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PROCEEDINGS OF THE ATLANTIC CANADA WORKSHOP ON METHODS FOR THE PRODUCTION OF NON-MATURING SALMONIDS: FEBRUARY 19-21, 1991. DARTMOUTH, NOVA SCOTIA.

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ATLANTIC FISHERIES
ADJUSTMENT PROGRAM

PROGRAMME D'ADAPTATION DES
PECHES DE L'ATLANTIQUE

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EDITOR'S COMMENTS

The Atlantic Canada workshop on methods for the production of non-maturing salmonids was attended by: scientists who have been developing techniques to suppress maturation; producers who have had considerable experience in applying these techniques; and, legislators and resource managers who have responsibility for fishery management and aquaculture development. The workshop was convened as a means to determine the potential applicability of technology for suppressing salmonid maturation to the salmonid farming community and to wild salmonid fishery management.

At the request of workshop participants, these workshop proceedings are being published as a Canadian Technical Report of Fisheries and Aquatic Sciences for distribution to the Canadian aquaculture community. The papers that form the basis of this workshop have been exchanged within the scientific community represented at the workshop and therefore have received a cursory peer review, consistent with the standards of this report series. While I have edited all of the papers and discussion sessions to provide continuity of format, I have not changed their intended meaning. Any statements or views presented here are totally those of the speakers. The Department of Fisheries and Oceans advocates open dialogue on matters of fishery science and management but does not accept any responsibility for statements made by the speakers. Any mention of trade names does not constitute endorsement by the DFO.

The present Technical Report intentionally follows a science format. My own opening presentation (Motives, Actions and Goals...) constitutes an "Introduction" to this report. The workshop itself constitutes the "Materials and Methods" and the presentations and ensuing discussions constitute the "Results" being evaluated. These presentations, by both scientists and salmonid producers, together with the ensuing discussions, provide the objectivity of this "study". The final section of this report (Synthesis of Workshop Recommendations) is entirely my own interpretation of the facts of the workshop and is intended as the equivalent of the "Discussion" section of a scientific report. In this final section, I have attempted to interpret the workshop presentations and discussions and to come to conclusions that represent the achievements of the workshop, as well as to make workable recommendations for further technology development, refinement and transfer to industry. As an employee of the DFO Science Branch, I am guided by the principle that "In Science, we seek truth.". Thus, I make no claim to a comprehensive "Synthesis...", preferring rather to accept that I should present the facts and encourage you to come to your own conclusions.

The workshop was funded by the Aquaculture Science component of the Atlantic Fisheries Adjustment Program as project NCO058. This publication is intended as a compilation of present information on technology for suppression of maturation of salmonids. As a representative of the DFO, I hope to use this publication to inform the Canadian salmonid farming community about the technology for production of all-female, triploid salmonids. These proceedings also should convey to salmonid farmers the concerns DFO has, regarding long-term interactions between aquaculture salmonids and wild stocks, as well as the stability of Canadian aquaculture. This publication is part of an initiative to establish a cooperative liaison between the aquaculture industry and the federal and provincial agencies that have regulatory responsibilities for management of fishery resources. I am hopeful that further government/industry dialogue will proceed from this publication to establish a program of salmonid broodstock development and maturation suppression that will benefit the aquaculture industry and our natural resources.

ABSTRACT

Pepper, V.A. (ed). 1991. Proceedings of the Atlantic Canada workshop on methods for the production of non-maturing salmonids: February 19-21, 1991. Dartmouth, Nova Scotia. Can. Tech. Rep. Fish. Aquat. Sci. No. 1789. vi + 152p.

There is concern among salmonid resource managers that accidental release of aquaculture fish may cause changes in genetic structure of wild populations. In consideration of the present uncertainty on this issue, the Newfoundland Region of the Department of Fisheries and Oceans convened an Atlantic Canada Workshop to determine the "state of the art" for suppressing maturation of salmonids.

Participants were in favour of salmonid reproductive control and concluded there are potential advantages to the salmonid farming industry and resource managers. The option that was preferred for Atlantic Canada was all-female, triploid stocks. Workshop endorsement was contingent on research to provide adequate resolution of "coincident" phenomena as well as attempting to deal with the needs of the industry. Regional programs are required to demonstrate this technology to industry.

RÉSUMÉ

Pepper, V.A. (éd.). 1991. Travaux de l'atelier des provinces de l'Atlantique sur les méthodes de production de salmonidés qui n'atteignent pas la maturité : du 19 ou 21 février 1991, Dartmouth (Nouvelle-Écosse). Can. Tech. Rep. Fish. Aquat. Sci. No. 1789. vi + 152 p.

Les aménagistes s'inquiètent du danger que la libération accidentelle de saumons d'aquiculture entraîne des modifications dans la structure génétique des populations à l'état sauvage. Compte tenu de l'incertitude entourant cette question, la région de Terre-Neuve de Pêches et Océans Canada a convoqué un atelier des provinces de l'Atlantique pour déterminer les plus récentes techniques propres à empêcher la maturation du saumon.

Favorisant le contrôle de la reproduction des salmonidés, les participants à l'atelier ont conclu que l'industrie de la salmoniculture et les aménagistes y trouveraient probablement des avantages. La préférence des intervenants de la région de l'Atlantique a porté sur des stocks triploïdes formés uniquement de femelles. La position des participants était toutefois conditionnelle à l'aboutissement des recherches en vue de résoudre adéquatement les phémonènes "simultanés", et ce, tout en essayant de satisfaire aux besoins de l'industrie. Des programmes régionaux seront nécessaires pour démontrer cette nouvelle technologie aux industriels.

PRODUCTION OF NON-MATURING SALMONIDS: MOTIVES, ACTIONS AND GOALS, USING NEWFOUNDLAND REGION AS A MODEL

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ABSTRACT

Potential interactions between wild salmon and escapees from marine salmon cages have been of considerable concern to resource managers. The Newfoundland Fishery Regulations and the provincial Aquaculture Act impose regulatory requirements for these concerns. Local salmon farmers wish to import late maturing stocks into Newfoundland to provide 'domesticated' salmon that will impart economic viability to Newfoundland salmon farms. Unfortunately, there currently is no satisfactory technology to confirm if there is or is not a significant genetic risk to natural stocks from imported salmon.

Production of non-maturing salmonids has intuitive appeal to salmon resource managers who must contend with potential genetic influence of aquaculture salmonids on wild populations. However, this technology must be proven under practical fish farm conditions if salmonid farmers are to accept this approach. The purpose of this workshop is to identify the level of interest in Atlantic Canada for an action plan to evaluate performance of reproductively sterile Atlantic salmon. The present workshop is a Newfoundland Region initiative that was implemented as a result of the Aquaculture Strategy.

INTRODUCTION

Aquaculture developments have placed new demands on fishery resource managers. Historically, fisheries science has focused on matters of significance to the ecosystem and to sustainable harvest of wild fishery resources. More recently, the federal Justice Department for Canada has interpreted that aquaculture is a fishery and therefore falls within the mandate of the Department of Fisheries and Oceans (DFO) under legislation governing wild fisheries. As a result, DFO became the federal lead agency for aquaculture.

This interpretation of DFO legislative responsibility is of considerable significance. Some legislation has had to be modified to recognize that individuals can have property rights over the aquatic resources they are raising. Concurrently, concerns of resource managers also have had to expand to recognize that aquaculture activities deserve equal consideration. The Newfoundland Fishery Regulations (Section 4. "No person shall have in his possession at any time, in an unpreserved state, more fish of any species taken in any inland waters than double the daily catch limit for that species...") provide an example of the historical regulation problem. The traditional "thou shalt not" legislative framework is no longer appropriate considering the Department of Fisheries and Oceans Aquaculture Strategy goals (i.e., "advocacy and dialogue to promote sustained growth and development of the aquaculture industry").

Canada is a relative newcomer to aquaculture endeavour. This may have resulted in loss to the international community of an uncertain amount of market opportunity. However, it has had the advantage of providing an opportunity for selective technology importation and a better understanding of previously unforeseen consequences that are not desirable, either from the viewpoint of the fish farmer or the resource manager.

The business of aquaculture is a high risk endeavour. This risk prevails at all stages in the development of an aquaculture enterprise and during each growing and marketing cycle. The aquaculturist must contend with these business risks. Concurrently, the resource manager must consider potential risks to the environment and to local wild fishery resources. This does not mean that aquaculture must not pose any risk to the environment but that the magnitude of this risk must be weighed against potential social and economic benefits of industrial development.

The historical regulatory orientation towards the "thou shalt not" philosophy gradually is being infused with the notion of risk assessment. If potential environmental risk is low and expected benefit is high, there is ample reason to proceed with development. However, at the opposite extreme, if risk is high and potential benefit is low, caution is advisable. This does not necessarily dictate that caution preclude development. Under these conditions, specific risks have to be identified and steps taken to impose conditions that minimize risk factors inherent in the initiative. These extremes are easily recognized by aquaculturists and resource managers alike. However, it is not usually the extremes that present conflict. The more usual situation is when potential benefits appear attractive but the risks are not easily quantified. One example of potential risk is the inadvertent escape of various salmonids from sea cages.

BACKGROUND TO WORKSHOP

Potential interactions between wild salmon and escapees from marine salmon cages have been of concern to resource managers in Newfoundland. The Newfoundland Fishery Regulations, Section 5(a), subsection (2), provide the regulatory base of these concerns:

> "The Minister may authorize the introduction into the waters of the Province, or the transfer from any waters of the Province to any other waters thereof, of any fish that
> (a) do not have any disease that may be harmful to the protection and conservation of fish in the Province; and
> (b) will not have an adverse affect on
> (i) the stock size of fish in the Province; or
> (ii) the genetic characteristics of fish or fish stocks in the Province.".

As well, the provincial Aquaculture Act (Section 3) states:

> "The Minister shall not approve the introduction into or transfer to a body of water or aquaculture facility in the province of a species or strain of aquatic plants or animals not present in that body of water or that aquaculture facility unless the impact of that introduction or transfer has been assessed in accordance with the provisions of The Environmental Assessment Act, whether or not that introduction or transfer is an activity of the type otherwise requiring assessment under that Act."

There is ample international experience with marine salmon farming indicating that escapement from marine cages is an increasing problem and has occurred at very significant levels. Norwegian authorities estimate 25% of spawning salmon in their rivers are domesticated fish that have escaped from sea cages. Unfortunately, present technology is not sufficient to undertake meaningful risk assessment that would identify how potential industry benefits compare with potential environmental or wild stock costs from such releases. At present, there is no sound biological substantiation for an exemption from the Aquaculture Act.

The recent (1990) Norwegian meeting on influence of aquaculture on wild stocks resulted in recommendations that reinforce concerns about potential influence on local wild salmon populations. The meeting produced evidence that:

> "... when salmon escape at the smolt stage from freshwater they will return with relatively high precision to that river. When smolts and post-smolts escape from fjord areas in most months they will tend to return to the general area and stray to nearby rivers. However, when post-smolts escape in winter they will stray further away. When fish escape at an adult stage they will enter rivers in the vicinity they are in when for physiological reasons they have to move into freshwater. Small fish escaping in the winter may have poor survival. Comparison of the migratory behaviour of wild and hatchery reared salmon showed that about 6% of wild fish were caught in the sea while only 3% of hatchery fish were caught. Straying into rivers other than the Imsa was greater in hatchery fish and although both wild and hatchery adults returned to

coastal waters at the same time, wild fish entered freshwater earlier than hatchery fish and stayed for a longer period of time. While most wild males appeared to spawn up to one third of hatchery reared males did not. Hatchery fish also exhibited marked movements up and down stream and had a higher incidence of injury... Due to later spawning by farmed fish they can overcut redds made by wild fish.".

