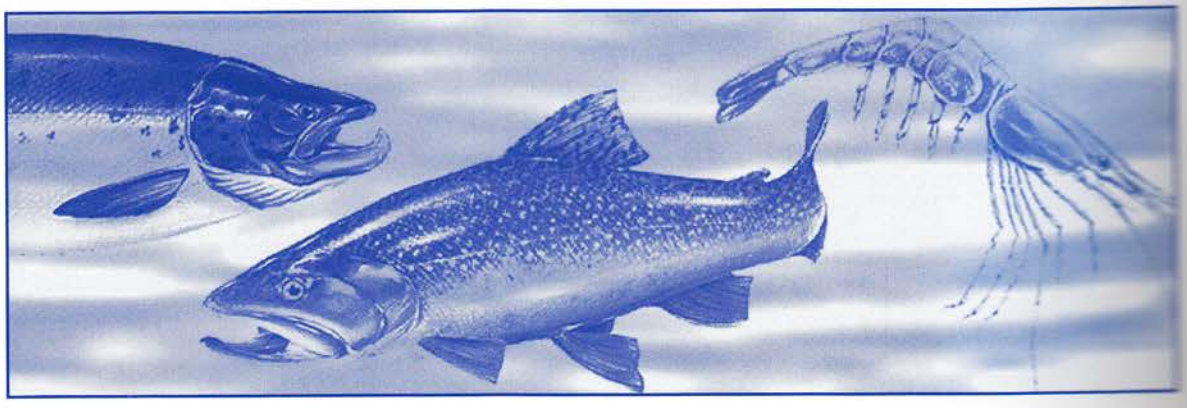


Use of all-female and triploid salmonids

Edition 96-2
June, 1996

ASTOS™ Stabilized Vitamin C.

The aquaculture business was never this healthy.



ASTOS™
Stabilized Vitamin C

Working Around The World

Learn how ASTOS™ can help your aquaculture business.

Pfizer

Food Science

Food Science Group
Pfizer Canada Inc.
17,300 Trans-Canada Highway
Kirkland, Quebec H9J 2M5

Tel (514) 426-6892 ; 1-800-363-7928
Fax (514) 426-6895

Bulletin
of the
Aquaculture Association of Canada

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

Officers

Cyr Couturier, President
Joseph Brown, President-Elect
Chrisitine Hodgson, Vice-President
Al Castledine, Past-President
Brenda Bradford, Secretary
Jay Parsons, Treasurer

Directors

Becky Field
Maureen McInerney-Northcott
Irwin Judson
Tillmann Benfey

Bulletin Staff

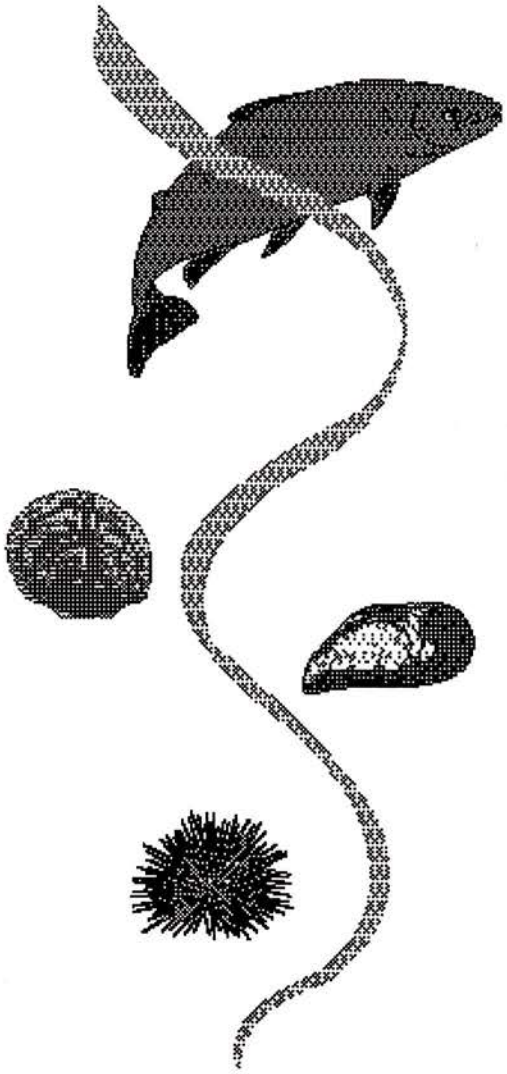
Managing Editor — S. Waddy
Contributing Editors — D. Aiken, J. Parsons, C. Couturier
Editorial Assistant — Theresia Fawkes

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

ISSN 0840-5417
Printed by Tribune Printing, Sackville, N.B.

Aquaculture Canada 96

2-5 June 1996
Byward Market, Ottawa



Triploid female brook trout (Salvelinus fontinalis) reared at the University of New Brunswick (2.5 years, 2 kg). — Tillmann Benfey photo

Contents

proceedings of the special session

Use of All-female and Triploid Salmonids for Aquaculture in Canada

— Aquaculture Canada '95, Nanaimo, B.C.

Tillmann J Benfey, editor

Master of Science in Aquaculture

UNIVERSITY of GUELPH

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

Studies may encompass:

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

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

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

Financial Supporters of Aquaculture Canada 95:

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

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

British Columbia Ministry of Small Business, Tourism and Culture

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

City of Nanaimo Regional Economic Development Office

National Research Council (TIP)

Moore-Clark Ltd.

Hoffman-La Roche

Syndel Laboratories

Pfizer Animal Health

Bamfield Marine Station

Connors Brothers Ltd.

EWOS Canada

BIOMED

Organizational assistance provided by:

MEL-Mor Science

B.C. Ministry of Agriculture, Fisheries and Food

Department of Fisheries and Oceans

B.C. Salmon Farmers Association

B.C. Shellfish Growers Association

Malaspina University–College

Coregan, Conley and Associates

Donors of seafood for the opening reception:

BC Packers Ltd.

Fanny Bay Oysters Ltd.

Intercan III Aquaculture Corporation

Island Scallops Ltd.

Mac's Oysters Ltd.

Madrona Shellfish Ltd.

Mid-Island Marine Ltd.

Pacific National Group

Stolt Sea Farms Ltd.

Proceedings of the Special Session

Use of All-female and Triploid Salmonids for Aquaculture in Canada

Introduction

This issue of the AAC Bulletin contains the proceedings of a special half-day session entitled "Use of All-Female and Triploid Salmonids for Aquaculture in Canada", which was held in Nanaimo on June 7, 1995, as part of the 12th Annual Meeting of the Aquaculture Association of Canada. Interest and controversy surrounding this topic are presently high in Canada, as various agencies attempt to limit perceived genetic impacts of escaped fish from salmonid aquaculture facilities. Several provinces have implemented, or are in the process of implementing, legislation requiring the use of "non-reproductive" stocks of fish under certain specific aquaculture conditions. This strategy has received little support from people within the salmonid aquaculture industry, who cite the clear need for production-scale data on the performance of such stocks under commercial culture conditions prior to the legislation of their use.

The purpose of this special session was not to dwell upon whether non-reproductive fish must be used by industry, but rather to call upon various experts to outline how such a strategy might be implemented, and to provide up-to-date information on the performance of all-female and triploid salmonids in an aquaculture setting. Papers are included from several perspectives: government, industry, academic and conservation organizations were all involved, and speakers came from British Columbia, New Brunswick, Nova Scotia, Newfoundland and Scotland.

In 1991, Vern Pepper, on behalf of the federal Department of Fisheries and Oceans, organized the Atlantic Canada Workshop on Methods for the Production of Non-Maturing Salmonids. The proceedings of this workshop were published as Canadian Technical Report of Fisheries and Aquatic Sciences No. 1789 (March 1991). The present proceedings, although less comprehensive, add a more current picture to the still very useful proceedings of the 1991 workshop. One thing that has not changed since 1991 is that a clear impression of whether all-female and triploid salmonids can be used effectively by the Canadian aquaculture industry is still lacking. All-female diploid chinook salmon have done well in British Columbia aquaculture, as have all-female triploid rainbow/steelhead trout in commercial culture operations in Cape Breton (Nova Scotia) and Bay d'Espoir (Newfoundland). Triploid Atlantic salmon are a very different picture: commercial data to date from both New Brunswick and Scotland have not been encouraging.

I would like to take this opportunity to thank the Aquaculture Association of Canada, and specifically Al Castledine (Past-President), for including this special session in the annual meeting and for publishing the proceedings as a full issue of the Bulletin. I would also like to thank Irene Johnston of the Department of Biology at the University of New Brunswick, and Susan Waddy and Theresia Fawkes of the AAC Office, for their assistance in putting this Bulletin together. Financial support from the AAC and the Government of British Columbia to Robin Stuart and Ray Johnstone, respectively, allowed them to attend the meeting, and is gratefully acknowledged. Finally, I would like to thank all the participants in the special session for what was, I believe, a very productive afternoon.

—Tillmann J. Benfey

Use of all-female and triploid salmonids for aquaculture in Canada

Tillmann J. Benfey [1]

The intended use of all-female and triploid stocks for aquaculture was initially to eliminate problems associated with early maturation. More recently, the emphasis has switched to the prevention of spawning in genetically-altered and/or non-native fish. All-female populations are usually produced by hormonal masculinization of genotypic females and subsequent use of their sperm to fertilize normal eggs. Gynogenesis can also be used to produce all-female stocks. If inbreeding problems are avoided, individuals within an all-female population do not differ from females within a normal mixed-sex population. Triploidy can be induced easily and effectively by means of pressure, heat or chemical treatments applied to eggs shortly after fertilization. Triploids differ from diploids in their heterozygosity, cell size and number, and reproductive abilities. Female triploids put less energy into gametogenesis than do male triploids, and triploidy is generally induced in all-female stocks. All these reproductive technologies have been sufficiently well developed that they can be applied on a commercial scale. Certain triploid-specific problems have been reported, including lower jaw deformities, increased mortality, reduced tolerance to chronic stress and poor growth. These problems can likely be addressed through a better understanding of the basic biology and culture requirements of triploids.

Rationale

Research on the application of monosex (female) and triploid stocks to the salmonid aquaculture industry was initially based on the need to develop methods to circumvent early sexual maturation of production fish. Sexual maturation typically leads to reduced flesh quality as lipid and protein energy stores are depleted in the muscle and replaced by water, and as pigments are mobilized from the muscle for deposition in the eggs. Sexual maturation is also associated with external darkening and changes in head morphology (secondary sexual characteristics), and increased aggressiveness, susceptibility to disease, and mortality. Male salmonids tend to mature earlier and at a smaller size than females, and in some species these problems associated with early sexual maturation can be eliminated by the use of all-female stocks. In other species, where a significant proportion of females may also mature prior to reaching market size, sterilization by induced triploidy is a possible solution.

Improvements in fish husbandry and stock selection have largely addressed problems associated with early maturation of production fish. However, there now is increasing concern about the possible genetic and/or ecological effects of escaped farmed fish on wild salmonid stocks. This is based on the recognition that all farmed fish are to some extent genetically different from wild populations, be they exotic (non-native) species or simply domesticated popula-

tions of native species. In the case on non-native species, the use of monosex stocks should eliminate the risk of establishing wild populations, assuming that there are no individuals of the opposite sex present in the wild and that hybridization with native species will not lead to the production of fertile offspring. With the use of native species, sterilization via induced triploidy is necessary to prevent any interbreeding with wild populations.

Production of all-female stocks

In any species where females are homogametic (i.e., with only X-chromosomes, in contrast to heterogametic males that possess both X- and Y-chromosomes), as is the case with salmonids, gynogenesis can be used to produce all-female populations.[2] In this process, sperm are irradiated in such a way as to inactivate their DNA without affecting their ability to swim and penetrate eggs. Eggs thus activated will begin developing in the absence of the paternal genome. If their chromosome number is duplicated, they will possess the normal (diploid) number of chromosomes, but because their chromosomes are entirely of maternal origin, these fish will always be female. Chromosome duplication is typically done by applying thermal or hydrostatic pressure treatment to eggs shortly after fertilization. Although gynogenesis has been induced successfully in several salmonid species, it has had only limited application in commercial aquaculture. Problems associated with this technique include reduced heterozygosity (due to uniparental inheritance), reduced fertility and difficulties in ensuring proper treatment of the sperm.

All-female populations can be produced relatively easily by the direct administration of estrogens to production fish early in development, prior to the completion of sexual differentiation.[3] Such fish cannot be sold for human consumption in Canada, due to regulations on the use of steroids in production fish. Even if the sale of these fish were allowed, it is unlikely that the salmonid aquaculture industry would want to risk the potential consumer rejection of steroid-treated fish. As an alternative, indirect hormonal feminization can be achieved by treating potential broodstock with androgens early in development, thereby converting genotypic females into functional males.[3] The sperm of these fish will invariably carry an X-chromosome, allowing for the production of all-female stocks by crossing normal females with these sex-reversed females. This method circumvents problems associated with the use of steroids, as steroid treatment is limited to broodstock which never go to market. Indirect feminization is presently the best method available for the production of all-female salmonids, and is already used widely in Canada and elsewhere.

Production of triploid stocks

Triploid salmonids are typically produced by using various treatments[4] to retain the haploid second polar body that is normally lost from the egg at the completion of meiosis, just after fertilization. Common treatments include the use of heat shock, hydrostatic pressure shock, or gaseous anaesthetics such as nitrous oxide. Although all three have given high triploid yields, the general consensus is that hydrostatic pressure shock gives the best results. Commercial-scale systems for the production of triploid salmonids by hydrostatic pressure shock are available in Canada and elsewhere.

It is also possible to produce triploid salmonids by crossing tetraploids with diploids.[4] Although difficulties exist at present with producing sufficient numbers of viable tetraploids for broodstock development, this method should be pursued because it would allow simple triploid production by normal hatchery mating procedures. It should also be kept in mind that while triploidy is at present the preferred method for producing sterile salmonids, other techniques that are better suited to aquaculture may be developed in the future.

Biological differences between triploids and diploids

Triploids differ from diploids in three basic ways: they are more heterozygous, they have larger but fewer cells in a variety of tissues and organs, and they are sterile.[5] Increased heterozygosity, which in this case results from retention of the second polar body, is generally considered to be beneficial. Increased cell size in the absence of a change in cell shape leads to a reduction in cell surface area to volume ratio. This may affect processes limited by cell surface area, such as nutrient, metabolite, ion and gas exchange. Since cell numbers are reduced, these effects should be seen at the level of the

tissue as well as the cell. However, these cells should have an advantage in reduced energetic costs associated with the production and maintenance of cell membranes and in maintaining ion and water gradients across these membranes. Numerous studies of the physiology of triploids have demonstrated little difference compared to diploids,[5] indicating that these changes in cell size and number either do not affect general physiology, or that they are compensated for in triploids.

Triploids are sterile because their cells generally cannot complete meiotic development to yield mature eggs or spermatozoa. Triploids develop large testes due to the huge numbers of spermatozoan-precursors produced, which are larger in size than spermatozoa themselves. As a result, triploid males appear to mature, and are of no added benefit to aquaculture. Triploid females, on the other hand, have tiny ovaries at the normal time of sexual maturation and retain the characteristics of immature fish. There is some evidence that triploid females may occasionally produce small numbers of eggs later in life, beyond the normal time of harvest in aquaculture. These eggs are likely aneuploid, as are occasional spermatozoa obtained from triploid males, indicating that egg production by triploid females should not restrict their use as sterile fish for management purposes.

Problems encountered with triploids in aquaculture

Although triploids typically are no different from diploids in controlled, laboratory experiments, they do not appear to perform as well as diploids in commercial aquaculture. Problems encountered with triploid salmonids include reduced survival, especially during early incubation and during seawater grow-out,[6] reduced ability to withstand various types of chronic stress,[5] and increased incidence of various abnormalities including lower jaw deformities, cataracts, shortened opercula and constricted and divided red blood cell nuclei.[6-15] Reduced survival during early incubation is generally associated with poor technique in triploidy induction, and can be eliminated with proper handling of eggs. Problems related to chronic stress may reflect limitations of triploidy physiology. Morphological and hematological abnormalities may be related to nutritional deficiencies. It should not be assumed that triploids of a given species will have the same requirements as diploids of the same species. The best approach to using triploids in aquaculture may be to consider them as a new species, therefore taking a slower, more cautious approach. It is apparent from results in Canada to date that triploid rainbow trout *(Oncorhynchus mykiss)* perform well in aquaculture,[9,16] but that triploid Atlantic salmon *(Salmo salar)* do not.[6,7] Similar results have been reported in the United Kingdom.[17] It is unlikely that this is due to species-specific differences in the performance of triploid salmonids in aquaculture, but rather that the optimum rearing conditions for triploid Atlantic salmon have not yet been determined.