A theoretical model, that was presented at the Norwegian meeting, suggested "... where intrusions of farmed fish are as massive as already observed in some instances, the characteristics of native stocks could become extinct after only a few generations... the model predicts that if cultured salmon constituted more than 30% of the spawning population and if they enter a river system to spawn at frequent intervals then the native genomes would become extinct within four generations.". The conference identified a multitude of studies that are providing data on various aspects of behavioral and genetic interactions between stocks. However, there currently is insufficient data from which to draw sound conclusions on the real influence of fish farm escapees on wild salmon populations. There is empirical evidence that releases of hatchery fish have resulted in gene changes in wild populations. A reduced natural productivity appears to accompany introgression or displacement of natural stocks (NASCO Council Paper CNL(90)28). Further evidence is required to substantiate this interpretation. Accordingly, resource managers find themselves with few options. This has resulted in a 'damage control' approach. Importation of salmon eggs from outside Newfoundland has been limited to a level that is not likely to result in 'swamping' local wild salmon production.

Newfoundland salmon farmers wish to import late maturing salmon stocks into Newfoundland to provide 'domesticated' salmon that will impart economic viability to Newfoundland salmon farms. The basis of the present Newfoundland Region concern is that a Newfoundland salmon stock may have closer genetic ties to wild stocks in the Bay d'Espoir area than any stock from mainland North America. Newfoundland Region does not wish to place its wild salmon stocks at serious risk (i.e., the Conne River salmon stock that is in the vicinity of the Bay d'Espoir salmon cages and which is considered by the Council of the Conne River Micmacs as the base of their Native food fishery). Development potential for Atlantic salmon farming in Newfoundland has been evaluated as marginal (DPA Group Inc., 1990), even under the best of environmental conditions for this region.

DFO concerns are with relative magnitude of potential impact under worst case scenarios. Estimations of natural salmon smolt production in the Conne River range from 60,000 to 78,000 (Dempson et al 1987; Dempson 1988). Corresponding adult spawning escapement has been 7,500 to 10,000. What would happen if some natural disaster, or some sort of accident with the marine cages, resulted in escape of hatchery smolts into the Bay d'Espoir estuary? Of course, no one can say for sure. Accidental release of all marine cage smolts in the Bay d'Espoir area is unlikely. However, the concern is that, should some 100,000 smolts escape from the sea cages, there could be close to a 1:1 ratio of foreign:local smolts emigrating from Bay d'Espoir for subsequent return to the area. Even worse, if as many as 60,000 adult salmon were to escape from the sea cages, the ratio of foreign:local stock in the Conne River spawning escapement could be even more biased in favour of the influx of foreign genetic material into the Conne River salmon stock.

Newfoundland Region has opted to confine risk by limiting importations. In the absence of technologically sound approaches to quantify relative risks, Newfoundland Region concluded it would not be prudent to exceed the 100,000 egg importation limit until such time as potential risks can be quantified and subsequently minimized. By virtue of the fact that DFO has allowed limited importations of salmon eggs into Newfoundland, the department has not insisted on no risk to wild stocks but rather on minimizing the potential risk to these stocks.

In an attempt to evaluate industry potential and aquaculture technology, the Bay d'Espoir Salmon Hatchery Ltd. imported 50,000 eggs of LaHave River stock in 1985 and, each year since 1988, has imported 100,000 Atlantic salmon eggs of St. John River origin. This limitation has resulted in conflict between salmon farmers and resource managers. The Bay d'Espoir Salmon Growers Cooperative has estimated that a minimum of 200,000 eggs per year is required for economic viability of the Newfoundland marine salmon farming industry. Ultimately, the salmon farmers wish to develop their own salmon brood line, from the St. John River stock, and to increase their annual egg supply to as high as 0.5 million per year.

These problems have persisted in Newfoundland since 1985 when the Bay d'Espoir Salmon Hatchery Ltd. collected its first wild salmon brood from the Grey River on the south coast of the island. In 1990, the DFO Aquaculture Strategy focused departmental attention on aquaculture development. The present workshop is a Newfoundland Region initiative, that was implemented as a result of the Aquaculture Strategy, to pursue a potential solution to the dilemma of a brood stock for Newfoundland salmon farmers.

NEWFOUNDLAND SALMON FARMING RESEARCH INITIATIVES

Newfoundland Region priorities for research in support of addressing stock research and management deficiencies are as follows:

1) development of biochemical genetics monitoring techniques that will support genetic typing and genetic tracing of stocks and risk evaluations;
2) design of a long term salmon brood selection program to develop stock characteristics conducive to salmon farming;
3) development, perfection and demonstration of technology for suppression of maturation of Atlantic salmon grilse and imported two sea-year stocks ; and,
4) implementation of a brood selection program that will recognize the needs of industry together with the legislative responsibilities of both levels of government in Newfoundland.

Newfoundland Region activities on salmon brood stock have attempted to limit risks while still encouraging interim aquaculture industry development. This has evoked concurrent endeavours on all of the above items.

Biochemical Genetics.

Under the present concept of fish stocks (Simon and Larkin 1972, Billingsley 1981) an imported stock is more likely to have a gene pool that differs from that of the Conne River stock than is some other local Newfoundland salmon stock. Local stocks within the same geographic area have a higher probability of similar evolutionary pressures. As well, there is likely to be greater gene flow (i.e., spawner straying) among local rivers.

Cross and Healy (1983) provided evidence for discrete stocks of Atlantic salmon. Similar evidence was presented by Stahl (1983) for Baltic salmon where separate stocks were identified both within and between river systems. We know that variations in life history parameters exist within and between different river populations of Atlantic salmon. Some of these variations are interpreted as adaptations. These traits include morphology, migration patterns and developmental timing.

We have evidence to suggest adaptation within individual stocks to the conditions that prevail in riverine environments. There is indication of heritable patterns of homing precision (Ricker 1972) within stocks. However, as yet we have no unequivocal data to confirm genetic differences among salmon from different North American rivers. We do not yet know if local adaptations within our Atlantic salmon stocks are part of an Evolutionarily Stable Strategy (Maynard Smith 1982) that has developed for individual river systems. These may be simply environmental adaptations by a phenotypically plastic species. It is recognized by the international scientific community that "The details of these adaptations need to be clarified by appropriate genetic studies, and by empirical studies of controlled introductions." (NASCO, Council Paper CNL(90)28).

Our present technology is not adequate to quantify "genetic distance" between our various North American salmon stocks. It is known that electrophoretically detectable protein variation in this species is low. However, the value of protein electrophoretic methods alone in stock discrimination of Atlantic salmon is not sufficient to yield clear cut differences between Atlantic salmon stocks (Verspoor 1986; Davidson et al. 1989). We are in need of genetic typing techniques and general survey work to identify stocks that may be of a 'minimal risk' nature. Accordingly, Newfoundland Region supported a Science Subvention research project in 1989 to examine the possibility of genetically typing salmon from different North American rivers using the technique of polymerase chain reaction/direct sequencing of mitochondrial DNA. The initial research results were astonishing. As described in the preliminary research report (W. Davidson, Memorial University of Newfoundland, pers. comm.):

> "There were no differences detected that could be used to differentiate between salmon from North America and Scotland. Indeed, only one base substitution was observed among all the salmon that were examined. This represents the lowest level of genetic variability of all the species that have been examined in my lab. It indicates that salmon have probably undergone a major bottleneck in the near past and that this constricted their genetic pool. Only four base substitutions separate the Atlantic salmon from their close relative the brown trout and none of these changes cause a difference in the structure at the protein level.".

This interim result is potentially of overwhelming significance. All of our current salmon management philosophy (both Atlantic and Pacific) is founded very strongly on the stock concept. While more recent research has provided a means to identify the continent of origin of Atlantic salmon (Cutler et al, in press), there is as yet insufficient evidence to conclude a unique genetic composition for Atlantic salmon from different watersheds of North America.

Identification of differences in the DNA sequences would provide us with a prototype technique with which to select a minimal risk stock for aquaculture development (i.e., one that is genetically similar to local wild salmon). This goal may be of benefit to salmon farmers since we do not know if there are specific adaptations within local stocks that confer survival advantages under harsh environmental conditions. If this is so, imported stocks may not do well under Newfoundland winter marine conditions. Experience to date in Roti Bay (Bay d'Espoir) has indicated only that larger salmon have had lower overwinter survival than smaller fish. This may have some bearing on the fact that most Newfoundland rivers produce grilse.

<u>Brood selection program design</u>.

Development of an Atlantic salmon farming industry in Newfoundland has been constrained by marginal environmental conditions and lack of a suitable local salmon brood stock. Under the Canada-Newfoundland Inshore Fisheries Development Agreement (NIFDA), Newfoundland Region evaluated alternative breeding designs for Atlantic salmonid brood stock development in support of local salmonid farming. Essential elements of this industry development planning study include consideration of Newfoundland aquaculture industry goals and government regulatory requirements.

This NIFDA contract resulted in a generalized breeding program design (Peterson et al. 1990) for pursuit in the long-term, and technological options for stock performance improvement in the short-term. We know that some stocks perform better than others under aquaculture conditions. We know also that some stocks represent greater threat of genetic swamping. The NIFDA contract was implemented largely in recognition of these sorts of concerns. Contract recommendations provide the framework to develop and implement a program for greater stability of fish farming industry development activities and encourage a systematic approach to problem solving.

<u>Production of non-maturing salmonids</u>.

Production of non-maturing salmonids is a technique of potentially significant value to salmon farmers and salmon managers alike. If reproductively sterile salmon are proven to perform similar to or better than present aquaculture salmon, salmon management concerns about genetic influence of aquaculture salmon on wild stocks will be solved. This also would produce enhanced economic benefits to the Canadian aquaculture industry. Considering the scope of potential benefits to managers and aquaculturists, this technology should be pursued on an integrated front with contribution and input from throughout Atlantic Canada.

Expertise currently is available within the Canadian scientific community to produce significant numbers of such salmon. Unfortunately, there is as yet no North American data to demonstrate how well these reproductively sterile salmon perform relative to a late maturing salmon stock under identical aquaculture conditions. Salmon farmers likely will continue to be reluctant to make use of this technology until reliable data are available.

Induced triploidy of all-female stocks currently is the only method available to sterilize salmonids on a commercial scale. Newfoundland salmon farmers were involved in a previous unsuccessful experiment on reproductive suppression with Atlantic salmon. This previous attempt was based on a now outdated procedure (i.e., heat shock) that produced a high incidence of deformities among resulting young salmon. As a consequence, the salmon farmers have expressed their reservations toward any further experimentation with this technology. More recently, there have been indications from fish farms in the Maritimes, and observations of non-maturing rainbow trout in Newfoundland, suggesting that growth performance among reproductively neutralized salmonids is inferior to that of salmonids with normal reproductive potential. This reproductive control technology must be proven under practical fish farm conditions if salmonid farmers are to support transfer of technology for the production of non-maturing salmonids to the aquaculture industry.

Local broodstock development.

There are stocks of Atlantic salmon within the province of Newfoundland and Labrador that may have genetic potential for brood stock development in support of local salmon farming. However, many Newfoundland salmon stocks mature after their first winter in the ocean. Such stocks are not desirable for salmon farming due to reduced growth rates, inferior food to salmon flesh conversion efficiency, and inferior flesh quality as a result of the metabolic demands of the maturation process. This in turn erodes the potential for economic viability of salmon farms.

Atlantic salmon stocks that are not native to Newfoundland may not be adapted to local environmental conditions (i.e., local diseases, very cold winter marine temperatures). Once imported, any stock that is foreign to Newfoundland may have to undergo additional selection to develop growth and survival characteristics that are compatible with the Newfoundland marine environment. It is likely that candidate stocks will have to be evaluated under Newfoundland conditions before they can be considered as "proven" for Newfoundland salmon farming.

The NASCO has recommended (Council Paper CNL(90)31) that "...local stocks ie stocks from the same river, or a neighbouring river with similar ecological conditions, should be used whenever possible.". Such efforts have been ongoing in Newfoundland since the first salmon aquaculture experiments in Bay d'Espoir in 1985. To date, the Bay d'Espoir hatchery has experimented with local salmon from the Grey River and the Conne River on Newfoundland's south coast, and with Exploits River salmon from the Northeast Coast of the island. All of these efforts confirmed that these natural grilse stocks started maturing during their first winter in the sea cages. Maturation was well advanced by the following summer. These salmon did not attain a satisfactory market size. More recently, work is proceeding in a renewed attempt to identify and develop a local Atlantic salmon stock that may have growth, survival and maturation characteristics appropriate to economic viability criteria for Newfoundland salmon farms.

Damman (1983) classified the Bay d'Espoir subregion as a small, sheltered valley system that is similar to the Western Newfoundland Ecoregion. Within the Western Newfoundland Ecoregion are several salmon rivers with small but self-sustaining populations of two-sea-year salmon. Considering that the Grand Codroy River also is a relatively sheltered valley system, its similarity to the Bay d'Espoir area makes it an attractive candidate as a brood source. Accordingly, with funding procured under the NIFDA, Newfoundland Region and the Bay d'Espoir Salmon Hatchery Ltd. collaborated on collection of Atlantic salmon from the Grand Codroy River.

Eggs from Grand Codroy salmon were transported to the Bay d'Espoir Hatchery in 1989. Juvenile salmon of this stock are being raised in the Bay d'Espoir Hatchery for performance comparisons with imported stock from New Brunswick (St. John River stock). Performance of these two stocks in the hatchery to date has been similar. Smolt performance evaluations for these two stocks in the Bay d'Espoir marine cages will be started in 1991. This work may provide a base from which to support a generalized breeding program for pursuit in the long-term, together with technological options for stock performance improvement in the short-term.

Identification of a 'minimum risk' salmon stock for Atlantic salmon farming in Newfoundland is a positive endeavour so long as the identified stock meets performance criteria that conform with industry requirements for economic viability. The NASCO recommendation of local stocks for brood development is being pursued. However, resource management concern must address the question of what genetic characteristics hatchery stocks will have after several generations of artificial selection.

It is a well known fact that hatchery propagation of salmonids typically results in the loss of genetic variability in favour of improving commercially desirable performance characteristics (Youngson et al 1989). Recognizing that the goal of Newfoundland's salmon farmers is to become self sufficient for brood stock, and that the Newfoundland salmon farming industry expects to expand to the level of 0.5 million eggs per year, it becomes questionable if a 'minimal risk' wild stock will result in a minimal risk aquaculture stock. Development of biochemical monitoring techniques should continue in an endeavour to provide means to quantify and evaluate potential environmental influence but will not in themselves provide any protection against such effects.

POTENTIAL FRAMEWORK FOR NEWFOUNDLAND MARINE SALMON FARMING

The question of influence of aquaculture on wild stocks has attracted international attention and concern. There is growing interest in identification of cultured fish and implementation of monitoring projects to document the occurrence, behaviour and reproductive success of fish farm escapees on wild populations. Efforts in this direction likely will be required under the Environmental Assessment and Review Process Guidelines Order.

Bay d'Espoir Hatchery fish have five "tags" by which the fish may be recognized as being of hatchery origin:

1) hatchery fish have a high incidence of fin erosion. Both the incidence and amount of fin erosion among hatchery origin salmon allows these fish to be distinguished visually from wild salmon in natural spawning escapements;
2) juvenile salmon are raised in the Bay d'Espoir hatchery for one year before they are transferred to sea cages. This is the only source of Age 1 salmon smolts in Newfoundland. Their growth pattern while in the hatchery, and the fact that these salmon smoltify at Age 1, can be readily identified by examining their scales, thereby distinguishing them as being of hatchery origin;
3) hatchery salmon have terramycin induced marks on the bony structures;
4) marine farm salmonids typically can be identified by their highly pigmented flesh, due to artificial pigments used in salmonid diets; and,
5) marine farm salmon typically are much larger than those in the wild. Few natural stocks in Newfoundland reach the size of those reared in cages. Most wild salmon in the Bay d'Espoir area are grilse.

The short term, interim approach to the brood stock problem has been to import limited numbers of 'foreign' salmon into Newfoundland. This importation allows the opportunity to evaluate the potential for local salmon farming while still limiting the risk of negative impact of imported salmon on local wild salmon populations. A broodstock selection program ultimately may be the long-term solution to developing an economically viable salmon aquaculture industry, but interim measures are needed to furnish suitable salmon stock to demonstrate industry potential.

Production of non-maturing salmonids may offer an interim solution to the brood stock question. If escapees from marine salmon cages are unable to reproduce, potential environmental influence of accidental releases will be limited to the more immediate consequences of predation and competition with wild stocks. It is likely that the five 'tags' identified above would facilitate environmental impact analysis, should the magnitude of any accidental release warrant such an assessment.

WORKSHOP ON NON-MATURING SALMONIDS

This workshop is intended to be the first step of a three phase undertaking, the latter stages of which are dependent on results of the earlier endeavours. The proposed phases are as follows:

1. Atlantic Canada Workshop. We are meeting to discuss the present state of technology for production of non-maturing salmonids and to determine what is known of the performance of these fish relative to reproductively active salmonids. The main goal of this workshop is to identify the level of interest in Atlantic Canada for an action plan to evaluate methods for the production of non-maturing Atlantic salmon.

It is my hope that these discussions will result in a positive evaluation. During the third day of our meeting, assuming both interest and support, I hope we will proceed to develop an action plan for production of non-maturing salmonids that will incorporate a performance demonstration program for consideration by the salmonid farming industry. Pending the outcome of our meetings, a workshop report, including the suggested implementation proposal, will be written and distributed to workshop participants and industry representatives in April, 1991. Industry response to the workshop report and suggested implementation plan will be requested, at the time of distribution, to be returned prior to the end of May, 1991.

2. Implementation. Techniques for production of non-maturing salmonids have been described by Donaldson and Benfey (1987). The procedure requires two generations. In the first generation, salmonid fry diets are supplemented with hormones to transform genetic females into phenotypic males. All genetic males subsequently are removed from this generation leaving only those fish that will produce sperm that has only female sex chromosomes. Once these altered fish mature, female sperm is used to fertilize normal salmonid eggs, thereby producing progeny that are all genetically female. These eggs from the second generation are subjected to pressure shock shortly after fertilization. This interferes with normal cell division and results in progeny that have three sets of chromosomes. These triploid salmonids are reproductively sterile. At present, there are Canadian commercial sources of reproductively sterile rainbow trout. There are no such supplies of Atlantic salmon. Accordingly, the second and third phases of this initiative likely will concentrate on Atlantic salmon.

A major goal of the phase 1 workshop, assuming workshop support for pursuit of this technology, is to decide on the level of effort (i.e., appropriate stocks, number of stocks, number of eggs per stock, number of grow-out sites to needed to evaluate genotype/environment interactions, etc.) required to support valid experimental quantification of stock performance. This could form the basis of an action plan for potential implementation in the autumn of 1991. The exercise also will serve to quantify costs, and identify a collaborative effort for implementing and operating such a program. An implementation proposal will be developed in June once industry input has been considered. Funding support for the recommended program will be pursued during the interval of July through September in an endeavour to establish the necessary program implementation infrastructure prior to the 1991 Atlantic salmon spawning season.

3. Performance Evaluations. Although this technology may be ready for transfer to the aquaculture industry, there is as yet no quantitative demonstration of the performance of reproductively altered salmon. Phase 3 of this research would address performance.

If there is sufficient workshop interest, Canadian researchers will be encouraged to develop a source (or sources) of female salmon milt. Recognizing that "Modified organisms should be evaluated on both their genotypic and their phenotypic characteristics rather than on the process used to produce them." (Devlin and Donaldson 1990), it is desirable for the demonstration phase of the work to take place concurrently at selected salmon farms throughout Atlantic Canada. This will require compliance with disease control precautions covered in the Canadian Fish Health Protection Regulations. The potential scope of performance evaluations will be identified as a result of this workshop.

Details of this experimentation will be highly dependent on industry interest. It is likely that much of the required performance testing will involve demonstration farms.

SUMMARY OF GOALS AND TIMEFRAMES

The present workshop will consist of nine additional presentations from researchers and producers who have kindly agreed to share their experiences with us. As a result of these presentations, and what I hope will be considerable discussion following each presentation, we will have the benefit of much of the world experience relative to the technology for production of non-maturing salmonids. Our challenge then will be to arrive at an objective conclusion as to what potential this technology offers to aquaculturists and resource managers and what needs to be done to perfect, demonstrate and disseminate the technology in support of aquaculture industry development. The third and final day of this workshop will be devoted to developing the workshop conclusions and, potentially, to decide on an action plan for potential pursuit over the next several years.

Anticipated events and their time sequence is as follows:

1. Workshop	- February, 1991
2. Workshop report and submission to industry	- March 30, 1991
- Industry input	- May, 1991
- Implementation plan and funding proposal	- June, 1991
3. Implementation	- October, 1991 (pending further funding support)

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PARTICIPANT DISCUSSIONS

Rapporteur: J. Pratt

Q: Benfey — What is natural spawning escapement to Conne River?

A: Pepper — There are two CAFSAC documents that identify historical spawning escapement as ranging from 7,500 - 10,000.

Q: Anderson — What stocks are being used at the hatchery?

A: Pepper — DFO has placed clear emphasis on evaluation of Newfoundland salmon stocks in preference to imported stocks. The DFO intent is to minimize risk of impacts on local wild stocks. At present there are two stocks in use at the hatchery. Most of the present hatchery salmon are St. John River stock but there also is a relatively small component of local stock from the Grand Codroy River located on the west coast of the island.

Previously, Grey River, Conne River and Exploits River stocks were used as local stocks but all were early (grilse) maturers that did not support any serious market considerations.

Grand Codroy on the other hand is a known multi-sea year component salmon stock. Eggs were taken in 1989 (all females were 2 sea year, several males were 1 sea year).

Q: Benfey — If we decide on St. John River stock, would we suppress maturation of that stock?

A: Pepper — Uncertain. It is likely to depend on several events that may happen during the next several years such as how many eggs the Newfoundland salmon farmers wish to import and what evidence is accumulated from around the world on the consequences of aquaculture stock interactions with wild stocks.

Q: Saunders — How much do we know about St. John River stock performance?

A: Sutterlin — We have had no post smolt maturation as with local Newfoundland (grilse) stock. We are not far enough along yet to make conclusions on that aspect.

Comment: Pepper

Vitelogenin levels have been looked at in Bay d'Espoir and in a landlocked fjord near St. John's (i.e., Holyrood Pond). All local stocks started maturing in January of their first winter in the sea cages. Thereafter, these fish advanced quickly to maturity and were not marketable. Unfortunately, among those St. John River origin salmon that did not mature during their first winter in the sea cages, most of the larger salmon were unable to adapt to sea cage conditions during the winter. A disturbingly large number of these salmon died.

Q: Saunders — Have you seen any difference between St. John and other (local) stock at Bay d'Espoir thus far?

A: Sutterlin — Some precocity noted in Grand Codroy at this point in time, but too early for any solid conclusion.