Notes and references

1. Department of Biology, University of New Brunswick, Bag Service #45111, Fredericton, New Brunswick E3B 6E1.
2. Ihssen PE, McKay LR, McMillan I, Phillips RB. 1990. *Trans. Amer. Fish. Soc.* 119: 698-717.
3. Donaldson EM, Benfey TJ. 1987. In, *Proceedings of the Third International Symposium on Reproductive Physiology of Fish* (Idler, D.R., L.W. Crim, and J.W. Walsh, Eds.), pp. 108-119. Memorial University of Newfoundland, St. John's.
4. Benfey TJ. 1989. *Can. Tech. Rep. Fish. Aquat. Sci.* 1682: 33 p.
5. Benfey TJ. 1991. *Can. Tech. Rep. Fish. Aquat. Sci.* 1789: 73-80.
6. McGeachy SA, O'Flynn FM, Benfey TJ, Friars GW. 1996. *Bull. Aquacult. Assoc. Can.* 96-2:24-28.
7. Sutterlin AM, Holder J, Benfey TJ. 1987. *Aquaculture* 64: 157-164.
8. Jungalwalla PJ. 1991. *Can. Tech. Rep. Fish. Aquat. Sci.* 1789: 47-71.
9. Sutterlin AM, Collier C. 1991. *Can. Tech. Rep. Fish. Aquat. Sci.* 1789: 89-96.
10. Hughes DR. 1992. In, *Proceedings of the Saltas Research Review Seminar,* 29th April 1992, pp. 17-64. Salmon Enterprises of Tasmania, Hobart.
11. King H, Lee P. 1993. In, *Proceedings of the Saltas Research Review Seminar,* 29th April 1993, pp. 37-44. Salmon Enterprises of Tasmania, Hobart.
12. Lee P, King H. 1994. In, *Reports from the SALTAS 1993-94 Research and Development Programme,* pp. 61-70. Salmon Enterprises of Tasmania, Hobart.
13. Wall AE, Richards RH. 1992. *Vet. Rec.* 131:553-557.
14. Stillwell EJ, O'Keefe RA, Benfey TJ. Unpublished observations, University of New Brunswick, 1993-95.
15. Biron M, Grant K, Stillwell EJ, O'Keefe RA, Benfey TJ. Unpublished observations, University of New Brunswick, 1990-95.
16. Stuart R. 1996. *Bull. Aquacult. Assoc. Can.* 96-2:29-31.
17. Johnstone R. 1996. *Bull. Aquacult. Assoc. Can. 96-2:* 9-13.

Experience with salmonid sex reversal and triploidisation technologies in the United Kingdom

R. Johnstone[1]

The chronological development of the use of sex reversal and triploidisation techniques in the UK trout and salmon aquaculture industries is described. Initially, the rationale behind the use of these technologies was to control maturity for reasons of commercial efficiency; more recently their use in minimising effects that might result from genetic introgression caused by escaped farmed fish has increasingly come to the fore. Currently, a 1.4 million ecu, three-country, four-year research programme on triploid biology is being funded by the EU Commission.

All-female stocks were first introduced into United Kingdom rainbow trout culture in the early 1980s. They have since become commonplace; according to official figures, ca. 85% of farmed rainbow trout stocks are now all-female. Sex-reversed "males" are produced directly by trout farmers using oral administration of hormones issued under prescription by veterinarians. Since most UK farmed trout are sold at "portion" size (500 g), early maturation problems were largely confined to males and the use of all-female stocks has eliminated this concern.

Sex reversal of Atlantic salmon was not attempted until after it had been successfully demonstrated in rainbow trout, and reversed salmon milt was not therefore widely available until the mid 1980s. It proved more difficult to optimise sex reversal treatments in salmon than in trout. At the first dietary dose used (3 mg/kg)[2] most animals were sterilised rather than reversed and the yield of reversed "males" was low. Yield increased when the dose was lowered (0.25-1.0 mg/kg).[3] Additional studies[3] suggested there was a very discrete window when immersion treatments were effective. It is possible that immersion treatments will result in more uniform effects than oral treatments since dose rates and degree of effect will presumably vary with amounts eaten. Immersion treatments also use less hormone and used solutions can be disposed of more easily in a responsible manner. There should be a degree of concern about uneaten hormone-laden food entering the environment where it might result in unplanned effects.

Reversed stocks must be carefully and separately managed to ensure they are not contaminated by normal males. Because dietary treatments alter testis duct formation, often making normal stripping impossible, most sperm is collected by dissection of the testes after slaughter. This phenomenon is not seen as a disadvantage, since it means that reversed sperm solutions can be carefully screened and tested prior to use. It also allows for the exclusion of normal males.

The identification of males of female genotype at the beginning of sex-reversal programmes when mixed sex batches are treated can be a problem. In trout, there is a good correlation of altered testicular development with sexual genotype. Blocked-duct rainbow trout "males" proved genotypically female and produced all females when back-crossed to normal females. In Atlantic salmon, precocious parr blocked-duct "males" are also genotypically female. With increasing age, however, this criterion for selection becomes less efficient. Approximately half of the blocked-duct grilse so far examined, but only a minority of those of salmon age, generated all females when back crossed. This presumably reflects variability of exposure, i.e., of food and therefore dose consumed, coupled with a degree of recovery and maturation of genotypic males whose earlier development had

been delayed by their treatment. If comprehensive progeny testing is to be avoided, an alternative method of selection of the genotypic females is required. Fortunately a convenient one exists, namely the selection of the obviously hermaphrodite animal. If a mixed-sex batch is treated with male hormones, any hermaphrodite is presumed to be of female genotype and to have been incompletely reversed. To date salmon hermaphrodites have always produced all-female batches when used as sires. Hermaphrodite animals are sufficiently common after dietary treatments to enable this criterion to be used to avoid the need to progeny test. Once the system has been cleaned up in this way, any male from a masculinised, otherwise expectedly all-female stock may be used to sire all-females. This is of course subject to the confidence that the stock has not become mixed with normal males — a constant threat on most commercial farms as fish appear to have a predilection to jump between tanks.

The alternative method of producing all females directly by gynogenesis is superficially attractive. Success demands an accurate knowledge both of sperm irradiation technologies in order that the second meiotic division of eggs is initiated at high frequency, and of ploidy manipulation such that the otherwise haploid condition may be diploidised. High yields have been reported,[4] but experience suggests the former is more difficult than the latter. Sperm solutions from different males are of variable opacity to UV and careful attention must be paid to the preparation and irradiation of sperm solutions to ensure optimal treatment and maximisation of yields. Over-irradiation will result in poor "fertilisation" rates while under-irradiation will generate animals of the XY genotype. The presence of the Y chromosome in a stock that is to be masculinised—albeit that satisfactory ploidy manipulation (100% rates are highly likely with optimised treatments) will cause these to become triploid—will unnecessarily complicate reversed "male" selection. In addition, gynogenesis generates a degree of inbreeding that has to be counteracted in subsequent crossing programmes. In brief, whilst these techniques can be managed in the laboratory they are unlikely to be within the scope of most farmers.

Although trout farmers readily adopted sex-reversal methodologies, they were never commonly adopted by the salmon industry; currently no commercial salmon farmers are using the technology. There were several reasons for this. Most importantly, salmon farmers never saw early male maturity as quite the problem that trout farmers had. Maturity of male salmon in freshwater was confined to 2-year-old smolts and these became increasingly less common as farmers became more efficient at producing one-year-old smolts.

Early salmon maturity problems in seawater, although largely a male phenomenon, were not confined to males. Because some females matured at grilse age most farmers thought the all-female approach was not worth the effort and believed that genetic selection to generate lower maturing stocks was the better route. Despite the fact that the use of sex-reversal techniques would not result in hormone residues reaching the animals destined to be marketed (only their fathers would have been treated) there was concern about the difficulties of presentation that might result from the use of hormones in the production of a luxury product. Some of those that did adopt the technology believed it wasteful of facilities and food because they grew reversed animals to maturity in salt water. However, Atlantic salmon will mature even if they spend their whole lives in fresh water; indeed in this medium their growth is more modest and reversed stocks can be more easily kept separate and uncontaminated—the single most important determinant of the success of the technology.

What salmon farmers wanted at that time was an immediate answer to their maturity concerns, regardless of sex. What scientists were able to offer them was a variety of techniques, the most promising of which appeared to be triploidy. Triploids occur naturally, albeit very rarely, and contain three instead of the normal two sets of chromosomes. Because of this they are unable to produce balanced sets of chromosomes when they attempt to mature and this makes them sterile.

Although triploids of both sexes are sterile, there are physiological differences between male and female triploids. Testes, as they divide and mature, have the potential to produce billions of sperm. In triploids, most of these divisions end in failure but because they make so many attempts, male triploids produce a thin watery sperm-like fluid (although this is incapable of fertilising eggs). Because they contain separate areas of hormone secreting tissue that

are independent of the germinal tissue, triploid males continue to produce hormones that cause the deteriorative changes associated with maturation. Triploid males are therefore functionally sterile but hormonally competent. In females, the growing eggs become invested with hormone secreting tissue at an early stage of their development. Females can only produce, at most, many thousands of eggs. In triploid females, the vast majority of eggs cannot grow and develop and, because the hormone secreting and germinal tissues are necessarily closely associated, neither does the hormone secreting tissue. Thus almost all triploid females are both hormonally and functionally sterile. This means that only female triploids have potential commercial benefit. By the late 1980s, we knew how to produce all-female salmon stocks, all that was needed to produce non-maturing stocks was a knowledge of how to make them triploid.

There are presently three methods of making recently fertilised salmonid eggs triploid, namely temperature (heat), pressure and anaesthetic gas (N_2O) treatments. Of the three, pressure has been shown to be the most efficient. Heat treatment is difficult to control exactly and yet exact control of timing and temperature is essential for success. Egg batches are also variable in their susceptibility to heat shock. During exposure to heat and N_2O the eggs must be held in thin layers and this makes reproducibility difficult and places limitations on throughput. Pressure treatments need special equipment but the technique is more "forgiving" in that eggs can be held in bulk (2-4 L) and the treatment parameters need not be so exact. For Atlantic salmon, pressures of 9-10,000 psi for durations of 3-10 minutes seem equally effective. Early treatments (before 25 minutes after fertilisation at 10°C) are damaging to survival; later treatments (after 50 minutes) are ineffective. There appear to be species-specific differences in the timing of optimal treatments; rainbow trout can be effectively treated at 40-50 minutes after fertilisation. If high quality eggs are used and if they are properly handled, it should be possible to achieve close to 100% triploid rates with survival similar to controls. At this stage of our understanding proper handling means that at all times eggs must be supported in water — they must not be moved from one container to another until after they have been triploidised and even then they should be laid down in their subsequent incubation facility by decanting under water so as to minimise disturbance. The recently fertilised egg is, after all, a valuable and fragile living organism.

Because the early commercial trials of triploid all-female stocks were encouraging, the technology was transferred to the industry in the late 1980s. In the peak year, 1989 (Fig. 1), some 6 million ova were triploidised in Scotland, ca. 7.5% of the total strip. Subsequently, triploids rapidly fell out of favour and today no triploid salmon are being reared commercially in the United Kingdom.

There were various reasons for the decline in the use of salmon triploids. They were introduced in the United Kingdom at a time of severe economic pressure for the industry. Profitability was being squeezed by poor survival, a consequence of increased prevalence of disease, and by alleged uncontrolled imports

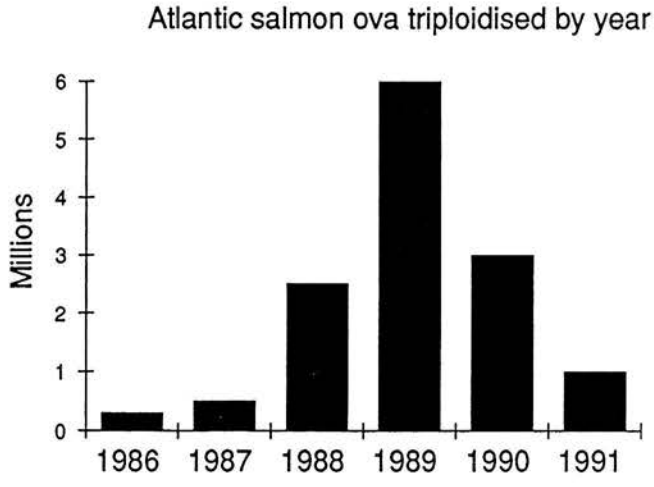


Figure 1. Atlantic salmon ova triploidized by year.

by non EU countries. Although this meant there was keen interest in any new technology that might improve efficiency, there was equal pressure to reject those practices that were not immediately successful. The initial commercial experience with triploids was not good. The problems cited by farmers were poorer growth and greater susceptibility to disease and cataract formation.

It is possible to explain the first of these: triploid fish grow differently than diploids.[5] As they mature, diploid fish are influenced by anabolic hormones produced by their gonads and grow more rapidly. Because triploid females lack these hormones they grow at the same rate as non-maturing fish. However, if triploids experienced similar survival rates, more triploids should grow to the larger, more valuable sizes. There should be a trade-off between less rapid, early growth but the production of more larger fish. In other words, no grilse but more salmon, and this was essentially the picture at the end of the research phase.

Subsequently, however, farmers came to believe that rather than giving increased yields, triploids gave poorer yields since they were more susceptible to disease. To date we have been unable to demonstrate any such disadvantage in scientific tests. However, triploids are more easily stressed and therefore more susceptible to those diseases that are in the environment, rather than being intrinsically more susceptible.

The cataract problem was first noticed in triploid populations shortly after sea lice treatments. Fish with cataracts cannot feed properly and soon die. The cataract problem may be related to the larger size of triploid cells — relative to a diploid, the triploid has larger cells but fewer of them. Despite this, no tests of relative physiological performance under normal conditions have shown this to be the cause of any measurable disadvantage. Nevertheless, gas diffusion processes in the larger triploid cell may more readily become rate limiting in critical situations. Fish farmers commonly expose their fish to periods of acute and chronic oxygen stress (e.g., during sea lice treatments or by overstocking) — if the problems with triploids are related to their greater susceptibility to stress they may demand different rearing techniques if they are to be reared at maximum efficiency.

Farmers in other countries where triploids were being reared also saw increased incidences of jaw deformities. These occurred in pressure-shocked Atlantic salmon in Tasmania and heat-shocked rainbow trout in Canada. In the United Kingdom, the incidence of deformities is very low. The reason is not known but genetics and nutritional causes have been suggested. It may also be related to the potential of the triploid condition to cope with increased development rates because animals are commonly growing very rapidly when jaw deformities first become noticeable.

In summary, trout farmers in the United Kingdom were satisfied with sex reversal as the solution to their maturity problems because they grew mostly smaller fish. They were not as interested in triploids — only 5% of trout stocks are triploid, these being primarily destined for restocking. UK salmon farmers were more interested in total maturity control, i.e., in triploidy and therefore in sex reversal. They remain, however, sceptical of the commercial advantages of triploids. Because of this and because all-females were only a means towards the end of all-female triploids, no all-female or all-female triploid salmon stocks are being reared in the United Kingdom.

And there the story might have ended, except that by the time that triploid technologies had been developed, a new concern was being expressed by fish stock managers. Many wild salmon and trout stocks had suffered large declines in abundance through the 1980s and some thought fish farm escapes might in part be responsible. Research showed that escaped fish had the ability, once mature, to return to rivers near the site of their release and to breed with wild fish. The concern was that interbreeding might lead to a deterioration of the genetic "quality" and therefore viability of wild stocks.

Clearly, the potential for genetic change by interbreeding is obvious, since part of the fish farming process is to "improve" stocks by selection. It was less obvious whether this potential for change had been or would be realised. One view suggested that "nature" would choose only those genotypes appropriate for particular river systems, i.e., there would be little change because most introduced genes would be less fit. Others saw the potential for improvement as evolution had new material to work with. Still others thought the changes could only be detrimental.

Proving which of these scenarios was most likely to happen or had indeed happened would

be technically difficult and time consuming. In the meantime, some thought the concern was so real that serious consideration should be given to compelling farmers to rear only sterile stocks. Evoking the precautionary approach, they thought that action should not necessarily await formal proof that the change had happened, for by that time the change might be irreversible. The concern in the United Kingdom related to the possibility of escape and interbreeding of an indigenous species — the Atlantic salmon. Rainbow trout do not breed in the UK and so any interaction of escaped fish would be "physical" rather than genetic and was presumed to be of less concern. In other regions such as the west coast of Canada, the breeding of escaped non-indigenous Atlantic salmon was the concern.

We can see from the above that fish farmers and fish stock managers will have different views on the desirability of the introduction of sex reversal and triploidisation techniques. What is therefore needed is some sort of comparative assessment of the overall effect, of the costs and benefits, that might follow from their introduction.

Sex reversal properly managed should not result in significant costs to the industry; in fact it has been of considerable benefit to the UK trout industry. This might become true for Atlantic salmon culture since recent research has shown that maturity in seawater can be delayed and growth advanced by appropriate photoperiod manipulation. Because females can be more easily persuaded to delay maturity than males, all-female stocks would be an advantage. In addition, all-female stocks would be essential for the most efficient means of "caviar" production were this to become a significant market.

Where the farmed species is not indigenous, releases of only females would limit "damage" to physical competition and to the consequences of interspecific hybridisation. These are less likely to be severe and may not be of any consequence (e.g., most Pacific/Atlantic salmon crosses are not viable). But where the problem is one of indigenous species escapes, introduction of an all-female policy would merely halve the problem. It would not eliminate the risk, as escaped females could still interbreed with indigenous males. Only triploid use can largely eliminate the risk — but Atlantic salmon farmers in the United Kingdom would presently conclude that they would become less efficient if they were asked to rear only triploids.

If that were true, the costs to farmers of introducing a triploids-only policy could be easily derived. They may comprise lowering of income, reductions in company viability, loss of jobs etc. However, the costs to the environment if fish farmers are allowed to continue to use diploids are much more difficult to calculate. Some, such as end-user costs, i.e., losses to anglers and the associated tourist revenue if wild stocks were affected, can be calculated. But, save for contingent valuation methods, the costs of, for example, the loss of genetic diversity that might arise from interbreeding cannot be immediately assessed.