Comment: Anderson

I doubt if there will be much difference in performance in different places for St. John River stock though I recognize the genetic concerns respecting releases of that stock in Newfoundland. As a physiologist, I feel we should attack the maturation process positively through the ploidy-sterility approaches.

Comment: Pepper

Possibly so. However, an unfortunate negative bias has developed among the sea farmers because of previous efforts with reproductive control using heat shock on Atlantic salmon in Newfoundland. There was a high incidence of jaw deformities within this experimental batch of salmon. We really have no idea if this was a coincident phenomenon (i.e., had nothing to do with the experimental procedure) or was a result of the heat shock treatment. This previous experience has resulted in an attitude among the salmon farmers that they really are not interested in further experimentation with this technology. Their attitude, toward both production of non-maturing salmonids and further experimentation with local salmon stocks, is now solidly entrenched in an attitude of "show us, (at your expense, not at ours) and we will determine if there is an advantage to our economic viability".

I perceive three possible outcomes of further experimentation and demonstration of production of non-maturing salmonids as follows: 1) better performance (i.e., growth, food conversion, quality of product; 2) equal performance; or, 3) inferior performance. The consequences of results in the first category are likely to benefit the industry though it ultimately may depend on how much better performance has to be to sustain the extra cost of the required treatments. The second category might be resisted by the industry due to the additional cost of securing triploid eggs. The third category can be expected to be rejected by industry though the question might still have to be addressed as to how much might be lost in terms of industry viability relative to environmental risks from non-local stocks.

In my dealings with industry, I simply have not had the answers to these questions and therefore have not been able to provide the positive advocacy you suggest. In truth, that is what I am looking for and is one of the main reasons why I have organized this workshop. I expect that much of the world expertise on this technology is assembled in this room and will provide us with the objectivity necessary to undertake some positive initiatives to integrate our various regional needs into a program that will meet the needs of the salmonid aquaculture industry as well as the resource managers who must concern themselves with wild fishery management problems.

Comment: Glebe

By picking the wrong stock for evaluation of maturity control techniques we may gravely affect the market and stability of the industry. We need to be assured of stability in the salmon aquaculture industry prior to applying sterilization techniques. It would not be reasonable to start a salmon farm with a stock that we know does not perform well.

Comment: Pepper

For Newfoundland, this is basically a Catch 22 situation. The Tri-Gen Report concluded that even with the best of salmon stocks for aquaculture, the Newfoundland salmon farming industry will still remain economically marginal. As a DFO biologist, I have responsibility to provide sound scientific advice to wild fishery resource managers but, in doing so, must not overlook both social and economic needs within the region to which I am assigned. This topic of production of non-maturing salmonids may provide a means to achieve these goals. Technology for the production of non-maturing salmonids, if applied to a local Newfoundland grilse stock, may provide a means to overcome the grilsing problem. In turn, improved stock performance could result in a desirable and marketable commodity that has not been attainable with local diploid stocks to date. On the other hand, if this technology is applied to imported salmon stocks, the resulting non-reproductive salmon cannot pose any threat of genetic swamping to local wild salmon stocks. Whatever technology is applied, we want to gain stock performance for sea farmers while protecting the wild stocks against genetic swamping. Therefore, I advocate comparative testing of techniques and various stocks to provide objective decision criteria for industry planning and management.

Comment: Donaldson

There are different groups of reproductive hormones that govern the diverse physiological processes of the animal. These hormones can have different affects on growth early in the cycle.

Brian Glebe

Q: Glebe — What is the likely reproductive success of wild fish in relation to possible releases (1:1 ratio of aquaculture:wild smolts) at Roti Bay cages. Would hatchery smolt have the same stamina in the wild as wild smolts? The 100,000 egg limit is very arbitrary. We should expect that cage-reared adult success in streams is not significant.

A: Pepper — The NASCO document I referenced in my presentation provides a considerable amount of information from around the world indicating that there have been interactions between aquaculture salmon and wild stocks. Currently, we simply do not know the consequences of these interactions. While the 100,000 egg limit may be arbitrary, it was set in consideration of the status of the Bay d'Espoir salmon stock most likely to be of concern should aquaculture salmon escape from the sea cages.

Comment: Johnstone

There is a concern that accidentally released farmed salmon may have temporally and spatially different movement patterns within rivers from that seen in indigenous stocks. Thus, redds of wild salmon may be overcut by farmed fish should the latter arrive later on the spawning areas. A Scottish example has been documented by Webb et al 1991. This is documented in my presentation to this workshop.

The extent to which active genetic transition takes place between wild and hatchery fish is not known but will be looked at in Britain and Scandinavia in the future.

Comment: Donaldson

Little is known about interbreeding between the two types of stock. Hybrids with time will likely take on wild characteristics in later generations, if numbers entering the river are not that great.

Comment: Pepper

The major goal of DFO in the Newfoundland Region is to minimize the spawning risk.

Comment: Johnstone

The extent to which actual genetic transfer between wild and hatchery fish takes place is not known but demands a measurable difference between the stocks in question. This situation existed in a Scottish example and is under continuing investigation. Any attempt to evaluate changes over time necessitates this kind of occurrence whether by accident or design.

Comment: Pepper

This is being done in England where the regional water authorities manage rivers. The Southern Water Authority has started a project to shift the salmon stock of the Test River (Southampton) from grilse to MSY. Natural production in the river has been about 25,000 smolts. The water authority has released 25,000 MSY Scottish farm smolts into this river. The project is being monitored by MAFF. The Lowestoft Lab nose tagged all introduced smolts and has been doing genetic typing on the two stocks.

Comment: Anderson

The grilse component of the Atlantic salmon population in Newfoundland is getting larger due to the selection of commercial fisheries which is a past phenomenon in Maritime rivers.

As long as we stock rivers we must be careful in matching/selecting stocks for enhancement purposes. Aquaculture is a different matter where one can experiment and manipulate stocks for breeding with greater flexibility. Hatchery adult returns tend to stay in the lower river. A fascinating situation.

In respect to marine salmon survival of escaped aquaculture stocks, there will be a symposium on marine survival sponsored by the Salmon Trust and the Atlantic Salmon Federation in 1992. The DFO now supports this initiative. It likely will be held in New Brunswick.

PRODUCTION AND PERFORMANCE OF TRIPLOID ATLANTIC SALMON IN SCOTLAND

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ABSTRACT

The chronological development of the means of production of all-female, all-triploid Atlantic salmon stocks in Scotland using pressure is reviewed. Their relative performance in both fresh and salt water is described. Triploids perform similarly in fresh water to diploids. In sea water, triploids grow less well for weight on length than maturing salmon but in a similar manner to non-maturing fish. Because they continue to grow steadily in length throughout their lives, they tend to have a characteristically long and lean appearance and are more like wild animals in their weight-length relationship. The existing information on the reproductive potential of Atlantic salmon female triploids is presented. Triploid female salmon have not matured at grilse age (1SW) in Scotland. A small minority of 2SW salmon have matured sexually and have produced eggs. On fertilisation with normal milt, a few embryos have developed to the eyed egg stage. Preliminary karyological analysis shows these embryos to have hyper-diploid, hypo-triploid chromosomal constitutions. Because none of the embryos so far obtained from triploid female crosses are expected to survive beyond hatch these results suggest that, for all practical purposes, all-female, triploid salmon stocks will be functionally sterile. It is concluded that triploid, all-female stocks will be of benefit both for commercial reasons and because they will minimise the effects of genetic transfer that might otherwise occur via accidental releases of farmed animals.

INTRODUCTION

The probable advantages that might follow from an ability to control maturity in farmed Atlantic salmon began to be appreciated during the very early stages of the development of the Scottish industry. This was because a relatively high percentage of fish were observed to mature after only one sea winter as grilse (industry average ca 30%), and this meant that a considerable proportion of total production had to be sold at small and less valuable sizes (< 2 kg) and in a narrow window of time (Johnstone 1989).

In the late 1970's it appeared that there were two differing approaches to the goal of maturity control. Some farmers in Scotland became involved in selection programmes in imitation of the earlier developed Norwegian industry (Gjerde 1984). This was in the expectation that age at first maturity had a genetic component and that later maturing stocks could therefore be bred selectively (for review see Refstie 1987). At the Marine Laboratory in Aberdeen an alternative approach was investigated which sought to describe the underlying physiological processes associated with maturity in this species and which attempted to identify more directly interventionist approaches to the production of later maturing or, ideally, non-maturing stocks. Attention was focused on those techniques which were thought most likely to be capable of relatively rapid industrial application.

SEX CONTROL IN ATLANTIC SALMON AND THE PRODUCTION OF ALL-FEMALE STOCKS BY STEROID HORMONE ADMINISTRATION

Since female salmonids were known to mature later than males, it seemed likely that later maturing stocks could be produced if it were possible to manipulate sex in the female direction. All-female stocks were expected to have two advantages in Atlantic salmon culture in Scotland. Firstly, the problem of male maturity in fresh water would be eliminated and, secondly, the grilse problem would be ameliorated since males were known to be more frequently represented in the grilse (1SW) fraction. As a result of the work of Yamamoto (1969) it was thought possible that all-female stocks might be produced either via the crossing of sex-inverted genetic females, produced by male hormone administration with normal females (assuming females are the homogametic sex), or by the direct administration of female hormones.

Contemporaneously with workers at our sister laboratory in England, and with others in Japan (Okada et al 1979), it was reported that the former approach could be applied to rainbow trout (Johnstone et al. 1979), this species being a particularly convenient experimental animal. Attention was next turned to Atlantic salmon and it subsequently demonstrated that a similar approach was applicable to this species (Johnstone and Youngson 1986). This technique has since been slowly adopted by several farmers in Scotland and the potential for producing all-female stocks by this method is expanding.

The administration of male hormones for sex inversion in Atlantic salmon was initially (Johnstone et al. 1978), and still remains to this day, associated with high levels of apparent sterility and of delay in age at maturation. An alternative direct approach to maturity control therefore suggested itself; that of the application of male hormone at oral doses higher than those necessary for sex inversion. Although it was demonstrated that this technique could substantially eliminate maturity at grilse age (Johnstone, unpublished observations), this approach was never accepted by an industry which was particularly concerned with maintaining a high value image for its product and which thought that the use of hormones in this way should be avoided. In any event, this kind of steroid hormone usage has subsequently been circumscribed by EC legislation. Thus direct feminisation using oestradiol, the alternative approach to all-female stocks alluded to above (Johnstone et al. 1978), was also rendered inappropriate although it has to be said that, in our experience, the feeding of oestradiol was more toxic and less uniform, under commercial conditions, in its effect than was the administration of methyl testosterone. Thus, oestradiol tended to be associated with lowered survival rates at optimal feminisation doses and to the production of bisexual gonads in animals of male genotype at lower doses. The male portion of these gonads tended to retain the male pattern of age at first sexual maturity and this reduced the usefulness of the technique (Johnstone, unpublished results.).

All-female salmon stocks have proved particularly useful in the elimination of the maturity problems associated with rainbow trout culture in the U.K. where most animals are grown only to portion size (250-500 g) (Bye and Lincoln 1986). In salmon culture, elimination of males comprehensively solved the problems associated with maturity in the fresh water phase at 1+ years of age but, since most Scottish farmers had opted by this time for the production of one year old smolts, this problem had diminished in importance. Additionally, all-female stocks were only ever likely to be a partial answer to the grilse maturity problem of Atlantic salmon because, although males were over represented in the grilse fraction, in high grilse years a proportion of females also matured. During the early 1980's, techniques for the production of triploid, expectedly sterile, stocks of salmonids, had been successfully demonstrated, initially via the use of heat shocks to recently fertilised ova (Chourrout 1980), and, subsequently, by the similar use of pressure shocks (Benfey and Sutterlin 1984; Chourrout 1984; Lou and Purdom 1984; Onozato 1984).

In contrast to male salmonid triploids, which are able to mature sexually but which are functionally sterile, female triploids were expected to be both endocrinologically and functionally sterile, and therefore to be of particular benefit to farmers. Since the production of all-female stocks of salmon had been demonstrated, the potential of producing sterile stocks via their triploidisation was next investigated.

THE PRODUCTION OF TRIPLOID STOCKS OF ATLANTIC SALMON.

The production of Atlantic salmon triploids in Scotland was first attempted in 1983 and, of the then available techniques, heat shock seemed the most likely to be capable of commercial application. The potential of heat shocks to produce triploidy in this species was demonstrated and the apparent relationship between temperature, duration and timing of initiation of shock described (Johnstone 1985).

However, since it subsequently was discovered that individual female fish differed in their susceptibility to a given heat shock, not only in terms of triploid rate but also in terms of survival rate and, therefore of triploid yield (Table 1), it was decided that heat shock was unlikely to be the method of choice for commercial application. Alongside the exploration of heat shock, the efficacy of pressure shock and of anaesthetic chemicals as triploidisation agents was investigated simultaneously. At the experimental level it soon emerged that pressure shocks, if of an appropriate intensity, duration and timing of application, could produce high yields of triploids in Atlantic salmon (Johnstone, unpublished results). Similarly, the efficacy of anaesthetic chemicals depended on similar variables and nitrous oxide was identified as the chemical of choice (Shelton et al. 1986; Johnstone et al. 1989). In experimental comparative trials with all three agents, nitrous oxide was intermediate in effectiveness of triploid yield generation, heat shock being poorest and pressure shock being best (Table 2.). The better performance of pressure was related primarily to the reduced variability in survival rates at high triploid rates following its use . It was therefore decided to explore the potential for commercial application of the high pressure triploidisation technique.

THE COMMERCIAL APPLICATION OF TRIPLOIDISATION USING PRESSURE.

Initial experience using a two litre vessel in the field, that is capable of treating eggs in commercial quantities, proved the effectiveness of the technique in terms of the generation of high (essentially 100%) triploid yields. The difficulties of moving from small batches of eggs at the experimental level, to large volumes under commercial conditions, initially led to markedly reduced survival rates relative to controls at some farms. These problems have now been overcome and the appropriate handling protocols for large volumes of eggs identified.

Since the sex inversion work referred to earlier had been adopted by several farms, it was by this time possible to couple the triploidisation process with that of fertilisation using milt from sex inverted females such that all-female, all-triploid stocks could be produced. Experience to date suggests that 90% yields of triploids at essentially 100% triploid rates are possible under commercial conditions at most Scottish farms.

Larger vessels (4L) have now been built and tested and the triploidisation process should not be rate limiting to the stripping process of females under commercial conditions except at the largest farms. Using a 4L vessel and the present operating parameters, and assuming a constant supply of eggs, some 16-20L (ca 80-100,000) eggs per hour can be processed. This technology has now been transferred to the industry and a triploidisation service is being offered by an Aberdeen based company. In the 1989 stripping season some 6 million eggs were triploidised at a number of sites throughout Scotland. The industry will increasingly, therefore, be in a position to evaluate their worth.

COMPARATIVE PERFORMANCE OF TRIPLOID AND DIPLOID SALMON IN FRESH WATER

Comparisons of all-female, 100% triploid stocks with equivalent diploid populations in the period from fertilisation to first feeding under closely controlled experimental conditions, usually demonstrate triploid egg batches to be some 30-50 degree days later in their development to this stage. In our experience, first feeding success is critically dependent on temperature and triploids may be not necessarily be disadvantaged by this later development, particularly if temperatures are low when the diploids start to feed. If temperatures are high at this time such that the diploids get off to a particularly good start and, further, if temperatures subsequently fall when the triploids start to feed, the latter may remain slightly behind their comparative controls throughout the freshwater phase and may produce fewer one year old smolts as a consequence. Smolt size seems little affected by triploidisation.

In one trial carried out under experimental conditions, 300 first feeding diploid or heat shocked triploid fry were reared in one metre diameter tanks. Duplicate populations were constructed by taking equal numbers of the survivors from an earlier heat shock variability trial involving ten different hens. Two months after first introduction of food relative survival rates were higher in the diploid replicates (94% and 96%) than in the triploids (91% and 87%). Thereafter survival rates in the period 2-7 months of age were again higher in diploids than in the triploids (94% and 96% vs 81% and 87%). Thus, overall to this point, survival rates were significantly ($p < 0.001$) greater in the diploid populations (89% and 92%, diploids; 75% and 76%, triploids).

All animals were measured at seven months of age when the populations had, as expected, become bimodal for length. The length frequency histograms constructed from this exercise are presented in Figure 1. Although diploid and triploid S1s were not significantly different in size at this time, triploid S2s were significantly shorter ($p < 0.05$). Although one year smolts were produced in greater yield in the diploid populations (42.5% and 44.8%, diploid; 39.4% and 35.1%, triploid), this difference was not significant (Chi square test $p = 0.17$).

No particular pattern of differential development has emerged under commercial conditions in those pressure treated populations monitored to date. Triploids have variously done slightly better than and slightly worse than their diploid counterparts both in terms of growth and survival. This might imply that in commercial practice, the presumed slight advantage of the diploid populations because of their faster development to the first feeding stage is counteracted by density related social factors at some later date. Nevertheless, the tendency towards later development of triploid eggs may indicate that triploidisation of eggs, stripped late in any one season, should be avoided except where facilities to augment the temperature of the water supply exist.

There is some anecdotal evidence that triploids generated by heat shock perform less well than those produced by pressure shock. This may relate to the more variable results seen, in our experience, as a result of heat shock procedures. It might be argued that the best agent for triploidisation is likely to be that in which all eggs are exactly and similarly exposed. This is most likely to be achieved if the exposure cycle has a "square wave" configuration since this is more likely to produce a uniform deformation of the meiotic spindle. For physical reasons, pressure treatments probably most closely conform to this ideal for salmon triploidisation. The biological variability data referred to in Table 2 would appear to support this contention. If a consequence of non-uniform spindle deformation were to be the induction of unbalanced chromosomal constitutions, these would certainly be associated with poorer survival. Although to our knowledge there is no karyological evidence for this hypothesis, it would be difficult in the current state of salmonid chromosomal analysis to detect very small disruptions to the normal genetic constitution.

COMPARATIVE PERFORMANCE OF DIPLOID AND TRIPLOID SALMON IN SEA WATER

The growth of triploidised all-female salmon stocks following transfer to salt water has been followed under both experimental (shore based tanks served by a pumped supply) and under fully commercial (cage culture) conditions. The relative growth rates of individually marked, experimentally reared heat shocked Atlantic salmon held in the same tank throughout the first year of culture are summarised in Table 3. Ploidy status of individuals was confirmed by an analysis of erythrocyte nuclear DNA content. The diploid animals in this experiment were, therefore, animals exposed to, but unaffected by, the heat shock procedure.

In the period between transfer and their first sea winter, growth rates were similar in all animals and there were no significant differences between diploids and triploids for either weight or length at the time of the October sampling 140 days after transfer (Table 3). The relative increase in weight gain of maturing and immature diploids and of triploid animals after this time is presented graphically in Figure 2. In March (day 299), immediately after their first sea winter, potential grilse were significantly heavier ($p < 0.1$) but not longer than triploids or those diploids which were destined to remain immature. Between 300-400 days after transfer, that is, in the spring and early summer period after their first sea winter, those diploid animals which subsequently matured after one sea winter (the grilse), experienced higher growth rates relative both to the potential two sea winter maturers (the diploid salmon) and to the triploids which grew at similar rates to each other. By day 435 (July) a majority of the grilse were obviously different in appearance; their original silver coloration having been replaced by the browner hues associated with their impending maturation. They were therefore sorted on this basis and put into a separate tank. Around this time they stopped feeding. Thereafter, in the same individuals, weight declined but length continued to increase slightly up to spawning time when they were culled.

At grilse age none of the triploid animals showed any secondary sexual signs of maturing. Coincidentally with the sorting of the diploid grilse to a separate tank, the diploid salmon and the triploids underwent a more rapid period of growth and by September (ca 500 days) were, once again, similar in size to the maturing grilse. Although the mean weights of the diploid salmon and the triploids continued to diverge towards the end of the experiment in February, they were not significantly different at this time. The growth comparison was abandoned at this point because it was thought that the number of diploid animals remaining (8) was too few to permit meaningful comparison. Although the assessment of their relative growth was terminated at this time, a proportion of the remaining animals (4 diploids and 11 triploids) survived to the beginning of the third sea winter and these were monitored for their comparative rates of ovarian development (see below).

Serum oestradiol levels in the different categories of fish in the period up to grilse maturation are presented in Figure 3. In contrast to the typical and prolonged rise in the sexually maturing grilse, levels in immature salmon and triploid animals remained low. In triploids, serum oestradiol levels were consistently determined to be at or below the limit of detection of the assay used.

Although there is considerable advantage in being able to monitor the growth of individually marked animals under experimental conditions, there are associated weaknesses in this particular experiment. The relatively small number of animals used in the various categories (8-22) had the inevitable consequence of making mean values overly susceptible to influence by the occasional accidental death or by greatly altered growth rates experienced by individuals. Thus, the apparent divergence of the triploid and immature diploid curves towards the end of the experiment, was exaggerated by the death of three larger than average triploids and by the death of two smaller than average immature diploid animals. Similarly, the apparent increase in mean grilse weight in the 560 day sample was a consequence of a period of very rapid growth in two 'late' grilse which were not originally categorised as such on day 435 (i.e., at the time when most grilse first became obvious). It can be argued further that not only might the relative performance of the different categories have been influenced by their being grown together but that, in addition, the overall general growth performance of the fish might have been reduced by their being grown in small ($2m^2$), shallow (0.5m) tanks.

Nevertheless, the relative growth picture of diploids that emerged from this experiment, was essentially similar to that described in farmed grilse and salmon by Youngson et al. (1988). Thus, in the spring and early summer months of the year of their maturation, maturing farmed animals experienced an elevation of presumed anabolic serum sex steroids and a coincident increase in growth rate relative to immature individuals. Some farmed grilse in this study apparently began to mature later, that is immediately after the first grilse to become obvious were removed, and the inference was made that the maturity of these 'late grilse' was, in some way, conditioned by the removal of the more dominant early grilse. The present study, involving individually marked animals, confirmed the late maturity phenomenon and it's coincidence, and that of an associated period of rapid growth, with the removal event. Because of the lack of suitable controls, we are unable to comment on the significance of that act in the initiation these processes.

This experiment suggested that triploids grew similarly for length and weight as did non-maturing salmon but less well in the late spring/early summer period than fish maturing as grilse. It also confirmed the expected sterility at grilse age of triploid females and offered the promise that triploid females would be of benefit. Experience with comparative performance of triploids under commercial conditions was clearly going to be necessary in order to assess their proper worth to the industry.

An analysis of the performance under commercial conditions of triploid salmon in sea cages awaited the arrival of significant numbers of fish and by 1988 these had been produced. In industrial practice, however, it should be remembered that scientists work under a different set of constraints that may serve to reduce the value of the information that can be gathered. Commercial considerations necessarily come first and, although numerically large numbers of animals can be measured, even apparently replicated populations can, in our experience, often be demonstrated to have performed significantly differently under commercial conditions. This can frustrate the extent to which meaningful conclusions can be drawn from data gathered under these circumstances. It is in this context that the commercial results presented below should be considered.