Nevertheless, in some locations, a comparative assessment might presently demonstrate that a reduction in the efficiency of the fish farming process was a price worth paying to protect the environment. In Europe, the concern about the potential genetic impact of farmed fish escapes has resulted in the EU commissioning a four-year, three-country project (Scotland, Norway and Ireland) designed to improve our understanding of triploid biology. It will look closely at differential performance, migration behaviour, developmental and disease biology, and swimming physiology. Clearly, if triploid biology could be better understood such that triploids could be as efficiently reared as diploids, farmers would have less objection to their introduction. Even if the rearing of triploids were shown to be necessarily less efficient, countries could be encouraged to harmonise their policies such that no one country was at a competitive disadvantage. Above all, it will be necessary to demonstrate to fish farmers, fish stock managers and to the public at large, as consumers of fish and users of the environment, that attempts are being made to strike the right balance.

Notes and references

1. The Scottish Office, Agriculture and Fisheries Department, Marine Laboratory, P.O. Box 101, Victoria Road, Aberdeen, Scotland AB9 8DB.
2. Johnstone R, Simpson TH, Youngson AF. 1978. *Aquaculture* 13:115-134.
3. Johnstone R, Maclachlan PM. 1994. *Aquacult. Fish. Mgmt.* 25:855-859.
4. Johnstone R, Stet RJM. 1995. *Theor. Appl. Genet.* 90: 819-826.
5. Johnstone R, McLay H, Walsingham MV. 1991. *Can. Tech. Rep. Fish. Aquat. Sci.* 1789:15-36.

Newfoundland experience with development of all-female and non-reproductive Atlantic salmon for marine aquaculture

V.A. Pepper,[1] A.M. Sutterlin,[2] T. Nicholls[1] and C. Collier [3]

There are potential strategic, economic and environmental advantages to using late-maturing or non-reproductive salmonids for aquaculture. Elimination of male salmonids from aquaculture strains is one strategy for reducing early maturation problems and for extending the market window for fresh product. Induction of triploidy is the preferred method to overcome the normal reproductive cycle. Non-reproducing salmonids are useful for avoiding undesirable consequences resulting from the onset of sexual maturation. As well, triploid salmonids diminish genetic concerns about potential breeding between domestic and wild fish. Experience to date indicates that pressure is the preferred means to produce triploid salmonids. Development of an all-female strain of salmon began in Newfoundland in 1989 and was based on sex-reversal using androgen treatment. Desynchronization of the maturation process between sex-reversed fish at the hatchery and normal brood fish in the estuarine cages in 1991 through 1993 delayed implementation of performance evaluations of non-reproductive salmon. Motile spermatozoa were obtained in 1994 after application of gonadotrophic releasing hormone treatments to potential sex-reversed spawners. Flow cytometry and microscopic analysis of red blood cell size of salmon parr produced from pressure treated eggs indicated the prototype pressure vessel was effective in inducing triploidy. After six years of endeavour to establish an all-female brood line in support of a Newfoundland program on non-maturing salmon, trials are now under way to evaluate performance of these reproductively incapacitated Atlantic salmon.

Introduction

Sustainable development of Canadian aquaculture requires careful attention to biological prerequisites, concurrent with consideration of economic constraints for successful competition in international seafood markets. In Newfoundland, where winter marine conditions are often severe due to the predominant effect of the cold Labrador Current, aquaculture of Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) has required considerable technical innovation. Although Newfoundland has an extensive coastline, cold winter marine conditions serve to limit the geographic scope of salmonid farming opportunities. A controversial and potentially expensive consideration for salmon farming in the province relates to the suitability of available brood stocks for aquaculture development. With a large number of historic Atlantic salmon producing rivers, the Newfoundland salmon farming industry has been constrained by the requirement to minimize potential environmental impacts of aquaculture operations on wild fishery resources.[4]

Economically viable salmon and trout aquaculture requires use of strains that grow well under the confined and artificial conditions of hatcheries and rearing cages. In addition, selected strains must be highly tolerant of confine-

ment and other human intervention. This is in marked contrast to wild species that must be extremely wary in order to survive. A challenge for salmon and trout aquaculture is to enhance strain characteristics that are desirable for artificial rearing and to suppress undesirable traits. Accordingly, salmon and trout that are used in an aquaculture operation, while they may be the same species as wild salmon and trout, typically are quite different in their genetic make-up.

Rainbow trout are not native to Newfoundland. As a result of trout farming activities, there is concern among fishery managers that inadvertent establishment of wild populations of this species could interfere with natural populations of Atlantic salmon and brook trout (*Salvelinus fontinalis*) by predation and competition. Similar resource management concerns with non-native strains of Atlantic salmon have resulted in limitations on importation of aquaculture strains of salmon eggs into the province. Fishery resource managers have been concerned about potential reproductive interactions between escapees from salmon aquaculture cages and natural spawners in Newfoundland rivers. Scientists have cautioned about preservation of genetic diversity of wild salmonid populations.[5] Such concerns culminated in international agreements on Atlantic salmon conservation[6] that have encouraged adoption of technology for reproductive control of Atlantic salmon by the aquaculture industry.

Induction of triploidy currently is the preferred method to overcome the normal reproductive cycle of salmon and trout. Protocols for inducing triploidy (time after fertilization for pressurization, pressure intensity, and duration of the pressure application) are well established for rainbow trout and, more recently, have been documented for Atlantic salmon.[7] Satisfactory protocols are not yet available within the public domain for species such as brook trout and Arctic charr (*Salvelinus alpinus*).

Non-reproducing salmonids have been shown to be useful in avoiding several of the undesirable consequences of sexual maturation (i.e., growth retardation, and poor flesh/skin colour and texture). Aksnes et al.[8] found that fat content of fillets of maturing salmon decreased from about 12% to 5%; protein content decreased from about 22% to 19%; and water content increased from about 66% to 74%. In Newfoundland, experience with steelhead (*Oncorhynchus mykiss*) has confirmed that triploidy is an effective strategy for production of 3 to 5 kg trout for marketing throughout the year. In addition to improved flesh quality, use of triploid salmonids for aquaculture operations negates resource management concerns about reproductive interactions between wild and aquaculture fish.

Techniques for inducing triploidy in salmonids have been reviewed.[9] While heat shock has been successful with rainbow trout, efficiency of this technique has not been entirely satisfactory for Atlantic salmon (69%).[10] Experience to date indicates that pressure is the preferred means to produce triploid salmonids.[9,11] The correct timing for administering pressure shock to fertilized eggs is temperature dependent and critical. The pressure probably alters the tertiary structure of contractile proteins (comparable to spindle fibres) involved in the exclusion of excess recombinant genetic material (i.e., the second polar body) from the egg during meiosis. This results in retention by the egg of an extra set of maternal chromosomes. The normal process of polar body exclusion begins at ovulation with elimination of the first polar body, and is completed shortly after fertilization with exclusion of the second polar body.

While pressure treatment has proven far superior to heat shock in the yield of triploid salmonid eggs and their subsequent survival, the cost of fabrication of pressure vessels has been excessive. Estimates of costs for such pressure systems have been as high as CAN $40,000.[12]

Other than one private aquaculture hatchery at Bay d'Espoir, on the south coast of Newfoundland, there are no salmonid hatcheries in Newfoundland and Labrador at which to conduct salmon aquaculture experiments. Accordingly, experiments on sex-reversal and triploidy have been confined to Bay d'Espoir (Fig. 1) where a 250 km^2 estuary provides considerable developmental potential for salmon farming.

The background to this paper is found elsewhere.[4,13] We describe here a low-cost, prototype pressure vessel system that has been tested over the past four years. The present paper also describes Newfoundland industry efforts towards establishing all-female, diploid and all-female, triploid lines of Atlantic salmon.

Much of the work described in this report was made possible with funding from the Aquaculture Science and Industry Development components of the Atlantic Fisheries Adjustment Program. Though the focus of this paper is on work

conducted in 1994/95, an overview of work since 1989 is included. All experiments were conducted with the Saint John River strain of Atlantic salmon. Salmon eggs for these experiments were procured from brood stock from marine cages located in Vyse Cove in the Bay d'Espoir estuary (Fig. 1).

Methods

All Newfoundland efforts towards sex-reversal prior to 1994 were based on a mixed-sex strain of Atlantic salmon. Due to severe limitations in hatchery space, experiments were limited to one rearing tank. This greatly constrained the opportunity to refine techniques in any quantitative way. Prior to 1994, the sex-reversal protocol used 3 mg of 17 α-methyltestosterone (MT)/kg of feed from first-feeding for 500 C°-days.

The original pressure vessel system was designed and fabricated for the Department of Fisheries and Oceans in 1991 with funding from the Industry Development component of the Atlantic Fisheries Adjustment Program. The components of the original system were assembled for $5,000. This first system was pressurized via a foot pump that utilized oil as the compression medium. A heavy rubber diaphragm in the vessel lid separated the oil from the water in the egg chamber. This proved unsatisfactory and the configuration of the apparatus was modified in 1992 to improve operational characteristics. The hydraulic foot pump was replaced by a low-volume, high pressure, hand-operated water pump (Teledyne-Sprague, Model S-525-200) capable of delivering up to 12,000 psi. Eggs are placed in a 1.8 litre, mesh egg basket that is inserted into the pressure chamber before the chamber is sealed in preparation for pressurization.

This Newfoundland prototype pressure system was designed to be portable for potential application at remote locations. It does not require electrical power and can be carried by two people. Figure 2 illustrates the pressure system components and their assembly. The pressure vessel system was tested in the laboratory prior to field application. An electronic pressure transducer (Omega Engineering Inc., Series PX302), with a certified accuracy of ±1%, was installed on the pressure release port and the system was pressurized in 1,000 psi increments to a maximum of 10,000 psi. At each 1,000 psi increment, the millivolt output from the pressure transducer was compared with the reading on the analog gauge.

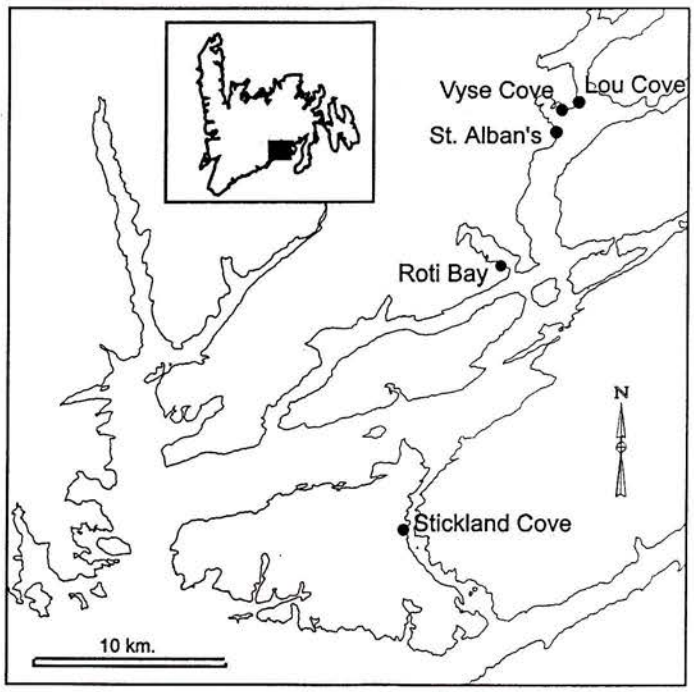


Figure 1. Bay d'Espoir, Newfoundland.

Field operation of the pressure vessel took place in the SCB Fisheries Limited salmon hatchery. Atlantic salmon eggs were procured from Bay d'Espoir marine cages, also belonging to SCB Fisheries Limited. Brood females were stripped in November, 1991 to 1994. Eggs were collected in batches so that each individual container transferred to the hatchery contained the eggs from at least three females.

Although the sex-reversal procedure employed since 1991 had

been successful in producing some obvious hermaphrodites (i.e., mature testes with immature ovaries attached), there has been a serious desynchronization of the maturation process between sex-reversed fish at the hatchery and normal brood fish in the marine cages. While there is some potential to heat water in the hatchery brood tank, there is no means to mimic natural autumn decreases in water temperature. As a result of this desynchronization experience, gonadotrophic releasing hormone (GnRH-A) was used in an attempt to synchronize spermiation of sex-reversed female salmon in the hatchery tanks with ovulation of normal female brood salmon from the marine cages. Of the 31 specimens that were available in the hatchery sex-reversal group in 1994, 26 fish were marked with individually-identifiable tags and were given GnRH-A. Fourteen received an intraparietoneal injection (immediately posterior of the pectoral fin) of 300 µg GnRH-A. Another 12 specimens received a pellet implant in the lateral musculature midway between the origin of the dorsal fin and the lateral line, again at a dosage of 300 µg

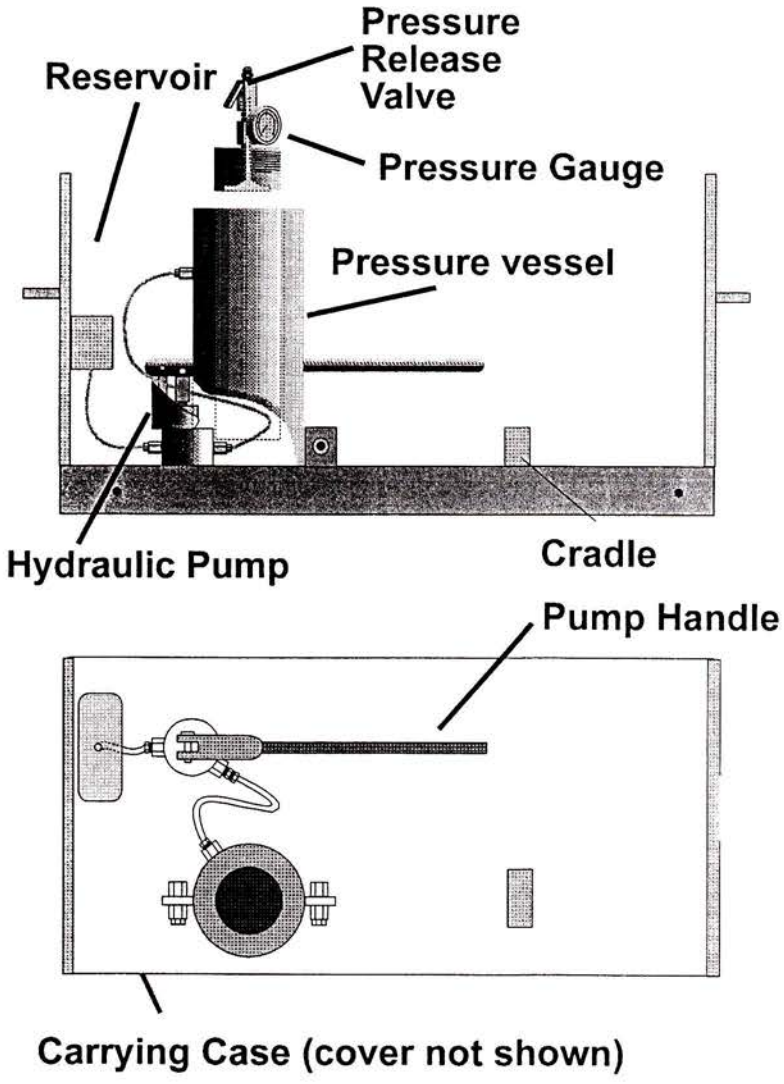


Figure 2. Newfoundland pressure vessel system.

GnRH-A. These treatments were administered 13 days before the anticipated stripping date of females from the marine cages.

Female brood stock in the marine cages were checked periodically to ascertain their stage of maturation. The brood fish were maintained in cages at the Vyse Cove site where there is a significant freshwater lens. Once spawning was imminent among normal brood salmon in the marine cages, presumed sex-reversed fish in the hatchery tank were killed with an overdose of anaesthetic. These specimens subsequently were thoroughly washed to remove anesthetic residues, dried with clean paper towel, then dissected to remove the gonads. The extracted sperm from obviously sex-reversed fish was mixed with a modified Cortland's sperm extender solution,[14] placed in separate storage containers with large surface-area-to-volume ratio, and stored in a domestic refrigerator for several hours before use.

Having deduced that spermatozoa were motile (microscopic examination), eggs from the Saint John River strain brood females were collected from the Bay d'Espoir estuary cage. In the most recent experiments (i.e., 1994), good quality eggs were transferred back to the hatchery where a random sample of some 161,000 eggs (subsets from about 100 brood females) was fertilized with presumed homogametic milt from the sex-reversed salmon dissected that morning. Fertilizations were conducted as per Figure 3. This breeding scheme was intended only to "homogenize" genetic variability among the experimental groups. After fertilization, about 40,000 eggs were removed from this experiment for use as a "control" population. These eggs were incubated in parallel with the SCB Fisheries Limited production line of salmon. Half of the remaining eggs were enumerated volumetrically and surface disinfected by a 10 minute immersion in an iodophor solution (300 mL Ovadyne/30 litres water). After surface disinfection, they were placed in appropriately labelled Heath trays. The other half received pressure treatment with the prototype pressure treatment apparatus (9,500 psi for five minutes at 300 C°–minutes after fertilization).