All-female, essentially 100% triploid stocks, were generated on site at several farms using inverted milt and our high pressure method. Triploids were compared at two sites with equivalent diploid batches that had not been subjected to the pressure shock. Fish were reared throughout using normal industrial practices and every attempt was made to treat triploids and diploids similarly. At sea they were maintained in similar and adjacent cages on the same raft (Farm A) or in similar cages on adjacent rates (Farm B).

At farm A, triploid and diploid smolts were stocked at a mean weight of ca 45g into 285 m^3 cages at a rate of 3,000 fish per cage. This meant that the populations remained ungraded throughout the first year. At farm B, 55g smolts were stocked at 6,000 per 330 m^3 and were thinned, as they grew, into adjacent cages. During sampling visits to both sites the cages being monitored were shallowed, divided and the lengths and weights of ca 50 randomly netted anaesthetized animals taken. When signs of maturity became evident in the diploid populations in mid-summer, the lengths and weights of 50 mature and 50 immature animals were taken.

At farm A, there was similar fish growth during the first 6-8 months post transfer (Table 4.). Although one of the triploid populations apparently outperformed the other cages in this period, there were no significant differences in the weight on length relationships of the populations under review at this time (Figure 4.). Weather conditions prevented the collection of data from one of the diploid populations during the April sampling at this farm, but analysis of variance showed indicated no significant differences in the mean sizes of those populations which were measured. Regression analysis of the weight-length relationship showed the diploid population which was monitored in April to have had a significantly different intercept, that is, to have been heavier for a given length than the separate triploid populations which were not significantly different in this regard (Figure 4.). The reason for this became obvious by July when those fish maturing as grilse were easily identifiable on the basis on their coloration. They were shown to have been significantly heavier and longer and to have been heavier for a given length than the immature diploid salmon from the same cage (Figure 4.). Regression analysis showed there to be significant differences in both the slopes and intercepts of the separate triploid and immature diploid populations at this time, the shorter triploids being generally slightly lighter for a given length than diploids.

The maturity rate in the diploid populations at grilse age in this farm was 12%. In contrast, only three of ca 5000 triploid fishes sorted showed the coloration typical of sexual maturation. No ploidy analyses were performed on these animals but they had the typical diploid weight on length relationship. They are presumed to represent either a residuum of animals not rendered triploid by the pressure treatment, or to be diploid animals that had been accidentally introduced into the triploid population at some time during their earlier rearing history.

The data collected from farm B are summarised in Table 4 and in Figure 5. Both diploid and triploid stocks grew better at this farm and, because they were retained longer, perhaps allow a more complete picture to be drawn of the comparative performance throughout the whole of the normal span of the farm cycle. Only two cages were compared at this site, one diploid and one triploid. The extent to which it is safe to compare un-replicated cage to cage relative performance should be borne in mind in the interpretation of the results. As before, cages were compared for relative size ("t" test) and their weight on length relationship was interrogated using regression analysis.

At the beginning of the monitoring exercise, in December 1988, some seven months after transfer to salt water, triploids were not significantly different in length but were less heavy than the comparative diploid population ($p < 0.1$). A similar situation was seen in April 1989. The significance of the difference in mean weight having increased ($p < 0.05$). In June 1988, at the time of the grilse sort, maturing animals were significantly heavier ($p < 0.01$), but not longer, than immature diploid potential salmon. Fish classified as immature were significantly heavier and longer than the triploids at this time ($p < 0.05$) but this comparison assumes a correct classification of maturity status of diploid animals. The inclusion of unidentified maturers (late grilse), which had not yet assumed their secondary sexual coloration, would necessarily have diminished the appropriateness of this comparison. As the fish entered their second sea winter in December 1989, 19 months after transfer, triploids were longer (n.s.) but significantly ($p < 0.05$) less heavy than the diploids which were destined to mature as salmon. In the following March and July the diploids maintained this weight advantage ($p < 0.025$) as they began to mature. The triploids continued to increase in length and remained longer than diploids (significantly so only in the March sampling; $p < 0.1$). At the time of the July sampling, some 26 months after their transfer to sea water, the diploids were 400g heavier in mean weight. Apart from those animals retained as broodstock, the majority of the diploid animals were marketed shortly afterwards. The triploids were retained for a further two months and continued to grow in both length and weight. The mean weight and length figures for the triploid population in September (28 months after transfer) were 5543 $\pm$139g and 76.4 $\pm$0.77cm respectively.

Regression analysis showed triploids and diploids to have different slopes and different intercepts in the period up to the grilse sort (Figure 5), diploids being heavier for a given length than triploids. At this time, 13 months after transfer to sea water, the mature animals, as at farm A, were heavier for a given length than were the immature diploids or the triploids. Thereafter regression analysis revealed the diploids destined to mature as salmon to be consistently heavier for a given length but to have had the same slope as the triploids. The triploids, because they tended to continue to increase in length throughout their lives, were characteristically long, relatively lean fish. They were more similar, therefore, in their weight-length relationship at this time to wild fish. On their return to mainland rivers in Scotland, wild fish are less heavy for a given weight than are triploids (see Fig. 5, data for 2SW river North Esk fish kindly supplied by D.Dunkely, SOAFD). The appropriateness of this comparison would be affected by the better fin condition of wild animals; farmed fish having shorter tail fins as a proportion of their body length than wild animals (Lund et al. 1989). Since any correction made for this phenomenon would effectively move the triploid curve in Fig. 5 to the right, it would have the effect of making the weight-length relationships more similar. The acceptability and palatability of triploid animals such as these, both to farmers and consumers, is only now being assessed.

COMPARATIVE SURVIVAL RATES AND DISEASE SUSCEPTIBILITY IN SEA WATER

To the extent that comparative survival rates were collected during these commercial trials no major differences were reported. Pancreas disease (Munro et al. 1984) was recorded at both farms. In farm B, the experience was said to have been more severe in the triploid population although affected numbers were low (< 5%). The pancreas disease episode was much more severe at farm A. This led to the premature culling of the stocks involved and to the unplanned termination of the experiment. Although diploids and triploids were reported by farm staff as having been similarly affected by the disease at this site, no systematic attempt was made to test the veracity of this communication.

Anecdotal information communicated to us from an additional site where we were not monitoring growth suggested that heat shocked triploids were more resistant to a chronic BKD outbreak that led to continual low level losses in the diploid controls. An attempt was made to test this observation experimentally but no significant differences in susceptibility to this disease were found in the populations tested (Bruno and Johnstone 1990). Triploids have been said to be slightly more susceptible to furunculosis at another site but it has not yet been possible to evaluate the significance of these findings. Observations of this kind do however suggest that there is a need to interrogate the relative susceptibility of triploid and diploid stocks. To be done properly, however, this demands not only a knowledge of the manner in which reliable infectivity trials may be carried out but also an understanding of the genetic make up of the stocks under evaluation and their ploidy status.

Triploid salmon have fewer, larger erythrocytes and it has been argued that they may therefore be more compromised by conditions of lowered environmental oxygen. While it can be argued that the competent farmer should not unduly expose the fish in his care to prolonged periods of oxygen depletion, there are inevitably periods when this may occur. Triploids might be considered to be at increased risk over diploids, for example during crowding, grading and sea lice treatments. In the last of these, which is considered to be highly stressful, the net is conventionally shallowed and surrounded by a tarpaulin in order to create a treatment "bath", into which an organophosphate is introduced. Additional oxygenation is provided during the treatment period. Although in the commercial trials reported above fish were regularly and successfully treated at both farms, it has subsequently been reported to us that triploids have an increased tendency to die during such treatments. It is assumed that this is an oxygen deprivation affect rather than an increased susceptibility to the chemical used and perhaps indicates a need for accurate monitoring of oxygen levels during treatment.

REPRODUCTIVE PERFORMANCE OF TRIPLOID ATLANTIC SALMON

The presumed advantage of triploid stocks for industrial use rests on the expectation that female triploids will be sterile. An additional advantage, were this to be proven true, would lie in the potential to reduce the genetic impact of accidental escapes from farms. Escaped fish may interbreed with naturally occurring populations and this possibility is a cause of some concern (Maitland 1989). That farmed fish escape during their culture in Scotland is not in doubt (Webb 1990). They also have been shown to migrate into and to spawn in a river adjacent to their site of release (Webb et al. 1991). The extent, however, to which farmed and wild fish actually interact genetically in Scotland is not yet known but is currently under investigation.

The ovaries of triploid animals held in fresh water commonly comprise an apparently sterile matrix of undeveloped oogonia containing the occasional oocyte. This is in complete contrast to the diploid ovary which is packed with oocytes. This picture is maintained in the early sea water phase and we have, to date, observed no proven example of a triploid animal maturing at grilse age. A small number of maturing individuals from both of the populations described above (the experimental, heat shocked population, and that held at Farm B) which have been maintained beyond their second sea winter have however been identified.

In the experimental population, four of eleven triploids looked capable of producing eggs at salmon age (i.e., after ca 30 months in sea water). Three killed prematurely had significantly ($p < 0.01$) lower GSI values (mean 8.3% range 7.3-9.4%) than did comparable diploids (n=4; mean diploid GSI 16.3%, range 15.5-18.1%). The remaining animal died before eggs could be obtained. After some 26 months of sea water growth, the remaining fish in the diploid population at Farm B were all showing signs of maturing as salmon. The majority were sold but some (120) were retained for broodstock purposes. Of some 3000 triploids which had been retained to this time, 50 including all those thought to be potentially maturing, were selected for further maintenance along with the diploid broodstock. By 30 months of age, 10 of these 50 animals including the only two obvious maturers, three possible maturers and five immatures, were taken to fresh water along with three control diploids. The two obvious maturers were subsequently proven to be diploids by erythrocyte nuclear DNA measurement and were presumed to have been animals that were untriploidised by the pressure treatment or to be diploid animals which had accidentally been transferred to the triploid population. The three possible triploid maturers were confirmed as such by DNA analysis and went on to produce eggs. Thus, only three of ca 3000 triploids underwent maturation at salmon age in this population in contrast to the diploid controls which had all matured by this time either as grilse (17.5%) or as salmon (the remainder). Serum oestradiol levels in triploids at salmon age were low (< 1ng/ml). Those few triploids that showed some evidence of an ability to produce eggs had higher levels than was typical of triploids in general but not as high as those seen in maturing salmon aged diploids.

Eggs from the maturing triploids were fertilised with normal sperm in December 1990. The diploids matured as normal and gave eggs which were readily fertilised and which generated high numbers of survivors (> 85%). In contrast to the diploid eggs which were of uniform size, the eggs stripped from triploids differed markedly in size and most eggs from triploid females underwent little obvious development. In the eggs of two triploid females a minority (ca 10%) of those eggs fertilised developed to the eyed egg stage. The embryos in these eggs were clearly malformed and none is, at the time of writing, considered likely to survive beyond hatching. Karyological analysis of a sample of these embryos showed them to be intermediate in chromosome number (hyperdiploid and hypotriploid). Eggs were also fertilised with UV irradiated sperm but there were even fewer survivors in these fertilisation batches and all were of a weak and malformed appearance. No evidence of an ability to form unreduced oocytes has so far been demonstrated.

Triploid axolotl females also produce eggs at an older age and in reduced numbers compared to diploids (Humphrey and Fankhauser 1949; Fankhauser and Humphrey 1950.). Almost all of the survivors of triploid female x diploid male crosses in this species had unbalanced chromosomal constitutions and rarely survived beyond hatch. Some individual females, however, had the ability to produce unreduced (triploid) oocytes and these resulted in tetraploid progeny when crossed with normal diploid males. Tetraploid females so produced also attained sexual maturity and, when crossed to diploids, produced triploids. Except for their possible ability to produce the occasional unreduced oocyte, these second generation triploids were expected to be as functionally sterile as those of the first generation.