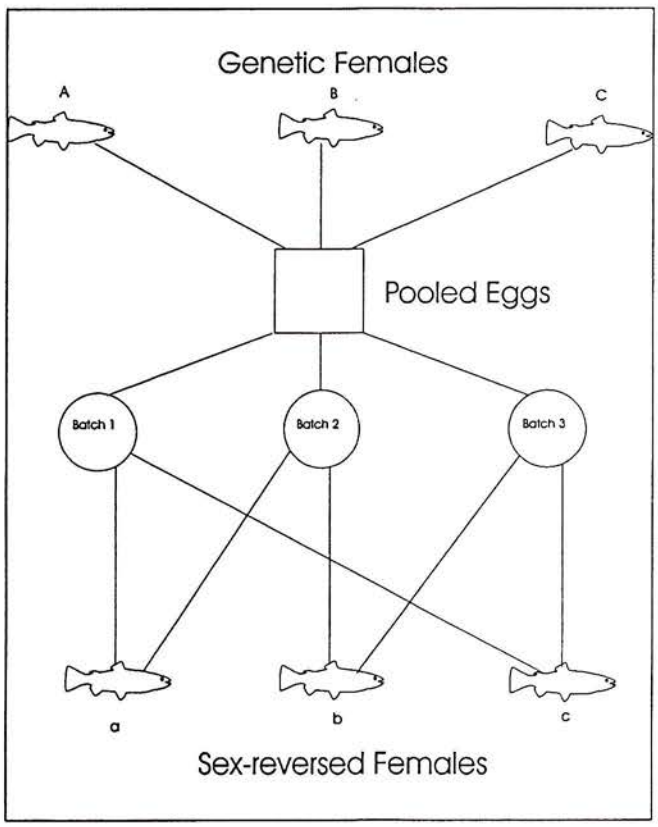


Figure 3. Batch matings for all-female lines of Atlantic salmon.

After water hardening, all pressure-treated subsets were thoroughly mixed, surface disinfected, and an estimated 53,000 were placed in Heath trays. Concurrently, SCB Fisheries personnel fertilized eggs for their annual production requirements with milt from normal male brood fish. Eggs of the experimental groups were incubated at 7°C. Thus, there were three sets of salmon eggs laid down in the Bay d'Espoir hatchery: 1) mixed-sex diploids; 2) all-female diploids; and, 3) all-female triploids. The first picking of dead eggs from the incubation trays occurred about eight hours after fertilization. Samples of eggs were taken from all experimental and production groups

throughout the incubation cycle and preserved in Davidson's Solution.

Eggs were monitored throughout the incubation cycle. Three different treatments were applied to the 1995 year class to achieve sex-reversal. Once the various groups of experimental eggs reached 50% hatch, two different hormone immersion treatments, motivated largely by Piferrer et al.,[15] were applied to attempt sex-reversal. On the advice of Dr. Ed Donaldson (West Vancouver Laboratory), these treatments were administered as follows:

1. two 2.5 hour immersion treatments of MT @ 2000 µg/L administered at 7°C, 10 days and again at 18 days after 50% hatch;
2. one 2.5 hour immersion treatment of 17α–methyldihydrotestosterone (MDHT) @ 2000 µg/L administered at 7°C, 10 days after 50% hatch.

In addition to these two groups of presumed sex-reversed fry, a third group of all-female fry was set aside for MT treatment at the time of first feeding as per the previous work to establish an all-female line.

Experimental groups of fry were ponded for first-feeding in separate early-rearing tanks. Batch weighings were conducted shortly after ponding to determine mean fry weight in each of the experimental groups. Weight and length data were taken from random samples of the experimental groups, plus one random sampling from the SCB Fisheries mixed-sex production line to support quantitative comparison of performance among the experimental and production fish.

Experimental groups of salmon fry were screened for ploidy status by erythrocyte smears and by flow cytometry. Flow cytometry was performed at the School of Medicine (Memorial University of Newfoundland, St. John's) using a FACSTAR PLUS flow cytometer (Becton Dickinson, CA).

Immediately prior to blood sampling, salmon fry were netted rapidly and anesthetized with 2-phenoxyethanol. These fry were bled by caudal puncture using syringes with 26 gauge hypodermic needles. Syringes were flushed with Na-heparin to prevent clotting. Samples of 3-4 µL of whole blood were added to 2 mL of propidium iodide solution (i.e., 50 mg/L propidium iodide in 0.1% sodium citrate) in a disposable polystyrene tube that fit into the flow cytometer. All samples were taken at the Bay d'Espoir hatchery, placed in prelabelled tubes, agitated with a vortex blender, and stored on slush ice for about 24 hours, during which time they were transported to St. John's for flow cytometry analysis.

Erythrocyte smears were made of a random sample of each of the presumed triploid and diploid experimental groups. Microscope slides were prepared by smearing a small amount (i.e., approx. 1 µL) of blood across the surface of the slide. Once dry, the smear was stained with Baxter "Diff-Quik® Stain Set" as per the supplier's recommended procedure. Stained blood smears were examined under a microscope at 500X magnification. Erythrocyte nucleus size was indexed by ocular micrometer measurement of both the major and minor axes of the ovoid nucleus.

Results

Laboratory testing of the prototype pressure vessel system in 1991 confirmed the precision of the analogue pressure gauge. At a pressure of 9,000 psi on the analogue gauge, the pressure transducer confirmed 100% accuracy. At 10,000 psi on the analogue gauge, the pressure transducer indicated the analogue gauge could be reading as much as 100 psi higher than actual. The pressure transducer was removed from the system prior to field use. While the egg basket that is placed in the pressure chamber is capable of holding about 1.8 litres of eggs, each separate lot of eggs was limited to about 1.5 litres to accommodate egg swelling that takes place while the eggs are in the chamber. The egg basket is placed in a bucket with sufficient clean water so that newly fertilized eggs can be poured into the basket with minimal mechanical agitation. Loading of the basket with salmon eggs takes <1 min. The pressure system is prepared by filling the pressure chamber with water from the intended incubation system, inserting the egg basket, and screwing on the lid. Precaution is taken to ensure that the pressure chamber is completely filled with water to minimize time for pressurization. This preparation takes less than two minutes. With vigorous pumping, the manual water pump injects about 75 mL of water into the pressure vessel to reach the desired 9,500 psi pressure. This process takes about 40 seconds.

Sex-reversed females that were expected to be used for fertilization were not ripe when eggs

were collected in 1991 and 1992. While these fish were sex-reversed, as indicated by hermaphroditic gonads, there was no evidence of spermatozoa motility when examined under a microscope. These conditions precluded meaningful use of the pressure vessel during these years.

All year-classes of Atlantic salmon that received hormone treatments at the Bay d'Espoir hatchery from 1989 to 1994 were from mixed-sex populations. Since there are no means to reduce temperature in the outdoor hatchery tanks, water temperature throughout final maturation was higher than would be experienced in the wild (i.e., 10 to 12°C relative to the preferred 3 to 5°C). Of the fish available at the hatchery to provide homogametic milt in support of the present experimentation, half were expected to be normal males, and therefore useless for triploidy. Only those fish that showed definite signs of developing secondary sexual characteristics (i.e., kype, colour) were dissected. The 31 fish ranged in whole wet weight from 1.6 to 4.2 kg (mean = 2.9 kg). Of the 21 candidates removed from the rearing tank at the time of brood stripping, milt was expressed freely from eight fish. These fish therefore had normal sperm ducts and were most likely genetic males. These specimens were discarded. Of the remaining fish, three were obvious hermaphrodites and therefore were genetic females that had been successfully converted to phenotypic males. An additional four specimens had deformed testes but no sperm ducts and were presumed to be successfully sex-reversed. Relative comparison of the two GnRH-A treatments is equivocal; four viable gonads were obtained from the injection group, three from the pellet implant. The remaining six fish, though they had no apparent sperm ducts, did have normal-looking testes and therefore were discarded, since there was doubt as to their genetic sex. Based on visual examination of the presumed sex-reversed fish that did not receive GnRH-A treatment, none had evidence of secondary sexual characteristics and therefore were judged to be immature. The gonads of the seven specimens of probable sex-reversed fish were individually macerated.

Motility of spermatozoa after dissection of sex-reversed brood fish was low relative to that for wild salmon. In fact, had there been any options, these seven lots of homogametic milt would have been discarded. Interestingly, after storage in the sperm extender and subsequent addition of water, spermatozoa activity increased. Samples of eggs taken at intervals following placement of eggs in incubation trays confirmed that fertilization had taken place. Egg quality of the approximately one million eggs taken from the marine cages was judged to be excellent. Fertilization, pressure shocking and surface disinfection all took place at the hatchery within hours of procuring eggs from the marine cages. The estimated 161,000 eggs used in the triploid evaluation experiment (including 40,000 all-female eggs placed in SCB Fisheries production incubators) were placed into incubation trays as per Table 1.

The first removal of dead eggs from the incubation trays, some eight hours after fertilization, revealed that SCB Fisheries production eggs had a normal level of mortality with some 300 to 500

Table 1. Incubation performance of experimental groups of all-female diploid and triploid Atlantic salmon.

Ploidy	Triploid				Diploid				
Hormone treatment							MDHT (bath)	MT (bath)	MT (feed)
Number of eggs planted	15020	15020	15020	7510	15020	15020	15020	15020	8261
Initial egg mortality	440	517	533	546	896	774	673	362	457
Survival to addling (%)	75.0	85.0	85.0	80.0	85.0	95.0	95.0	100.0	90.9
Survival to hatch (%)	42.9	57.2	54.9	60.6	72.1	81.3	81.9	84.8	74.0
Mean egg to alevin survival (%)	53.0				79.3				
Early rearing mortality (%)	28				7		7	6	18

dead eggs per Heath incubator tray. The all-female triploids had a similar level of mortality. The all-female diploids had a higher level of mortality as reflected in Table 1. Overall egg-to-alevin survival was lower for the pressure treated groups (53%) than for the all-female diploids (79%). Egg-to-alevin survival for the mixed-sex production line of Saint John River strain salmon was similar (78%) to that for the all-female diploids. Survival of triploid eggs was lower. First feeding mortality for triploid alevins was 10% more than for the worst of the diploid groups. Mean weight of alevins at the time of ponding for first-feeding was similar for all groups and ranged from 0.17 to 0.19 g.

Flow cytometry confirmed that pressure shocking was successful in achieving triploidy. All of the blood samples, for which there were sufficient erythrocytes in the sample, were in fact triploid. Unfortunately, not all of the blood samples obtained from the 1995 year class of fry contained sufficient erythrocytes for quantification of the incidence of triploidy. Measurement of erythrocyte nuclei, being generally ovoid in shape, were on average 18% longer on the major axis and 22.5% longer on the minor axis for triploid cells relative to diploid cells (Fig. 4).

Discussion

SCB Fisheries Limited, in a cooperative venture with the Newfoundland Region of the Department of Fisheries and Oceans during the past six years, has been working towards development of an all-female line of Atlantic salmon. This report documents initial efforts to produce reproductively incapacitated salmonids for use by the aquaculture industry in Newfoundland. The work undertaken to date is not intended as research but rather as technology transfer, demonstration and adaptation. In this context, experimental protocols have been adopted as per advice of leading experts in this field, especially Dr. Tillmann Benfey of the University of New Brunswick. Newfoundland experimentation is motivated also by recent events in the British Columbia salmon farming industry where the Ministry of Agriculture, Fisheries and Food has directed that all Atlantic salmon grown under aquaculture conditions in B.C. waters must be from totally female lines by 1998. Industry and researchers in British Columbia now are attempting to comply with this directive.

Experiments currently under way at the Bay d'Espoir hatchery have provided two groups of juvenile salmon that will be applied towards a rational decision process for Newfoundland industry planning; one group that is all-female diploid and will be used for future breeding purposes, and one that is expected to be all-female triploid for comparative performance evaluation and potential marketing.

Progress towards establishing an all-female line of salmon has been slow. The process started in 1989 with a mixed-sex strain of Saint John River origin salmon. Efforts to obtain viable homogametic milt have been proceeding for the past three years. Results of the present trials are illustrative of the experience of the previous two stripping seasons. In support of this project, homogametic milt was obtained in 1994 from seven of 21 fish dissected. While these results are confounded by desynchronization between hatchery and marine-cage fish, this low incidence of viable gonads suggests that

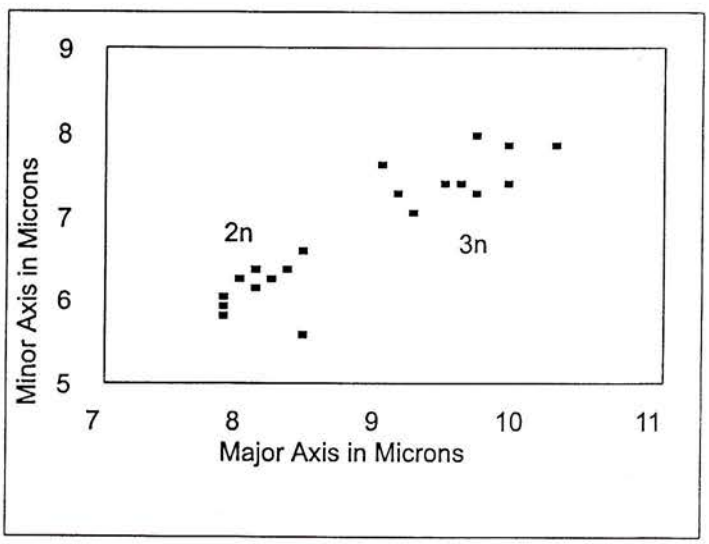


Figure 4. Atlantic salmon erythrocyte nucleus size distribution.

the scope of a program to develop all-female lines, to support a reasonable breeding program (i.e., effective breeding population of at least 100 individuals), would require a larger hatchery initiative than has been possible in Newfoundland to date. However, now that the initiative has progressed to the point of actually providing access to an all-female strain, there is a potential gain in economies of hatchery space in not having to deal with useless genotypic males. Present experience is that, relative to normal hatchery operations, there is little additional effort required to apply hormone treatments for sex-reversal, especially using the immersion technique.

Desynchronization of maturation between sex-reversed and normal females has been noted elsewhere.[16] The observation of minimal spermatozoa motility from sex-reversed fish in these Newfoundland experiments is cause for concern. This, together with the observation that the sex-reversed control group (no GnRH-A treatment) did not spermiate, suggests that the hatchery tank environment does not lend itself to proper reproductive synchronization with brood stock in the marine cages. Though preserved samples of zygotes confirmed that fertilization did take place, it is apparent that the all-female line of eggs, both diploid and triploid, was unusually sensitive. Improved motility of spermatozoa after storage in a sperm extender solution was noted also by Stoss.[17] Though a high incidence of fertilization was achieved with the 1995 year class at Bay d'Espoir, the quality of the resulting zygotes is questionable. There was a considerable incidence of egg mortality during initial egg picking just eight hours after fertilization. This is consistent with the observations of Disney et al.[14] who noted impaired survival of embryos after egg fertilization with extended sperm. The mixed-sex zygotes did not appear to have the same sensitivity to agitation at the eight-hour, post-fertilization picking.

At present, there is no absolute certainty that all of the presumably sex-reversed line did in fact produce homogametic milt. Obvious genetic males were eliminated to foster the beginnings of an all-female strain. Unfortunately, spermatozoa motility, even after storage in the extender solution, was markedly inferior relative to wild grilse males from Newfoundland salmon enhancement projects. Having undertaken to synchronize final maturation of the sex-reversed hatchery salmon with those in the marine cages by application of GnRH-A, it is apparent there was no obvious difference in spermatozoa motility between the GnRH-A hormone groups (i.e., two different treatments). In light of these observations, the 1993 year-class of sex-reversed fish has been transferred to a marine cage for final maturation in 1995 in parallel with the normal SCB Fisheries Limited brood stock. It is likely that GnRH-A treatment will be required again next November, but with a substantially greater number of fish.

The most recent Newfoundland efforts to maintain a line of salmon from which to procure homogametic milt are based on three different treatments for sex-reversal. Currently there is considerable uncertainty about the effectiveness of any single treatment in the Bay d'Espoir hatchery environment. Confirmation of the results of the different sex-reversal procedures will greatly simplify future work to maintain an all-female line for an Atlantic salmon aquaculture breeding program.

The original hypothesis for these experiments is one of no difference in performance of all-female diploid and all-female triploid Atlantic salmon under aquaculture conditions in Bay d'Espoir. The assumption is that the 10% grilsing rate of the present Saint John River aquaculture strain will be adequately addressed by simply switching to an all-female line of production fish. If this null hypothesis cannot be rejected, future industry interest in triploid Atlantic salmon will be minimal. However, this does not obviate the fact that potential egg deposition and introgression with wild populations of all-female aquaculture diploids may pose a greater threat than mixed-sex diploids. An all-female aquaculture strain of Atlantic salmon, were it to mingle with a wild salmon spawning escapement, would greatly bias the sex ratio and further erode effective spawning population of the wild stock. In addition to these concerns, there is considerable incentive to evaluate reproductive suppression technology in the context that it may have application if transgenic salmon being evaluated elsewhere impact on the Newfoundland salmon farming industry position in the international marketplace.

Bay d'Espoir salmon farming industry investors are aware of potential benefits of brood stock development and the application of technology for reproductive control. Benfey[18] made reference to decreased aggressiveness among triploid salmon and indicated that

triploids have decreased cell numbers in the brain and sensory systems. This should be of advantage in marine cages where aquaculture species must be docile and highly tolerant of human intervention in the daily activities of the fish. There is intuitive interest in the triploidy approach but this must be tempered by economic considerations and objective demonstration of how this technology applies under the specific conditions of the Bay d'Espoir estuary. There is a perception[4,19] that triploid salmon performance may not be adequate in marginal culture conditions. There is no certainty as to what this means in the context of the Bay d'Espoir estuary. The oceanography of the Bay d'Espoir fjord is unique in Newfoundland and requires that industry conduct evaluations of new strategies in a cautious and systematic fashion in order to secure ongoing industry viability.

The pressure chamber described in this paper was developed as a result of cooperative research and development efforts across a number of sectors. The government/industry partnership that has been pursued throughout this work was implemented to provide convincing alternative positions on how to deal with biological uncertainties of salmon culture within the constraints of business necessities. The prototype pressure unit has been used successfully in Newfoundland and is a demonstration that cooperative initiatives towards industrial development can be effective in resolving common problems that often are viewed from different and at times opposing perspectives. Since there are economic advantages to using non-reproductive salmonids for aquaculture operations,[20] and their use constitutes progress towards developing environmentally friendly salmonid stocks for aquaculture application, both industry and government have much to gain by wide scale use of triploid salmonids. While the cost of a pressure vessel may be too great for the individual fish farmer, it is well within the realm of investment among several fish farms, of academic institutions and of vested interest aquaculture associations.