Rainbow trout triploid males are capable of sexual maturity and produce a sperm with variably sized gametes. This sperm is incapable of producing normal embryos when used to fertilise normal eggs (Lincoln and Scott 1984). The maturing triploid female salmon referred to above are, therefore, similar in this regard to triploid salmonid males. This potential of female triploid salmon to mature sexually is a situation arguably more readily explicable than that of their previously presumed endocrinological non-functionality. Since diploid females also mature later than diploid males, it seems likely that a similar explanation is responsible for the difference in timing of age at first maturity in the sexes irrespective of their ploidy status (Meerburg 1986). The ultimate endocrine potential of any individual triploid female will presumably relate to its ability to form oocytes capable of surviving into the growth phase since the developing oocyte, with it's follicular cell envelope, is the endocrine unit of the salmonid ovary. The evidence currently available suggests that most triploid female salmon rarely form oocytes with this potential and may never achieve the hormonally active state or may remain endocrinologically inactive for prolonged periods of time. Rainbow trout females would appear to be even more refractory in this regard than are Atlantic salmon females since no mature females of the former species have yet been recorded. Similarly, in the Amphibia, certain species appear to be less able to mature as females than axolotls (Humphrey and Fankhauser 1949). Although rainbow trout female triploids may never reach sexually maturity, their presence would be noticeable only by their (probable) lowered GSI or the non-viability of their eggs on fertilisation. They may therefore have merely escaped detection. Artificially produced triploid rainbow trout females may not yet have been reared for long enough to assess their ultimate reproductive potential.

Naturally occurring triploid salmonids are presumed to result from the ability to produce an occasional unreduced oocyte and it must be assumed that triploids will retain this potential. The low rate of occurrence of this phenomenon in diploids, taken together with the markedly lowered facility to form oocytes capable of maturing in triploids, leads us to assume that the formation of unreduced oocytes in Scottish stocks will be an extremely rare event. Even if, as in salamanders, a proportion of triploids were demonstrated to be able to produce unreduced oocytes, their fertilisation would lead to alternating tetraploid and triploid generations assuming that continuity of fertilisation proved possible. It may therefore be concluded that triploid females are, for all practical purposes, functionally sterile. It is unlikely, therefore, that triploids will be capable of interacting with naturally occurring populations, save at the behavioural level.

ALTERNATIVE METHODS FOR THE PRODUCTION OF TRIPLOIDS

Triploids can be produced by the crossing of tetraploid animals with diploids. This has proved possible in rainbow trout (Chourrout et al. 1986) and demands a knowledge of the time at which the first mitotic division of eggs can be manipulated. Although close to identifying the timing of this event in salmon, we have not as yet produced tetraploids and are unable to predict whether triploid salmon may be produced in this way. In the French study, fertilisation rates of diploid females by tetraploid males were generally lower than those seen in normal males. This was dependent on sperm head diameter. The crossing of tetraploid females with diploid males might therefore be a more attractive option for producing triploid stocks. It remains to be seen whether tetraploid salmon females can be produced or whether they will achieve eventual functional maturity. Tetraploids are not expected to be easy to rear, however, and the reasons for this have been discussed by Chourrout and his colleagues (1986). Early maturing, land-locked strains of Atlantic salmon may be particularly valuable as experimental animals to enable a more rapid analysis of the potential for this approach. At the present state of our understanding, this seems unlikely to become an alternative means of producing triploids in the short term.

SUMMARY

The evidence presented in this paper, though necessarily of a preliminary nature, suggests that triploid female Atlantic salmon stocks have considerable potential for farming purposes. In Scotland the stocks so far triploidised have not matured to any significant extent during the normal length of the farming cycle as currently practised. Whether this will hold true for other stocks will need to be investigated. Triploids grow in sea water in a predictable manner and at a rate similar to that of non-maturing fish. Although they are characteristically long, relatively thin fish, market yields in terms of weight per unit of rearing volume per unit time in triploid stocks should be greater since more animals would be expected to grow to the larger and more valuable sizes. Their functional sterility should be of additional advantage in areas where the possible impact of accidental releases from farming operations is causing concern.

It is considered that further work is needed in the area of comparative disease susceptibility and on the best means of integrating triploids into the farming cycle. It is possible that triploids may be of especial advantage if staggered inputs are to become a reality in salmon farming practice. The present seasonal production of fish in Scotland of the most marketable sizes (2-4 kg; May to November) is an inevitable consequence of single input systems. Although some flexibility can be introduced by the use of two year old smolts, these currently are not highly regarded in Scotland because of their greater alleged tendency to mature as grilse. Inputs of triploid two year old smolts of a variety of sizes have the potential to eliminate this disadvantage. The ability to produce triploid female smolts of large size, without any attendant maturity problems and which are likely therefore, in our experience, to be tolerant of transfer to salt water over a wider period of time than smaller smolts, should further enable input times to be widened and output to be smoothed.

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Table 1. Variability in survival rates*, triploid rates and triploid yields in the eggs of ten different Atlantic salmon hens exposed to the same heat shock (30 °C x 10 mins. at 20 mins. a.f.)

Hen No.	Survival Rate (%)	Triploid Rate (%)	Triploid Yield (%)
1	21.7	100.0	21.7
2	63.0	96.0	65.6
3	81.5	96.0	78.2
4	79.2	98.0	77.6
5	0	-	-
6	82.6	100.0	82.6
7	35.8	95.9	84.4
8	39.7	90.2	35.8
9	11.9	96.2	11.5
10	53.0	98.0	51.9
MEAN ± S.D.	46.8 ± 29.9	96.7 ± 2.9	51.0 ± 26.4

* % relative to controls.

Table 2. Comparative triploid yields in the eggs of ten different Atlantic salmon hens using different agents.

Hen No.	Heat	Pressure	Nitrous oxide
1	33.3	97.9	73.5
2	25.9	97.4	85.2
3	97.4	98.5	77.1
4	93.2	98.2	81.4
5	94.3	100	76.5
6	99.6	100	92.9
7	24.6	100	75.7
8	6.3	100	76.6
9	26.6	100	75.9
10	98.3	100	89.5
MEAN ± S.D.	60 ±12.4	99 ± 0.3	80 ± 2.1

Triploid yields calculated as % relative to controls; all triploid rates essentially 100%.

Table 3. Comparative weights and lengths of diploid and triploid Atlantic salmon at different times after transfer.

Time after transfer (days)	Diploid mature (grilse) Length (cm ± SEM)	(No.)	Weight (g. ± SEM)	Diploid immature (salmon) Length (cm ± SEM)	(No.)	Weight (g. ± SEM)	Triploid Length (cm ± SEM)	(No.)	Weight (g. ± SEM)
0	12.0±0.2	(16)	—	11.9±0.1	(10)	—	12.5±0.2	(22)	—
140	24.3±0.2	(16)	170.5± 5.0	23.6±0.5	(10)	156.1± 10.4	24.0±0.4	(22)	156.0± 7.7
299	36.3±0.4	(16)	588.0±19.6	34.5±1.1	(10)	510.9± 51.0	35.1±5.4	(22)	500.5± 25.1
378	40.4±0.6	(16)	924.4±46.9	36.6±1.3	(10)	591.5± 57.6	37.2±6.3	(22)	603.4± 34.1
435	41.4±0.6	(16)	966.9±46.8	37.6±1.3	(10)	616.5± 72.0	38.1±0.7	(22)	632.0± 38.2
473	41.1±2.5	(16)	1039.7±36.0	42.0±1.6	(10)	971.0±105.0	42.6±0.7	(22)	918.4± 44.0
530	43.8±0.6	(16)	963.4±41.7	47.0±1.4	(8)	1344.0±129.0	46.0±0.7	(19)	1175.8± 69.1
574	44.2±0.6	(12)	962.5±48.7	49.3±1.4	(8)	1598.0±156.0	47.9±0.8	(19)	1369.0± 90.5
649	—		—	51.4±1.5	(8)	1686.0±189.0	49.7±1.0	(17)	1420.0±109.0

Table 4. Comparative weights and lengths of diploid and triploid Atlantic salmon at different times after transfer in two commercial sites in Scotland.

Farm A.

Time after transfer.		Length (cm ± S.E.M.)	Weight (g ± S.E.M.)
8 months	B9 diploid	39.11 ± 0.31	694.3 ± 16.9
	B11 triploid	40.11 ± 0.34	745.2 ± 18.0
	B12 diploid	39.99 ± 0.28	757.3 ± 15.9
	B13 triploid	41.33 ± 0.40	820.0 ± 21.9
11 months	B11 triploid	45.39 ± 0.32	956.9 ± 20.1
	B12 diploid	45.12 ± 0.42	998.4 ± 30.8
	B13 triploid	46.19 ± 0.40	984.7 ± 27.8
14 months	B9 diploid grilse	49.51 ± 0.32	1535.4 ± 34.1
	B9 diploid salmon	48.29 ± 0.47	1115.1 ± 32.9
	B11 triploid	50.25 ± 0.40	1195.4 ± 31.9
	B12 diploid grilse	50.31 ± 0.32	1613.3 ± 33.3
	B12 diploid salmon	47.71 ± 0.40	1029.2 ± 25.7
	B13 triploid	50.79 ± 0.41	1194.3 ± 29.8

Farm B.

Time after transfer.		Length (cm ± S.E.M.)	Weight (g ± S.E.M.)
7 months	diploid	40.52 ± 0.43	872.5 ± 26.2
	triploid	40.43 ± 0.52	805.9 ± 26.8
11 months	diploid	50.39 ± 0.35	1514.9 ± 36.9
	triploid	50.25 ± 0.41	1410.3 ± 35.8
13 months	diploid grilse	54.15 ± 0.43	1975.8 ± 52.0
	diploid salmon	53.97 ± 0.39	1711.8 ± 36.8
	triploid	52.48 ± 0.67	1505.7 ± 52.5
19 months	diploid	60.59 ± 0.66	2774.8 ± 90.3
	triploid	61.10 ± 0.42	2505.1 ± 60.3
22 months	diploid	66.97 ± 0.62	3889.8 ± 126.4
	triploid	68.71 ± 0.74	3672.9 ± 144.1
26 months	diploid	73.04 ± 0.64	5604.9 ± 153.2
	triploid	74.24 ± 0.52	5201.2 ± 134.3