We wish to express our appreciation for funding from the Industry Development and Aquaculture Science components of the Atlantic Fisheries Adjustment Program. This support was critical for fabrication of the prototype pressure vessel in 1991 and for implementation of the experiments undertaken in 1994. We also gratefully acknowledge the collaboration of Mr. Anthony Duarte, Engineering Department, Memorial University of Newfoundland, for modifications to the prototype pressure vessel to improve and simplify operational and safety features of the system. Dr. L.W. Crim, Director of the Ocean Sciences Centre (Memorial University of Newfoundland) prepared and administered the GnRH-A preparation. Bing Au, MUN School of Medicine, conducted the flow cytometry analyses of blood samples.

Notes and references

1. Department of Fisheries & Oceans, Science Branch, P.O. Box 5667, St. John's, Newfoundland A1C 5X1.
2. Fisheries & Aquaculture Services, Site 3, P.O. Box 19, Appleton, Newfoundland A0G 2K0.
3. S.C.B. Fisheries Limited, P.O. Box 400, St. Alban's, Bay d'Espoir, Newfoundland A0H 2E0.
4. Pepper VA [ed.]. 1991. *Can. Tech. Rep. Fish. Aquat. Sci.* 1789:vi + 152p.
5. Cloud JG, Thorgaard GH. 1991. *Genetic Conservation of Salmonid Fishes.* NATO Advanced Science Institutes Series. Plenum Press, New York. 314 pp.
6. Anon. 1994. *Amendments to the protocols for the introduction and transfer of salmonids. North Atlantic Salmon Conservation Organization/North American Commission.* NAC(94) 14, 27p.
7. Jungalwalla PJ. 1991. *Can. Tech. Rep. Fish. Aquat. Sci.* 1789:47-71.
8. Aksnes A, Gjerde B, Roald SO. 1986. *Aquaculture* 53: 7-20.
9. Benfey TJ, Bosa PG, Richardson NL, Donaldson EM. 1988. *Aquacult. Eng.* 7:147-154.
10. Sutterlin AM, Collier C. 1991. *Can. Tech. Rep. Fish. Aquat. Sci.* 1789:15-33.
12. Benfey, T. University of New Brunswick, personal communication.
13. Pepper VA [ed.]. 1991. *Can. Ind. Rep. Fish. Aquat. Sci.* 208:v + 26p.
14. Disney JE, Johnson KR, Thorgaard GH. 1987. *J. Exp. Zool.* 244:151-158.
15. Piferrer F, Baker IJ, Donaldson EM. 1993. *Gen. Comp. Endocrinol.* 91:59-65.
16. Boulanger Y. Pisciculture des Alleghanys Inc., personal communication.
17. Stoss J. 1983. In, *Fish Physiology* (WS Hoar, DJ Randall, EM Donaldson, eds.), Vol. IX, Part A. Academic Press, New York, p. 305-350.
18. Benfey T.J. 1991. *Can. Tech. Rep. Fish. Aquat. Sci.* 1789:73-80.
19. Graham MS, Fletcher GL, Benfey TJ. 1985. *Aquaculture* 50:133-139.
20. Boulanger Y. 1991. *Can. Tech. Rep. Fish. Aquat. Sci.* 1789:111-121.

Seawater performance of triploid Atlantic salmon in New Brunswick aquaculture

S.A. McGeachy,[1,2] F.M. O'Flynn,[1] T.J. Benfey[2] and G.W. Friars [1]

Two mixed-sex populations of pedigreed triploid Atlantic salmon were reared to market size in consecutive years. Performance comparisons with communally reared diploid contemporaries were made in terms of round (total) weight, dressed (gutted) weight, survival and number of deformities. At the time of seawater transfer, smolt sizes were similar for both ploidy groups. However, triploids outweighed diploids at market both before and after gutting. These weight differences were not significant ($P > 0.05$). Gutting losses were similar regardless of ploidy and were found to be approximately 5% the first year and 8% the following year. Any apparent advantage in market size for the triploids was offset by the survival rate and number of deformities. Seawater survival was 66% and 60% for the triploids in consecutive years. This was significantly lower ($P < 0.05$) than the 82% and 81% survival rates recorded for the corresponding diploids. The number of deformities was significantly higher ($P < 0.05$) for triploids than diploids. In particular, jaw deformities were found almost exclusively in triploids. Consequently, the seawater performance of triploid Atlantic salmon is currently considered unacceptable.

Introduction

Salmon farming in New Brunswick, Canada, is a 100 million dollar industry and represents the single largest food commodity produced in the province based on sales value of production.[3] The rapid expansion of this industry has led to concerns regarding the impact of cultured salmon on wild fish stocks. Reports of aquacultural escapees entering local rivers in relatively high numbers have been made by J.W. Carr, Atlantic Salmon Federation.[4] In an attempt to minimize the potential for genetic interactions between wild and cultured populations, non-reproductive salmon may be used by the industry.

The technology for producing sterile fish on a commercial level exists and is achieved through the induction of triploidy. Commercial-sized hydrostatic pressure vessels are currently being manufactured by TRC Hydraulics in Moncton, New Brunswick. These vessels have been highly successful in producing large quantities of triploid Atlantic salmon. However, commercial performance evaluations must be made in order to understand the impact that this technology will have on the salmon industry.

The freshwater performance of triploid Atlantic salmon has been found to be commercially acceptable in New Brunswick.[5] This is in agreement with reports from Scotland,[6] Tasmania[7] and the United States.[8] However, both freshwater and seawater stages of the production cycle must be evaluated before this technology will be adopted by the industry. In this study, performance of triploids was evaluated at sea under local conditions in the Bay of Fundy. Comparisons between diploid and triploid Atlantic salmon were made in terms of round

weight, dressed weight, survival and number of deformities after approximately 20 months of seawater rearing.

Materials and methods

Fish stocks and rearing conditions

Two pedigreed strains of mixed-sex diploid and triploid Atlantic salmon were evaluated in consecutive years. Smolts were obtained from the Atlantic Salmon Federation's Salmon Genetics Research Program in St. Andrews, New Brunswick. Triploids had been produced using hydrostatic pressure treatments.[5] The seawater facility was provided by the Atlantic Salmon Demonstration and Development Farm, in Lime Kiln Bay, New Brunswick.

In 1991, 40 diploid and 40 triploid smolts, from each of 10 families, were transferred to one 864 m^3 steel cage (400 of each ploidy group in total). Both the family and ploidy information were retained by using identifying brands and fin clips. An additional 2,400 unrelated diploid smolts were also transferred to the cage. Therefore the population consisted of approximately 12% triploids, 13% corresponding diploids, and 75% unrelated diploids. These unrelated diploids were not used in any analyses. Fish from this year-class will be referred to as Strain A.

A similar experimental design was used the following year. In 1992, 20 diploids and 25 triploids, from each of 20 families, were transferred to one 864 m^3 steel cage (400 diploid and 500 triploid in total). Again unrelated diploids were added to the cage so that the population consisted of approximately 20% triploids, 16% corresponding diploids, and 64% unrelated diploids. Fish from this year-class will be referred to as Strain B.

Data collection and analysis

Fish from Strains A and B were reared for 17 and 19 months, respectively, at which time broodstock selection data were collected. This involved taking weight and length data from every fish and then individually marking them with wing tags. Based on these data, broodstock were identified. Once this information was available (approximately 7 to 10 days later) selected broodstock were set aside while those fish destined for processing were returned to the cage. However, in order to collect weight data from market fish, a sample of triploids and corresponding diploids

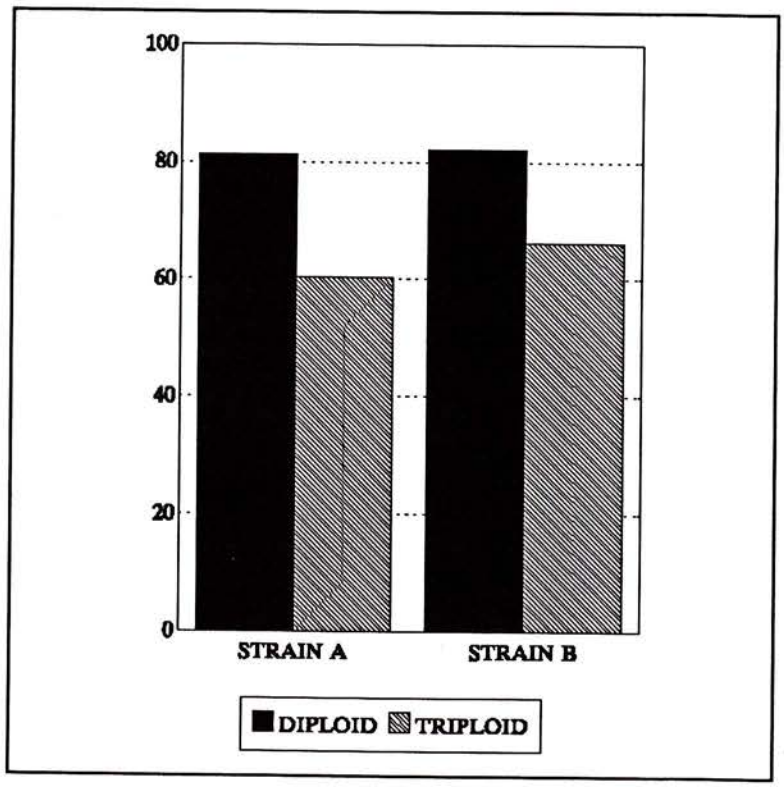


Figure 1. Percentage of diploid and triploid Atlantic salmon from two pedigreed strains that survived between the time of seawater transfer and broodstock selection (17 and 19 months for Strains A and B, respectively).

were also set aside. These market fish were reared for an additional two months in a 216 m^3 wooden cage prior to processing. All of the 292 surviving triploids from Strain A were set aside along with 100 (10 fish from each family) corresponding diploids. For Strain B, 120 fish (6 from each family) of each ploidy type were set aside in this small cage.

Survival was calculated from initial transfer to seawater until the broodstock selection process and was based on manual counts. Since conditions in the small wooden cage and the excessive handling during the selection process do not reflect those normally found in a commercial operation, survival was not considered during the final two months of rearing. All survival data were arcsine transformed prior to analysis. An analysis of variance was performed in order to determine any differences in survival.

At the time of processing, round and dressed weights were recorded along with any deformities. Weight data were log transformed prior to analysis. Again an analysis of variance was used to make comparisons between the two ploidy groups. The model used in the analysis of both survival and weight data included the effects due to strain, family and ploidy difference.

Differences in the number of deformed diploid or triploid fish were compared using a t-test. All statistical analyses were conducted using SAS version 6.04 (SAS Institute Inc., Cary, N.C.). A 95% confidence interval was used throughout this investigation.

Results and discussion

At the time of seawater transfer, diploid and triploid smolts were similar in size and appearance. Fish from both ploidy groups appeared to adapt to the seawater environment as there were minimal mortalities reported during the first 48 hours following transfer. However, by the time of the broodstock selection (17 and 19 months for strains A and B respectively) survival for triploids was almost 20% lower than the diploids (Fig. 1). This difference was found to be significant and no strain or family effects on survival were detected. These results are similar to those reported from France.[9] However, reports from Tasmania indicate that triploid Atlantic salmon fail to adapt to seawater resulting in exceptionally high numbers of "pinhead" salmon.[7] High mortality of triploids following seawater transfer has also been reported by Quillet and Gaignon,[10] but survival for the diploids was

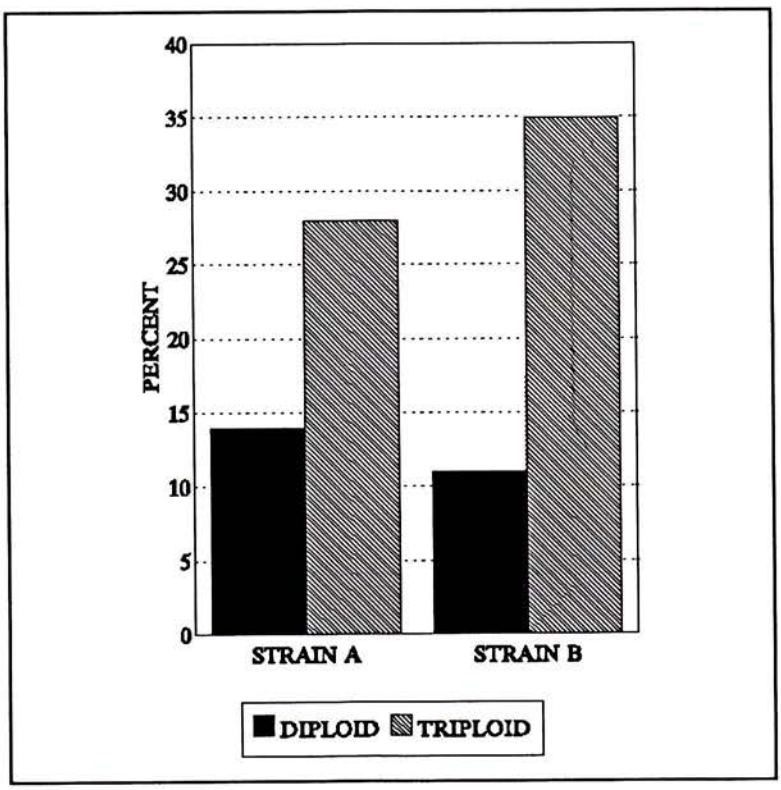


Figure 2. Percentage of diploid and triploid Atlantic salmon from two pedigreed strains with appearance problems that would reduce their market value.

also quite poor. Several reports indicate that triploids do not survive as well as diploids if environmental conditions are unfavourable.[7,10,11] This is most likely due to the lower oxygen carrying capacity of triploids; although they may be able to compensate during acute stress,[12] this mechanism may fail during times of chronic stress.[11] Those triploids that survived until market were found to have a significantly higher number of deformities than the diploids (Fig. 2). In particular, jaw deformities were found almost exclusively in triploids (Fig. 3). Although the cause of this deformity is still unknown, there have been speculations that it may be related to vitamin C deficiencies[13] and/or to the faster growth rate of triploids.[14] More work is needed to determine the cause of the jaw deformity before an attempt can be made to alleviate the problem. The poor appearance of triploid salmon will result in lower prices at market.

Despite these drawbacks, the growth of triploids should be commercially acceptable. Triploids weighed more than their diploid counterparts both before and after gutting (Fig. 4). This difference was not found to be statistically significant ($P > 0.05$). However, differences between the strains and among families within the strains were detected. These differences are not surprising since growth differences among families and strains form the genetic basis of selection. Gutting losses were similar for both ploidy groups and found to be approximately 5% for Strain A and 8% for Strain B. Reports on Atlantic salmon have typically found triploids to weigh less than their diploid counterparts.[6,9,10]

It should be stressed when interpreting these data that the triploids were reared communally with the diploids. It has been suggested that triploids are at a disadvantage when reared communally with diploids.[8,15,16] Perhaps seawater performance could be improved by rearing triploids and diploids separately.

Conclusions

Triploids grown to market size in seawater were inferior to diploids in terms of survival and appearance. Consequently, triploid Atlantic salmon are not considered commercially acceptable at present. However, the growth performance of these fish does appear to be acceptable. Those fish that survive to market are of a similar or slightly larger weight than their diploid counterparts and product quality remains high. As such, triploid technology may be considered by the New Bruns-

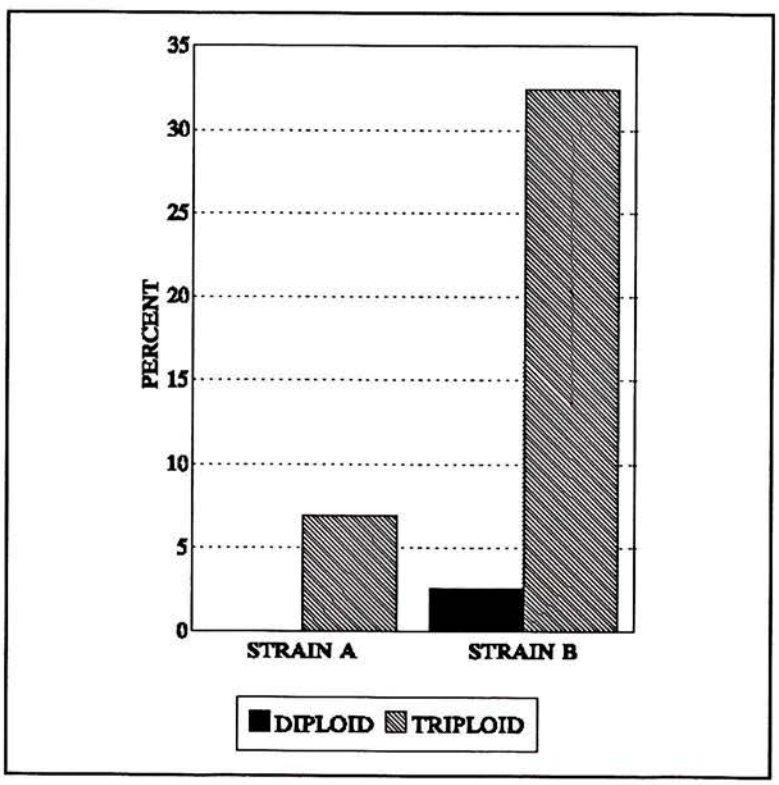


Figure 3. Percentage of diploid and triploid Atlantic salmon from two pedigreed strains that had jaw deformities.

wick industry if improvements in survival and appearance can be made.

Notes and references

1. Atlantic Salmon Federation, P.O. Box 429, St. Andrews, New Brunswick E0G 2X0
2. Department of Biology, University of New Brunswick, Fredericton, New Brunswick E3B 6E1
3. Anonymous. 1994. *New Brunswick Aquaculture Review.* New Brunswick Department of Fisheries and Aquaculture, Fredericton, New Brunswick, 31 pp.
4. Carr JK. 1995. *The 1994-95 Annual Report of the Atlantic Salmon Federation.* p.16-17. Atlantic Salmon Federation, St. Andrews.
5. McGeachy SA, Benfey TJ, Friars GW. 1995. *Aquaculture* 137:333-341.
6. Johnstone R. McLay HA, Walsingham MV. 1991. *Can. Tech. Rep. Fish. Aquat. Sci.* 1789:15-36.
7. Jungalwalla PJ. 1991. *Can. Tech. Rep. Fish. Aquat. Sci.* 1789:47-71.
8. Galbreath PF. St. Jean W, Anderson V, Thorgaard GH. 1994. *Aquaculture* 128:41-49.
9. Boeuf G, Seddiki H, LeRoux A, Severe A, LeBail P. 1994. *Aquaculture* 121:300 (abstract only).
10. Quillet E, Gaignon JL. 1990. *Aquaculture* 89:351-364.
11. Ojolick EJ, Cusack R, Benfey TJ, Kerr SR. 1995. *Aquaculture* 131:177-187.
12. King HR, Lee P. 1993. In, *Seeking & Solving, Papers from SALTAS Research & Development Review Seminar 1993*, p. 37-44. Hobart, Tasmania.
13. Hughes D. 1992. In, *Final report. Barriers & Breakthroughs — Papers from the SALTAS 1992 Research & Development Review Seminars,* p. 17-32. Hobart, Tasmania.
14. Lee P, King HR. 1994. In, *Reports from the SALTAS 1993-94 Research and development programme,* p. 61-69. Hobart, Tasmania.
15. Cassani JR, Caton WE. 1986. *Prog. Fish-Cult.* 48:184-187.
16. Lincoln RF, Bye VJ. 1987. In, *Proceedings of the Third International Symposium on the Reproductive Physiology of Fish* (DR Idler, LW Crim, JM Walsh, eds.), p. 134. Memorial University of Newfoundland, St. John's.

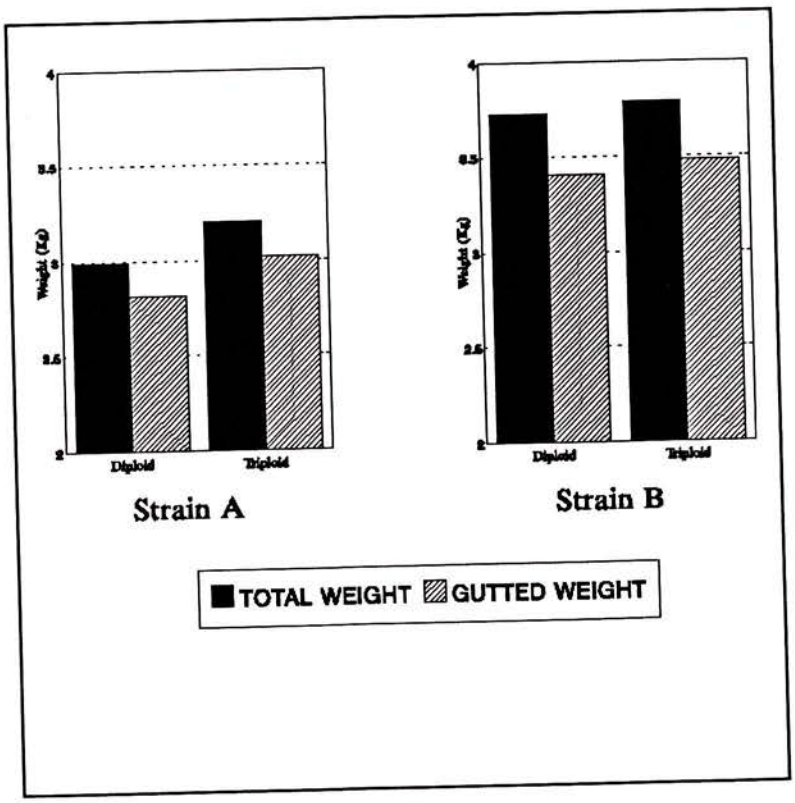


Figure 4. Market weight (total and gutted) of diploid and triploid Atlantic salmon after 19 (Strain A) and 21 (Strain B) months in seawater.

This research has been supported by the National Research Council, the Atlantic Canada Opportunities Agency, the Atlantic Fisheries Adjustment Program, the New Brunswick Salmon Growers' Association, the Department of Fisheries and Oceans, the Natural Sciences and Engineering Research Council, The University of New Brunswick and the Atlantic Salmon Federation.

Triploidy: a commercial application in Canada

Robin Stuart

The performance of all-female triploids is influenced by many factors that are not well understood. Additional work is required to define the environmental tolerances to temperature, salinity, pH, and alkalinity. Triploids respond differently to their environment than do normal fish and may not be suitable for every farm. Triploids are particularly susceptible to stress and good husbandry is critical to their success. The industry would like to see proof of the negative effects of escaped fish and their influence on wild stocks prior to banning the use of diploids.

Introduction

Rainbow trout have been cultured on a commercial scale in marine net-pens in Cape Breton since the mid-1970s. But it was not until the early 1980s that an attempt was made to grow large sea-reared trout instead of smaller pan-sized fish. The growth and survival of these large trout were encouraging, and the fish were very similar to Atlantic salmon in taste and appearance.

One major obstacle that kept appearing each year in early October was the extremely rapid and blatant onset of sexual maturation. The males, especially, seemed to change overnight from a silvery hue to black, ugly, kipe-ridden bucks. This severely hampered the marketability of the product, as the external appearance of the fish deteriorated as both fat levels and pigmentation declined.

Several measures were employed to counteract this problem, including marketing prior to maturation, reconditioning mature fish over winter, filleting fish, and using different genetic stocks. This problem also occurred in salmon grown throughout the 1980s. It was originally felt that the high water temperatures of the Bras d'Or lakes accelerated maturation, but early onset of maturation was a problem even in areas with lower temperature regimes.

During this time I was following the development of techniques for inducing sterility, but they were either commercially impractical or illegal, such as the use of androgen steroids in fish being sold for meat. In the mid-1980s, Dr. Arnold Sutterlin co-authored a paper on the application of heat shock and pressure methods for inducing triploidy in salmonids. This work spearheaded the use of triploids as a tool for salmonid farmers.

Loch Bras d'Or Salmon Farms Ltd. and Nova Aqua, a Norwegian company, conducted trials in the late 1980s on heat shocking salmon and trout eggs. The work was abandoned because of the discouraging results — the growth and survival of triploid fish were far inferior to diploids.

Since 1989, however, SCB Fisheries in Bay d'Espoir, Newfoundland, has used triploid trout. In this instance there was little choice, as government regulations prevented the introduction of diploids because of fear of introducing a threatening exotic species to the province. Once again, Dr. Sutterlin conducted the initial trials with a significant measure of success. Although the salinity and temperature profiles at Bay d'Espoir differed somewhat from Cape Breton, they were similar enough to warrant further work. I also felt that perhaps the use of heat shock, instead of the pressure shock used in earlier studies, may have been an important factor in their success.

In March of 1993, 200,000 all-female triploid eyed eggs were purchased from Pisciculture des Alleghanys in Quebec. By October the fingerlings were 40-50 g. Of these, 75,000 were

shipped to a marine wintering site in Whycocomagh and the remainder were kept in the hatchery.

Whycocomagh site

Fish at Whycocomagh were overwintered under the ice with very little attention. Temperatures went as low as – 0.4°C in January of 1994, but later stabilized at + 0.4°C until March. During the last week of March, the site was struck by a major algal bloom that killed 200,000 salmon of all sizes from 1 kg to 3 kg. The triploid all-female trout were the only group of fish that did not suffer heavy casualties — they moved to the deeper part of the cage and escaped the algae at the surface.

The triploid all-females grew well in the summer of 1994 with few mortalities. They increased in size from 59 g in March to 1 kg by November, with the loss of only 1.7% of the stock. A low incidence of water belly syndrome, in which the stomach fills with salt water, was evident during the warmest period of the summer, but there were few mortalities associated with this phenomenon.

In January and February of 1995, water bellies became prevalent among fish in all the cages. Divers removed many mortalities (14.5% of the remaining fish), all of which had water in the stomach and the air bladder. It was felt that a sudden change in temperature at these low temperatures (< 0.5°C) may have triggered osmoregulatory failure, resulting in water belly. Once the fish were removed from their feed and the temperature increased in March to 2.3°C, the process reversed and the incidence of water belly declined to very low levels.

Upweller pumps were used in this second winter and this resulted in elevated temperatures and salinities in the net-pens, eliminating the ice cover. By April of 1995, 60,616 (80.8%) of the initial 75,000 fish remained, with an average weight of 1.1 kg.

Small triploid trout introduced to the site in November of 1994 experienced high mortalities within one week of transfer. These same fish experienced mortalities in the hatchery two weeks prior to shipping. No pathogens were found and the losses were attributed to physiological problems associated with a rapid drop in temperature.

Seal Island site

In the spring of 1994, 65,550 all-female triploids remaining at the hatchery from the initial purchase in 1993 were moved to a summer marine net-pen site at Seal Island. Their performance at this site was remarkable. Growth was comparable to diploids even through the warm water period (20°C). There was no reduction in growth rates in September and October, when growth is usually reduced in diploids due to maturation. The fish grew from 65 g to 1.27 kg in seven months (May to November) — a growth rate as good or better than any previous growth of diploids at this site — with 94.9% survival.

There was good resistance to *Vibrio anguillarum* by the triploids. The losses of salmon smolts were 5% higher than the losses incurred by the triploid trout. At peak high temperatures water belly syndrome was again encountered and about 3% of the population was affected during the worst period. Reducing the amount of feed seemed to help and the problem diminished once temperatures dropped below 20°C; however, even in the autumn it did not completely disappear.

Triploid trout could not take the rigours of grading in the autumn as well as the salmon could. Mortalities of 1.5% of the stock forced us to stop this procedure.

Lingan site

In December of 1994, all-female triploid trout were introduced from Seal Island to the land-based marine farm at Lingan, which utilizes cooling water from a power generator to maintain temperatures at 10-11°C all winter. This was the first time that triploids were exposed to full marine salinity. The fish were transferred to Lingan in early December at an average size of 1 kg. They were moved by tanker truck from Seal Island after being pumped from the cages by a Transvac™ fish pump.

These fish still displayed some water belly when they were moved from Seal Island. Three per cent of the fish died in the first month after transfer, all due to water belly. Initial growth rates were poor and this was attributed to very high stocking densities (> 20 kg/m^3). The growth of salmon was also poor at this time, due to high levels of suspended solids in the water from storm activity. Once fish densities were

reduced, growth rates improved. The average size of the triploids was 1.5 kg by March, with a total mortality of 6.1%. When the fish were marketed, virtually none were the second grade fish normally experienced with diploids due to maturation.

Several hundred of these triploid fish were harvested for a study conducted by the Technical University of Nova Scotia to evaluate shelf life and quality when various freezing techniques were used. The results indicated a superlative product in both taste and appearance.

Glace Bay Hatchery

Survival to the fry stage at the Glace Bay Hatchery of the all-female triploids purchased in 1993 was about 65%. Their growth was comparable to that of diploids, but their survival was lower. In May of 1994 another 200,000 eyed eggs were purchased from the same source with similar results, although the survival in the second batch was 20% higher than in the first year. In March of 1995, 200,000 eggs were again purchased, but this time the result was high mortality (90%) during hatching. There was no clearly defined cause of death, although water pH of 6.2 may have been a contributing factor —triploids are extremely sensitive to pH below 6.5. There is also some indication that water hardness and alkalinity may have played a role in mortality. These are environmental parameters for which limits have yet to be defined for these fish.

Pisciculture des Alleghanys replaced many of these fish free of charge in 1995. The group of eggs may have experienced rough transit and this was reflected in the heavy mortalities during hatch. Hatchery staff have consistently found that triploid fish do not tolerate handling nearly as well as diploids and that the best results occur in fish that experience the least handling. Even fungal treatment routinely used on other eggs causes excessive mortality in triploids.

Pubnico site

The first all-female triploid trout sold to a full marine net-pen site went to a site in Pubnico. Fish were removed from Whycocomagh in May when water temperatures were in the 7-12°C range and salinity was 18 ppt. Fish averaged 1 kg in size and were pumped by Transvac™ pump to oxygenated tankers. The temperature and salinity at Pubnico were 6°C and 32 ppt.

Fish were taken off feed 5 days prior to shipment. The trip by tanker took about 9 hours and, for the most part, oxygen levels were kept at 10-11 mg/L. Normal mortalities, in the 5-6% range, were experienced. There was one anomaly that resulted in a supersaturation problem as the oxygen level soared to 25 mg/L over an hour. The result was burst corneas and hemorrhaging throughout the body cavity, with 74% mortality in this load of fish. Mortalities ceased after ten days at the new site. Such a mortality would not have occurred with salmon. Hyperactivity is the usual reaction to such elevated oxygen levels, but this was not the case with the triploids.

Summary

- Performance of all-female triploid trout is dependent on many environmental factors and may differ from site to site.
- Triploidy is only one tool in fish farm management. Due to site differences it may not be suitable for all farms. It does not eliminate the need for a broodstock development program.
- There are still many unknown factors affecting the performance of triploids; therefore legislation of their use would be premature.
- The industry would like to see proof of the negative genetic effects of escaped fish and their influence on wild stocks prior to any banning of the use of diploids.
- Further work is needed to define the environmental limits on performance of all-female triploids (temperature, pH, salinity, alkalinity, etc.).
- All-female triploids are more susceptible to all forms of stress imposed through normal commercial fish farm practices. Good husbandry is more vital for the success of triploids than diploids.
- The cost of triploids, including egg price and higher mortality, has to be weighed against the benefits of zero maturation.

Robin Stuart is with Eskasoni Fisheries Ltd., P.O. Box 186, New Waterford, Nova Scotia, B1H 4K4.

Position of the British Columbia Ministry of Environment, Lands and Parks on the use of non-reproductive Atlantic salmon

Bryan Ludwig [1]

In April 1995 the British Columbia Ministries of Environment, Lands and Parks (MELP) and Agriculture, Fisheries and Food (MAFF) announced an Action Plan for provincial salmon aquaculture that involves a move toward the use of non-reproductive Atlantic salmon by 1998. Implementation of the policy to mandate use of all-female Atlantics depends on an assessment of the results of additional reseach and monitoring to determine whether escaped Atlantics pose an ecological threat, an analysis of the industry's economic competitiveness, and the outcome of an environmental assessment. Although requiring the use of non-reproductive Atlantic salmon by 1998 does not provide immediate protection for wild stocks, it is the earliest date that broodstock can be developed and it lessens the impact on the industry.

Introduction

On April 13, 1995 the British Columbia Ministry of Environment, Lands and Parks (MELP) and Agriculture, Fisheries and Food (MAFF) announced the establishment of an action plan for provincial salmon aquaculture. This plan was the culmination of months of development work involving consultation with stakeholders and negotiation within government and includes:

- An environmental review of salmon farming activities by the Environmental Assessment Office, using the Broughton Archipelago as a study area;
- Development of a provincial salmon aquaculture policy to guide future sitings and operations of fish farms;
- A commitment to improved consultation with industry, environmental groups, fishers, First Nations and local government in development of policy; and,
- A move toward the use of non-reproductive Atlantic salmon by 1998.

This paper will focus on the last point — a move toward use of non-reproductive Atlantic salmon by 1998.

Action plan on non-reproductive salmon

In the action plan, the statement pertaining to non-reproductive Atlantic salmon reads as follows:

> *Government and Industry will conduct the necessary research and technology transfer to ready industry to switch to production of non-reproductive (all-female) Atlantic salmon. The target for this is 1998. Government will review the progress in achieving this target annually and confirm or adjust the date accordingly.*

This work is to include:

- Continuing the baseline monitoring program for escaped Atlantic salmon in the commercial fishery;
- Enhancing the voluntary freshwater monitoring program;

- Conducting a survey of rivers and an assessment of the ecological impacts of Atlantic Salmon (in communication with the Department of Fisheries and Oceans (DFO));
- Notifying all Atlantic salmon licence holders that use of all-female Atlantic salmon by the 1998 brood-year is the government's target;
- Managing the broodstock development and research activities so that they are completed on schedule to allow for full implementation of the policy by 1998.

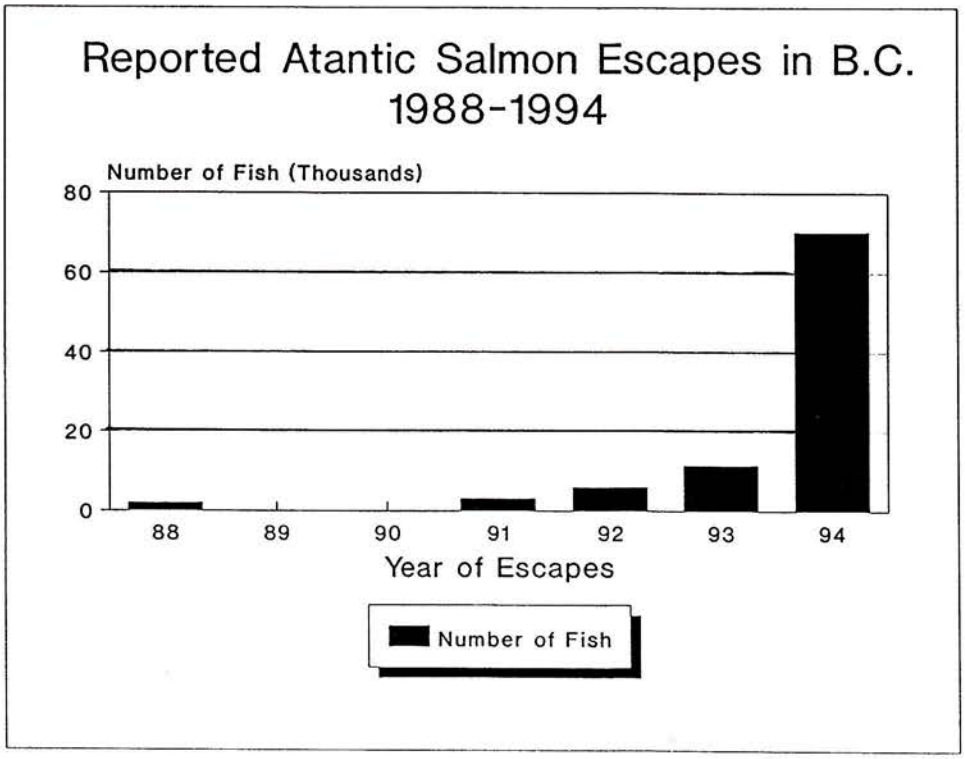
Implementation of the policy to mandate use of all-female Atlantic salmon by 1998 will depend on an assessment of the results of additional research and monitoring to determine whether escaped Atlantics pose an ecological threat, an analysis of the impacts on industry's economic competitiveness, and the outcome of the environmental assessment.

Concerns

Atlantic salmon are an exotic species on the West Coast. Escaped Atlantics were first reported in 1988 (Fig. 1). Since then, there has been an increasing trend in the number of reported escapes and in 1994, close to 70,000 fish were reported. The average size of these fish has varied from smolt size up to 3.3 kg, but many of the escaped fish are two to three kilograms in size. Although the industry is concentrated in Broughton, Campbell River and Clayoquot Sound there have been few reported escapes of Atlantic salmon from Clayoquot Sound (Fig. 2).

Escapes are caused by predators, human error, and storm or tide events. Obviously some of these are preventable, but it is the view of MELP staff that despite the good intentions of industry to prevent escapes, they will continue to occur.

Figure 1



Where are escaped Atlantic salmon found?

The first report of Atlantic salmon caught in the British Columbia commercial fishery was in 1991 when a single fish was reported (Table 1). In 1993, 4,500 Atlantics were caught in the commercial fishery and in 1994, 1,037 fish were reported.[2,3] In 1994, Atlantic salmon were also reported in commercial fisheries in Washington State and Alaska despite the fact that Alaska does not have any commercial culture of Atlantic salmon in the state. Stomach samples collected from over 100 fish in 1994 indicate little evidence of feeding. Of the 70 Atlantic salmon checked for maturity in 1994, 36% of the males and 10% of the females were maturing.

Table 1. Commercial recoveries of Atlantic salmon over the period 1991-1994.

Year	Number of fish reported (by location)		
	Alaska	Washington State	British Columbia
1991	5	n/a	1
1992	1	165	359
1993	23	227	4,529
1994	29	337	1,037

Figure 2. Reported sightings of Atlantic salmon in freshwater streams in British Columbia for the period 1990-1995.

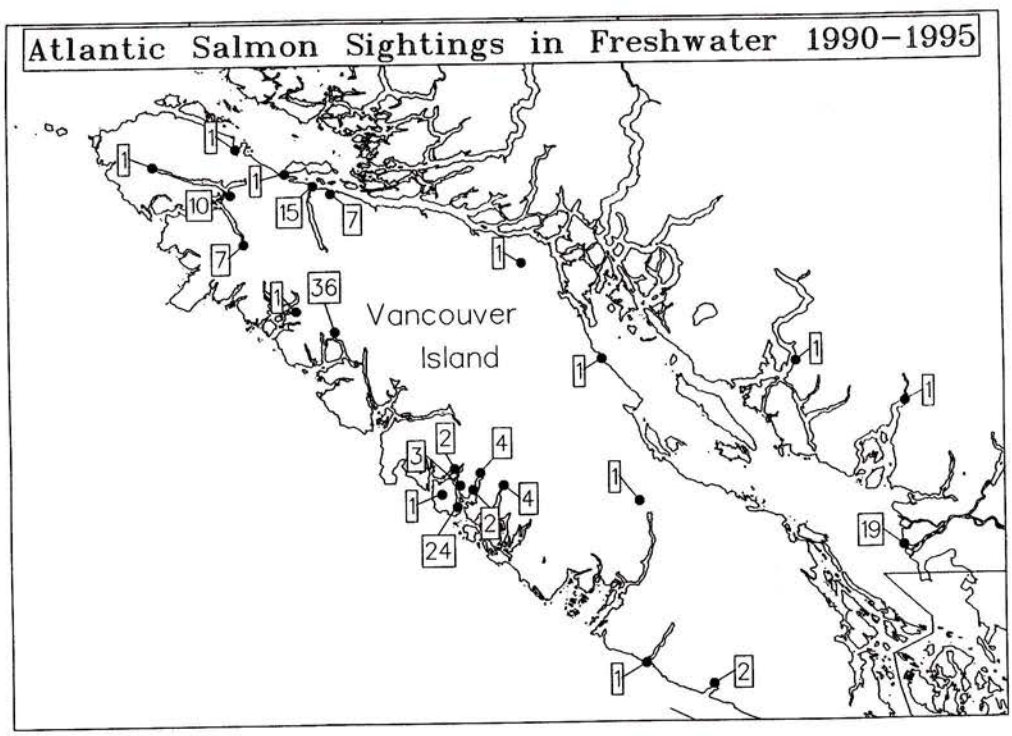


Table 2. Atlantic salmon recovered or sighted in freshwater.

River system	Year 1990	1991	1992	1993	1994	1995*
West Coast Vancouver Island (south)						
Gordon River				2		
Nitinat Lake Narrows				1		
Stamp River					1	
Clayoquot Sound						
Ahousat (Flores Island)			24			
Arnette Creek (Flores Island)				1		
Atleo River				2	1	
Bedwell River				1	3	
Megin River				1	1	
Moyeha River					4	
Small stream in Herbert Arm				2		
Quatsino Sound						
Colonial/Cayeghle River	3	1	2		1	
Goodspeed River			1			
Marble River					10	
Esperanza						
Zeballos River						36
Kaouk River						1
East Coast Vancouver Island (North)						
Cluxewe River				1		
Glenlyon Creek					1	
Kokish River		4		1	2	
Nimpkish River			9		6	
East Coast Vancouver Island (central)						
Oyster River			1			
Roberts River		1				
Mainland Coast						
Fraser River		1	8	9	1	
Harrison Creek			1			
Squamish River			1			
Treat Creek			1			
Totals	3	7	48	21	31	37

*The sightings and capture for 1995 are for up to and including May 14, 1995.

There have been a small number of adult Atlantic salmon recovered in freshwater (Table 2); most of the fish are found in the vicinity of salmon farms. However, since 1991, 19 Atlantics have been found in the Fraser River (Fig. 2). Although some of the Atlantics found in freshwater were maturing, no evidence of spawning (kelts or fry) has been detected.

Why is MELP concerned?

1. Since Atlantics are exotic fish, their behavior in this new environment is unpredictable and thus impacts on wild stocks are not fully understood.

2. Habitat requirements of Atlantic salmon overlap with those of native species.(4) A

recent literature review on competitive interactions between Atlantic salmon and rainbow trout indicates that Atlantics are likely to be outcompeted for the most desirable habitat by both steelhead and coho, but this result may be size dependent.(5) Atlantics may emerge from the gravel two to four weeks earlier than steelhead and thus Atlantic fry would be considerably larger than steelhead fry.(6)

3. Some native stocks of fish are in decline. Vacant habitat is available. Should Atlantic salmon become established, it may be more difficult for wild stocks to recover.

4. It will take some years before the impacts on wild stocks are scientifically determined. One of the risks of waiting is that the effects may be irreversible.

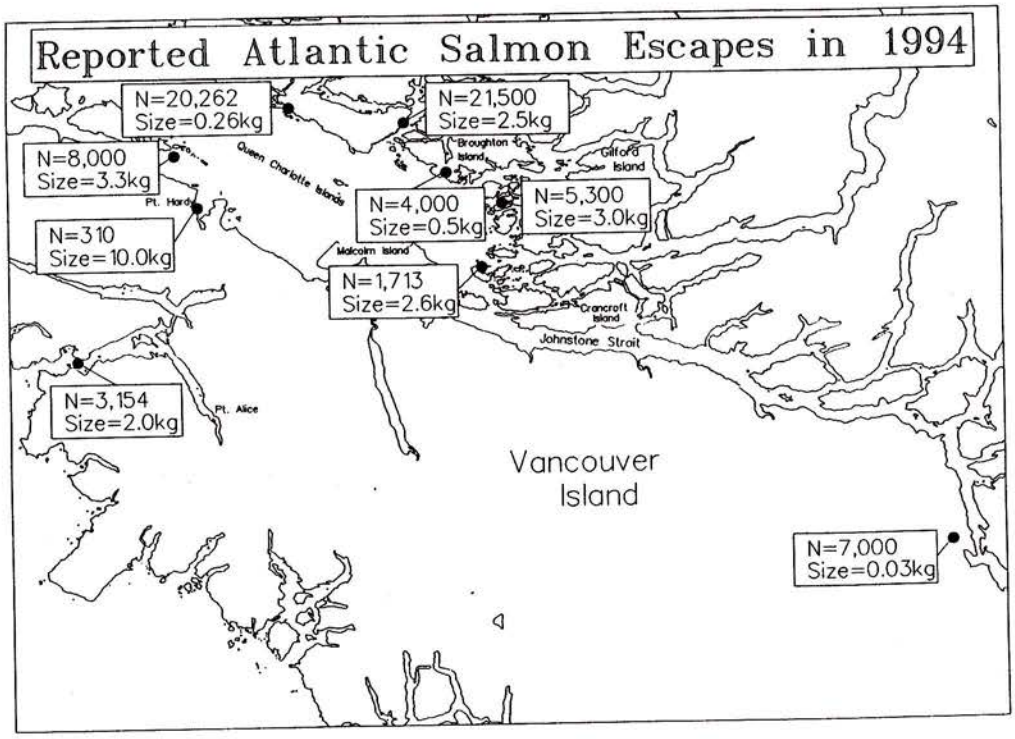
Why worry?

1. Why worry... Atlantics are heavily domesticated and unlikely to survive.

MELP Response: Without much doubt, the survival of domesticated Atlantics will be poorer than their wild counterparts within the native range. The results outside the range are less predictable. The fish obviously are capable of making a long migration to Alaska. Even though domesticated fish will not likely survive as well, there will still be some measure of survival.

2. Why worry... In the early 1900s millions of juvenile Atlantics were released into British Columbia and runs did not become established. Fisheries workers have tried to transplant Atlantic salmon and results indicate they do not do well outside their native range.

Figure 3 Reported escapes of Atlantic salmon in British Columbia in 1994.



MELP Response: Comparing the performance of egg, fry or smolt plants with that of 2-3 kg sub-adults is not valid. Even if progeny from these adults did not survive in salt water, there is still a ready supply of adult escapees to have a competitive impact in freshwater. Runs do not necessarily have to become established for there to be an impact.

3. *Why worry ...* If Atlantics were going to survive, we would have seen spawners by now.

MELP Response: Establishing an exotic stock could take numerous introductions before the species takes hold. There are thousands of streams in the province. Looking for a small, developing population is the proverbial needle in a haystack. Conditions that were not right four years ago for Atlantic salmon may be right now. Certainly, in some systems there will be little competition from native stocks.

Solutions

A range of solutions were considered by MELP biologists including restricting industry to use of native stocks, and immediate sterilization of all Atlantic salmon. Although these suggestions would have provided immediate protection for wild stocks, the impact on the industry would have been severe. The solution we were looking for was one that provided strong protection for wild stocks despite the uncertainty of effects, and allowed the industry to continue. Although requiring the use of non-reproductive Atlantic salmon by 1998 does not provide immediate protection for wild stocks, this is the earliest date that broodstock to produce all-females can be developed and the impacts on industry are not as severe.

In July 1994, MELP, MAFF and DFO notified industry of the intention to move toward all-female Atlantic salmon. Government and industry agreed on a schedule for development of the broodstock and set up a research committee to design studies to compare performance of all-female, sterile all-female and normal Atlantics, and to work with industry on developing all-female broodstock.

In case you feel that MELP's approach to resolving the issue of escaped fish is a result of the opinions of a few "misinformed biologists" and a "conservative Minister", as we have read in various media of late, I would like to wrap this up with a short description of our Ministry's philosophy in regards to resource management.

The Ministry's goals and objectives include:

- Protection, conservation and restoration of a full range of biological and physical diversity native to British Columbia, and,
- Clean, healthy and safe land, water and air for all living things.

The primary objective of the Ministry's fisheries program is to conserve and protect wild fish stocks and their habitat.

The Ministry recently published a document on goals and objectives for strategic planning. The document stresses that we are stewards of a public resource and as such we must champion its protection. The Ministry advocates the "precautionary principle" adopted in 1992 in the Rio Declaration which means we have a responsibility to take precautionary measures to anticipate, prevent or minimize adverse effects. This will require a long term view that considers the needs of future generations. The precautionary principle clearly indicates that a lack of full scientific certainty as to impacts is not adequate reason to postpone measures that will protect the resource. This is a fundamental point that should guide all those involved in developing policy for protection of public resources.

In 1998, we should be in a position to weigh the results from monitoring for escaped Atlantics, with the economic analysis of the performance of all-females, and make a decision whether to fully implement the policy.

Notes and references

1. Fisheries Branch, B.C. Ministry of Environment, Lands and Parks, 2nd Floor, 780 Blanshard Street, Victoria, B.C. V8V 1X4.
2. Thomson AJ, McKinnell S. 1994. *Can. Manuscr. Rep. Fish. Aquat. Sci.* 2246: 35 pp.
3. Thomson AJ, McKinnell S. 1995. *Can. Manuscr. Rep. Fish Aquat. Sci.* (in press).
4. Fausch KD. 1988. *Can. J. Fish. Aquat. Sci.* 45:2238-2246.
5. Chiasson AG, Gibson RJ, Bastien-Daigle S. 1990. *Can. Tech. Rep. Fish Aquat. Sci.* 1610:vi + 51p.
6. Ludwig B. Unpublished data.

Non-reproductive Atlantic salmon: cost of industry compliance

David Egan [1]

In 1993 over half the value from the British Columbia salmon farming industry's production was from sales of Atlantic salmon. Potential interactions between wild Pacific salmon and escapees of Atlantic salmon from sea cages have been of considerable concern to resource managers since Atlantic salmon were introduced in the mid-1980s. As a result, the Province of British Columbia is proposing to introduce regulations requiring the production of only non-reproductive salmon by the year 1998. This paper summarizes an industry consultation study that addressed incremental industry costs and potential revenue implications resulting from the development and use of non-reproductive Atlantic salmon. A reporting system to capture industry compliance costs associated with the proposed regulations is outlined, as well as linkages with existing programs.

Purpose and scope of the study

Salmon farming has been a growth industry in British Columbia since 1980 and introduced Atlantic salmon have become the predominant production stock. Concerned about potential interactions between indigenous salmonids and escapees of Atlantic salmon, the Province of British Columbia is proposing to introduce requirements for use of non-reproductive Atlantic salmon by the year 1998 pending further monitoring. Use of all-female stocks would prevent the establishment of Atlantic salmon in British Columbia.

In British Columbia, the technology for producing non-reproductive Atlantic salmon must be adapted from laboratory scale efforts and proven under practical farm conditions. Two options for non-reproductive Atlantic salmon are being investigated by industry: the production of all-female and all-female triploid stocks (which would be sterile) (Fig. 1). A strategy of producing all-female populations has been successful with chinook stocks farmed in the province. A similar strategy with Atlantic salmon is made more difficult because a DNA sex probe does not yet exist for this species and because of differences in anatomy. Sterilizing fish using triploiding techniques has been most successful with female fish, particularly rainbow trout.

A cooperative industry/government research project, which began in 1994 and is taking place over a four-year period, is being spearheaded by the B.C. Salmon Farmers Association (BCSFA). This developmental or experimental phase involves:

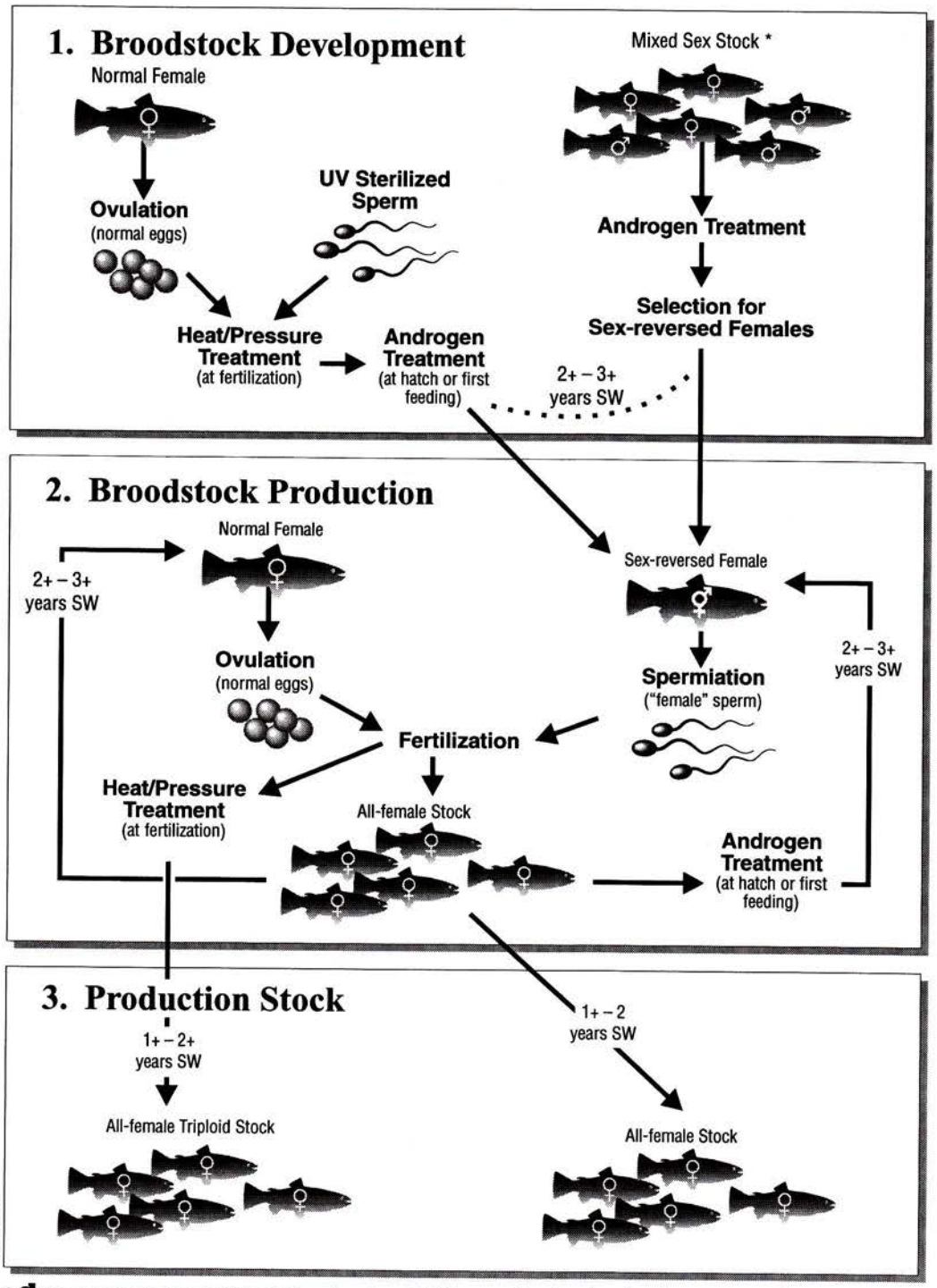
- A field trial comparing the grow-out performance of all-female and all-female triploid Atlantic salmon to regular Atlantic salmon; and
- Development of sex-reversed fish to provide industry with sufficient supplies necessary for all-female Atlantic salmon production by the year 1998.

The B.C. Ministry of Agriculture, Fisheries and Food, the lead provincial agency for aquaculture development, contracted Coopers & Lybrand Consulting to develop a reporting system to capture industry costs associated with the proposed use of non-reproductive stocks.

Key findings

As perceived by the industry, the main advantage of all-female Atlantic salmon production is

Production Scheme for Non-Reproductive Atlantic Salmon



* ♀ Represents sex-reversed female (i.e., females which become functional males after treatment with androgen).

the potential to reduce grilsing rates, without negatives effects on growth and survival. With all-female salmon, significantly higher production costs are likely during the development phase relating to broodstock and maintenance of all-female lines.

Provided there are no other options, the all-female stock option is likely to be preferred by producers over the triploid stock option. The main impact of a requirement to produce triploid Atlantic salmon is perceived to be the lost productivity of marketable fish. Higher production costs and lower revenues are therefore likely to be realized with the production of sterile salmon, as triploid Atlantic salmon have experienced slower growth rates in commercial farming situations in other regions.

Recommendations

Measurement of incremental industry costs in the experimental phase can be partially accomplished through on-going data collection in the BCSFA project. Costs will need to be collected for the hatchery and grow-out phases. Additional biological performance data will be required to measure possible changes in productivity. A gross margin approach for measuring both incremental production costs and changes in revenues is recommended.

The B.C. Salmon Farmers Association's Cooperative Assessment of Salmonid Health (CASH) Program, which currently collects biological performance data from Atlantic salmon producers on an on-going basis, is seen as the best vehicle to capture combined incremental costs and changes in productivity during the experimental phase. The CASH data can be supplemented with other data in order to estimate incremental costs and revenue changes at an industry level as a result of producing non-reproductive Atlantic salmon.

Recommendations for data collection, storage and analysis reflect the practical difficulties in carrying out a field trial. A planning meeting involving a Scientific Committee, the CASH Program Coordinator and the participants in the three-way evaluation should occur prior to the planned smolt transfer to seawater in the spring of 1996.

Participants in the three-way comparison should agree to harvest fish within a predetermined harvesting window. A log book should be maintained by each farm participating in the three-way evaluation in order to describe any unusual events or occurrences with the field trial.

David Egan is with Coopers and Lybrand Consulting, 1111 West Hastings Street, Vancouver, B.C. V6E 3R2.

The swimming performance of triploid brook trout (Salvelinus fontinalis)

Evelyn J. Stillwell and Tillmann J. Benfey[1]

Triploid fish, as a result of their larger erythrocytic size, may have a reduced aerobic capacity which might impair their swimming performance. In the present study the critical swimming velocity (CSV) of a group of diploid and triploid brook trout *(Salvelinus fontinalis)* was measured in order to assess aerobic swimming capacity. No significant difference in the CSV was found, suggesting that the aerobic capacity of triploids is not impaired.

Introduction

Triploid fish have three sets of chromosomes in the nuclei of their somatic cells instead of the normal diploid number of two. In order to accommodate this extra genetic material the cell nuclei of triploid fish are larger than those of diploid fish, as are triploid cells.[2] The organ and body sizes of diploids and triploids are equivalent since, in triploids, the increase in cell size is accompanied by a decrease in cell number. The presence of a smaller number of larger cells has been established in a number of triploid tissues and organs (e.g., cartilage, epithelium, kidney, liver, brain, retinal tissue) as well as in the blood where both erythrocytes and leukocytes are affected.[3]

Larger cells have a lower surface area to volume ratio which, in the case of larger erythrocytes, may lead to a reduction in the efficiency of gas exchange. This in turn may result in a reduction in the aerobic capacity of triploid fish. Conflicting results have been reported in two studies that examined the aerobic capacity of triploid salmonids: Virtanen et al.,[4] using indirect biochemical indicators to assess aerobic capacity, concluded that the aerobic capacity of triploid rainbow trout (*Oncorhynchus mykiss*) was lower than that of diploids. Small and Randall[5] found the aerobic capacity of diploid and triploid coho salmon (*O. kisutch*) to be equivalent based on critical swimming velocity (CSV).

The conclusions of the Virtanen et al.[4] experiment must be viewed with scepticism because the triploids tested had a significantly lower haematocrit than the diploids at the beginning of the experiment. This is an atypical finding; many studies report equivalent hematocrits in diploids and triploids.[6-8] Jones[9] found that a reduction in haematocrit to 47% of normal levels resulted in a 34% reduction in maximum swimming speed. It is therefore not surprising that the triploids in the Virtanen et al. study had a lower aerobic capacity than the diploids. To conclude, based on these atypical triploids, that triploids in general would have a reduced aerobic capacity is not justified.

Assessing aerobic capacity by measuring CSV (as was done in the Small and Randall[5] experiment) assumes the mechanical swimming ability/efficiency of diploids and triploids to be equivalent. Stillwell and Benfey[10] found that diploids and triploids required an equivalent tail beat frequency to maintain the same swimming speed (relative to body length). These findings suggest that the aforementioned assumption is

correct thereby validating the use of CSV tests to assess aerobic capacity. Small and Randall,[5] however, questioned whether their own results might be specific to their experimental species (coho salmon).

The objective of the present experiment was to replicate the Small and Randall experiment using brook trout (*Salvelinus fontinalis*) as the experimental species, in order to determine whether Small and Randall's[5] findings are species or genus–specific or whether their conclusions are valid across salmonid genera.

Materials and methods

A Blazka-type respirometer[11] served as a swim tunnel in this experiment. The speed at which the fish were swimming could be maintained within a narrow margin and the temperature and dissolved oxygen level of the water within the respirometer were held constant.

The critical swimming velocity test was conducted with a 3-4 month old mixed-sex group of 22 diploid and 17 triploid brook trout that were of the same mean size (forklength and weight were respectively 6.8 ± 1.1 cm and 4.3 ± 2.5 grams for diploids and 7.1 ± 0.9 cm and 4.6 ± 1.7 grams for triploids, $p > 0.05$ for both). Diploids and triploids came from the same egg lots (i.e., the same parents); triploidy was induced by subjecting fertilised eggs to a hydrostatic pressure shock.

These fish were conditioned for one month prior to the experiment to ensure that all fish were at the same fitness level at the beginning of the experiment. Conditioning involved placing the fish in a circular "doughnut"-style tank in which they swam against a water current of 1 body length (BL, equal to forklength in this study) per second; diploids and triploids were held in the same conditioning tank to eliminate tank effects.

The experimental protocol was as follows: A fish was randomly removed from the conditioning tank and, after forklength had been estimated, placed into the respirometer for a 1-hour habituation/training period. During this period the fish was allowed to adapt to the novel surroundings and trained to swim against a 0.5 BL/second water current (i.e., the downstream screen was electrified and fish resting against this screen received a mild electrical shock). Visual and auditory disturbances were kept to a minimum; a dark covering was placed over the respirometer and a light was shone onto the front portion of the respirometer to help the fish to orient itself.

The test period immediately followed the habituation/training period: water speed was increased in 0.5 BL/second increments every 30 minutes until the fish was fatigued. Fatigue was defined as an inability to escape from the downstream grid after three consecutive electrical shocks. Once fatigued the fish was removed from the swim tunnel and after a short recovery period was anaesthetised. Weight was determined, forklength verified and a 10 µL blood sample was taken for ploidy confirmation via

Table 1. A comparison of the critical swimming velocity of a group of diploid and triploid brook trout tested (a) at various times of day and (b) at the same time of day.

Time of Test Period	Ploidy	Number of Fish	Critical Swimming Velocity (body length/second ± 1 SD)	p
(a) Various	Diploid	22	2.3 ± 0.3	0.11
	Triploid	17	2.2 ± 0.3	
(b) 4 PM	Diploid	6	2.3 ± 0.2	0.42
	Triploid	6	2.2 ± 0.3	

flow cytometric measurement of erythrocytic DNA content. The critical swimming velocity, a measure of the maximum sustained swimming ability, was calculated according to the mathematical formula of Brett.[12]

Results are presented as means ± 1 standard deviation and were statistically analysed using a non-parametric one-way Wilcoxon test.

Results and discussion

The critical swimming velocity of the diploid and triploid brook trout tested at various times of day was found to be equivalent (Table 1, section a). There was some concern as to whether testing fish at various times of the day might have affected the swimming speed of the fish. A sub-group of these fish that had been tested at the same time of day (4 PM) were therefore examined separately (Table 1, section b). Within this group of fish, the critical swimming velocity of diploids and triploids was also the same. These findings suggest that the aerobic swimming capacity of triploid brook trout is not impaired relative to diploids, thereby supporting the findings of Small and Randall.[5]

Conclusions

The present findings are in agreement with those of Small and Randall[5] indicating that their results were not in fact genus-specific but may apply to salmonids in general. The Small and Randall experiment was also replicated by Parsons[13] using a non-salmonid species: Parsons also found no significant difference in the critical swimming velocity of diploid and triploid white crappies, *Pomoxis annularis*, indicating that triploids in general may show this trend.

The results of the present study suggest that, although triploids do have larger erythrocytes, they are not impaired in their aerobic swimming capacity. The reduction in the efficiency of gas exchange of larger triploid erythrocytes may be compensated for by the reduced oxygen requirements of triploids[10] such that aerobic capacity is not impaired. The findings of the present experiment suggest that the performance of triploid fish in an aquaculture situation should not be restricted by their aerobic swimming capacity.

This project was supported by funding from an NSERC research grant. We would like to express our thanks to Mr. Donald Hornibrook and Ms. Robyn O'Keefe for their technical assistance, Drs. R.L. Saunders and A.J. Wiggs for their comments on the manuscript, and Dr. R.L. Saunders for loan of the respirometer.

Notes and references

1. University of New Brunswick, Department of Biology, Bag Service #45111, Fredericton, New Brunswick Canada. E3B 6E1.
2. Dunham RA. 1990. *Rev. Aquat. Sci.* 2:1-17.
3. Benfey TJ. 1991. *Can. Tech. Rep. Fish. Aquat. Sci.* 1789:73-80.
4. Virtanen E, Forsman L, Sundby A. 1990. *Comp. Biochem. Physiol.* 96A:117-121.
5. Small SA, Randall DJ. 1989. *Can. J. Fish. Aquat. Sci.* 46:243-245.
6. Benfey TJ, Sutterlin AM. 1984. *J. Fish Biol.* 24:333-338.
7. Graham MS, Fletcher GL, Benfey TJ. 1985. *Aquaculture* 50:133-139.
8. Biron M, Benfey TJ. 1994. *Fish Physiol. Biochem.* 13: 153-160.
9. Jones DR. 1971. *J. Exp. Biol.* 55:541-551.
10. Stillwell EJ, Benfey TJ. 1994. In, *High Performance Fish — Proceedings of an International Fish Physiology Symposium* held at the University of British Columbia in Vancouver, Canada, July 16-21, 1994. (DD MacKinlay, ed.), pp. 288-293. Fish Physiology Association, Vancouver.
11. Kutty MN, Saunders RL. 1972. *J. Fish. Res. Bd. Can.* 30:223-227.
12. Brett JR. 1964. *J. Fish. Res. Bd. Can.* 21:1183-1226.
13. Parsons GR. 1993. *Trans. Am. Fish. Soc.* 122:237-243.

The senior author of this paper, Evelyn Stillwell, was the recipient of the Best Student Paper Award at Aquaculture Canada '95 — the 12th annual meeting of the Aquaculture Association of Canada. Moore-Clark Ltd. provided the $250 prize.

Calendar

•**Successes and Failures in Commercial Recirculating Aquaculture,** 19-21 July 1996, Roanoke, Virginia. Topics: successes and failures of recirculating systems, fish health, economics and farm management, product quality, waste management and by-product recovery, system design and management, business plans and farm management, shellfish production. Contact: C. Meck, 110 Shenandoah Avenue NE, Roanoke, VA 24016 (tel 540 857-6055).

•**International Astacology Association,** 11th Symposium, 11-16 August 1996, Lakehead University, Thunder Bay. Sessions on crayfish science and field trips to crayfish habitats. Information: Dr. W. Momot, Dept. Biol., Lakehead Univ., 955 Oliver Road, Thunder Bay, Ontario P7B 5E1 (tel 807 343-8277; fax 807 343 8023; e-mail Walter.Momot@lakeheadu.ca).

•**Coastal Zone Canada 96,** 12-17 August 1996, Université du Québec, Rimouski. Theme: Integrated Management and Sustainable Development in Coastal Zones. Papers and case studies from coastal zone stakeholders, community-based organizations, scientists, engineers, primary resource users, industry and business. Information: Prof. M. El-Sabh, Groupe de recherche en environnement côtier, Univ. du Québec, 310 allée des Ursulines, Rimouski, Québec G5L 3A1.

•**Conference on Aquaculture Development in Eastern Europe,** 1-5 September 1996, University of Budapest, Hungary. Information: EAS, Coupure Rechts 168, B-9000 Gent, Belgium (tel 32 9 223 77 22; fax 32 9 223 76 04).

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

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

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

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

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

• **Health of Coastal Ecosystems through Shellfish Restoration**—An International Conference, 20-23 November 1996, Hilton Head, South Carolina. Remediation/pollution abatement; habitat restoration; and stock enhancement/aquaculture. Invited oral presentations, contributed posters. Information: E. Knight, S.C. Sea Grant Consortium, 287 Meeting Street, Charleston, SC (tel 803 727-6406; fax 803 727-2080; e-mail knightel@musc.edu).

AQUACULTURE CANADA '96

13th Annual Meeting of the Aquaculture Association of Canada

Theme: ***DIVERSIFICATION***

June 2-5, 1996 Holiday Inn, Byward Market - Ottawa, Ontario

PLANNED SESSIONS:

Urchin Culture & Enhancement
Gov't Industry Relations
Human Resource Issues
Bottlenecks in Juvenile Production
Fed'l Aquaculture Strategy Review
Regulatory Issues
Therapeutants

SOCIAL EVENTS

June 3 - Student Barbeque

June 4 - Banquet

IMPORTANT DEADLINES

Abstracts due April 1st
Conference pre-Registration April 20th
Hotel Registration April 22nd

Student travel assistance is available through the **AAC Student Endowment Fund.** Students are elegible for the **Student Presentation Award.**

For more information contact:

Aquaculture Association of Canada Office
P.O. Box 1987
St. Andrews, N.B., Canada
E0G 2X0

Ph: 506-529-4766
Fax: 506-529-4609
e-mail:aac@wolves.sta.dfo.ca