Figure 1. Length frequency histograms of duplicate triploid and diploid tanks

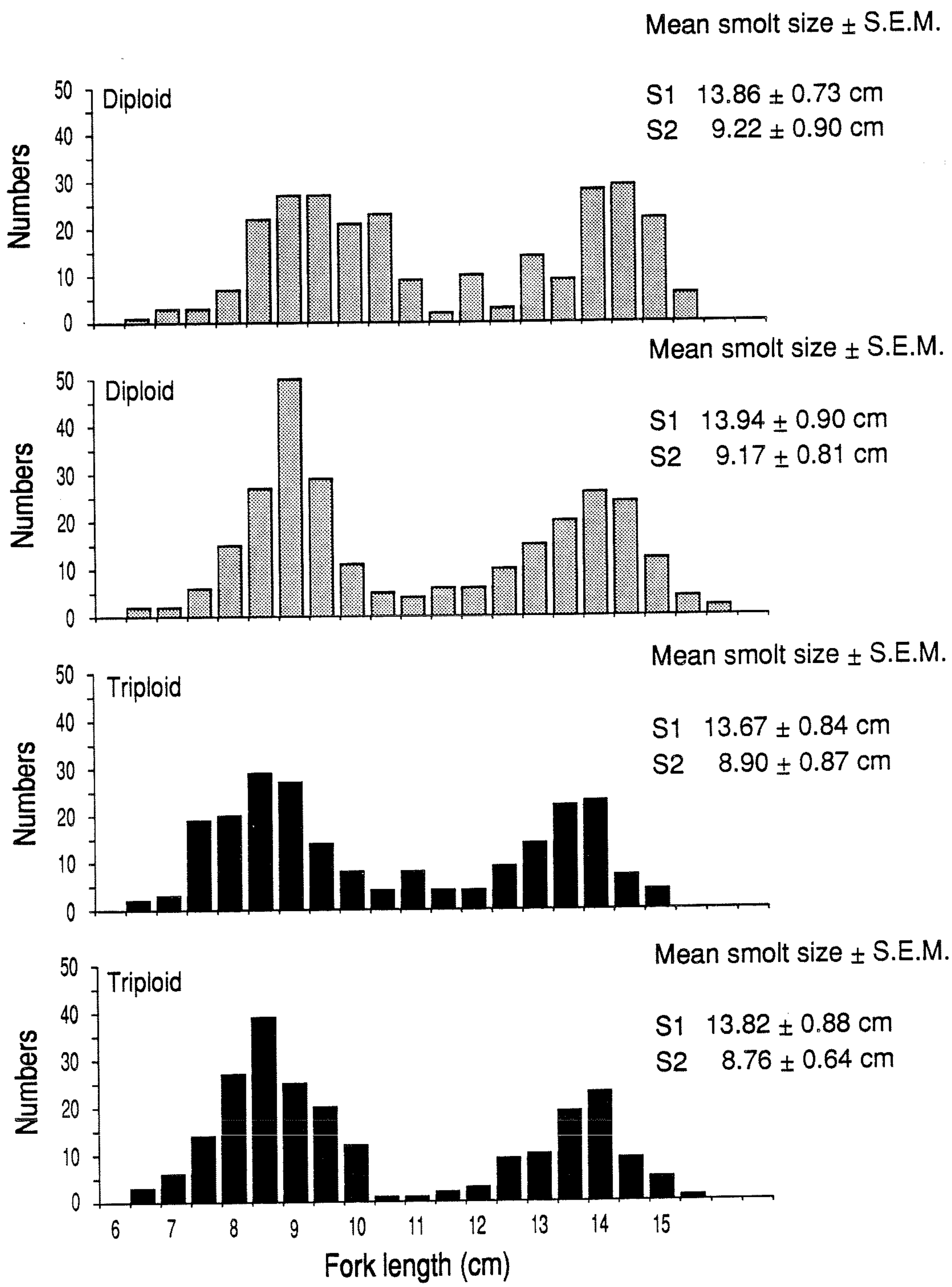


Figure 2.

Comparative increase in weight of all - female diploid and triploid Atlantic salmon following transfer to salt water.

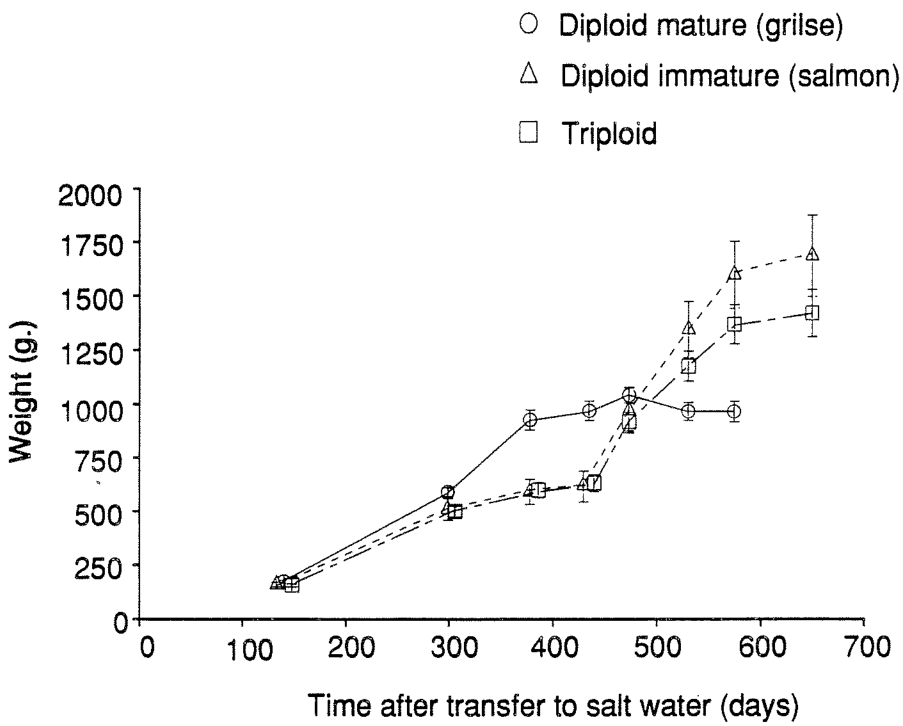


Figure 3. Comparative serum oestradiol levels in maturing diploid and immature diploid and triploid Atlantic salmon after transfer to salt water.

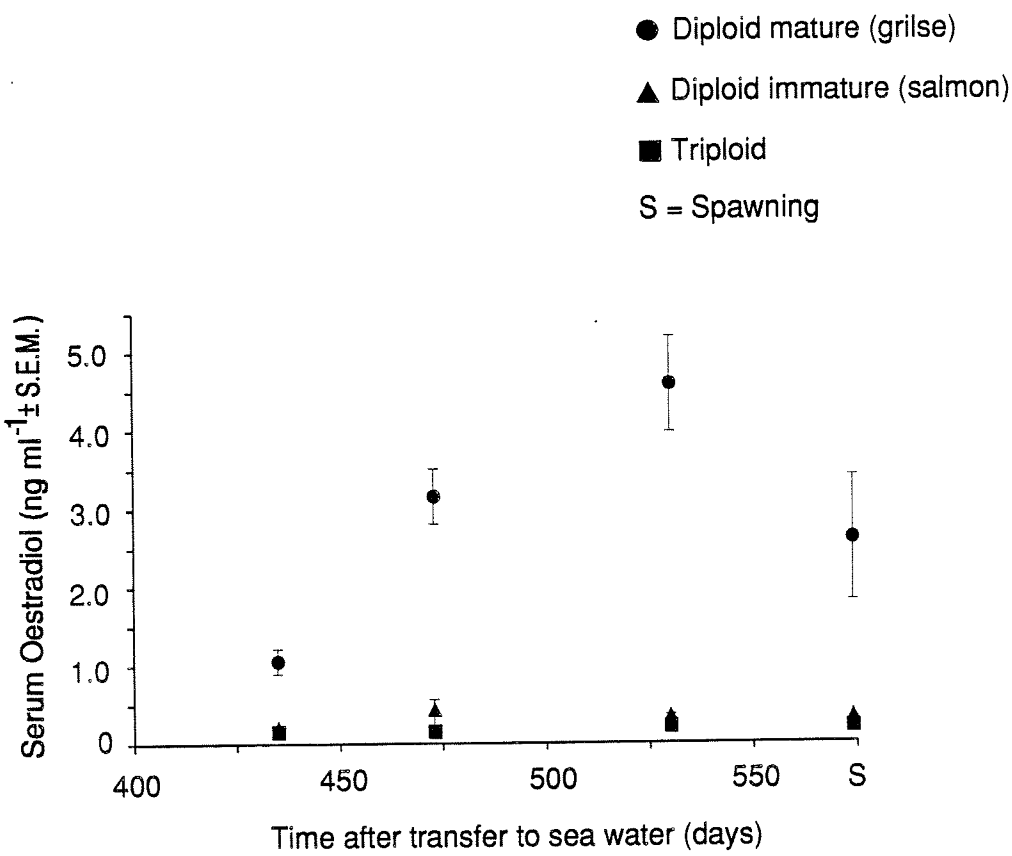


Figure 4. Farm A. Weight length relationships at different times after transfer in replicated cages.

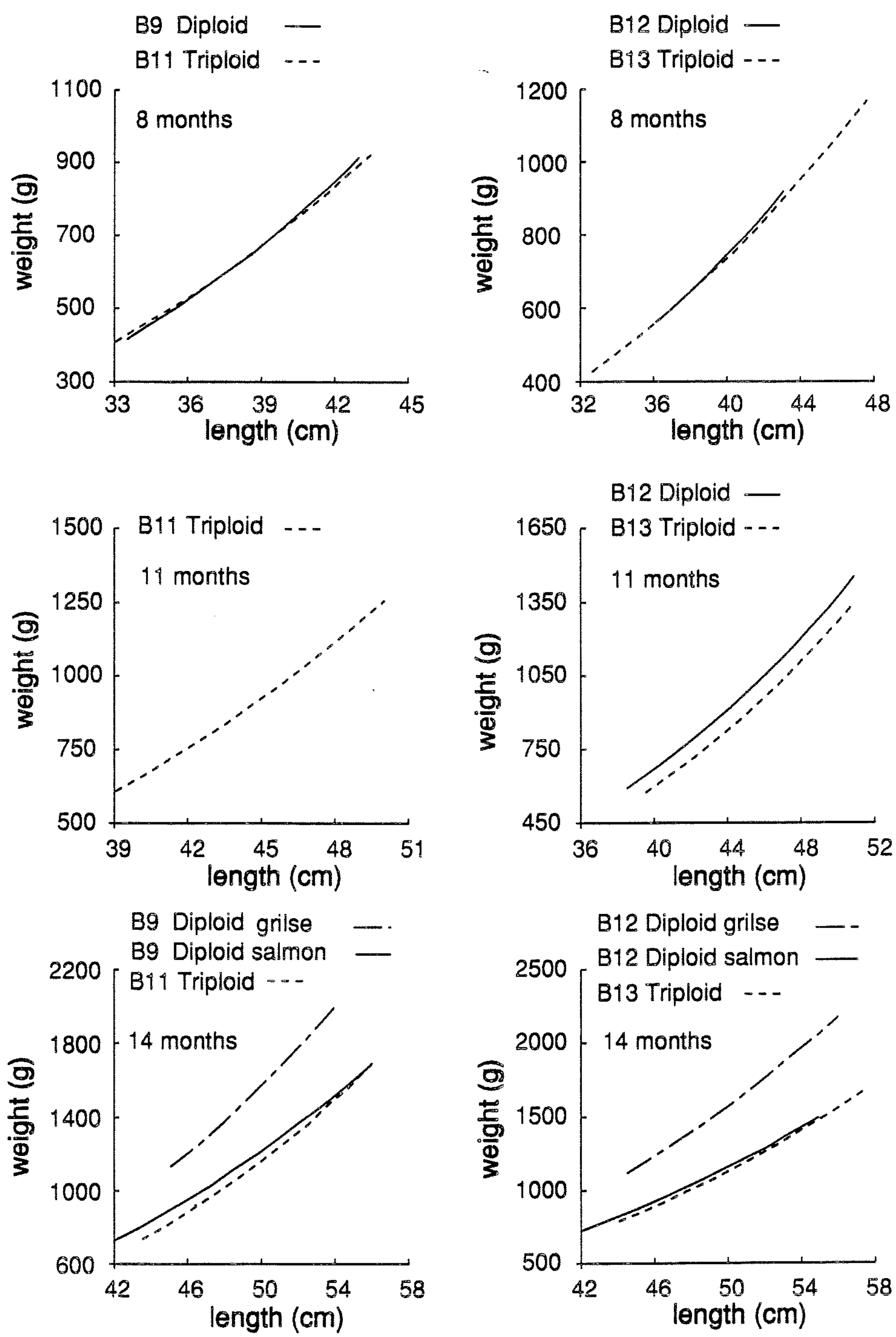
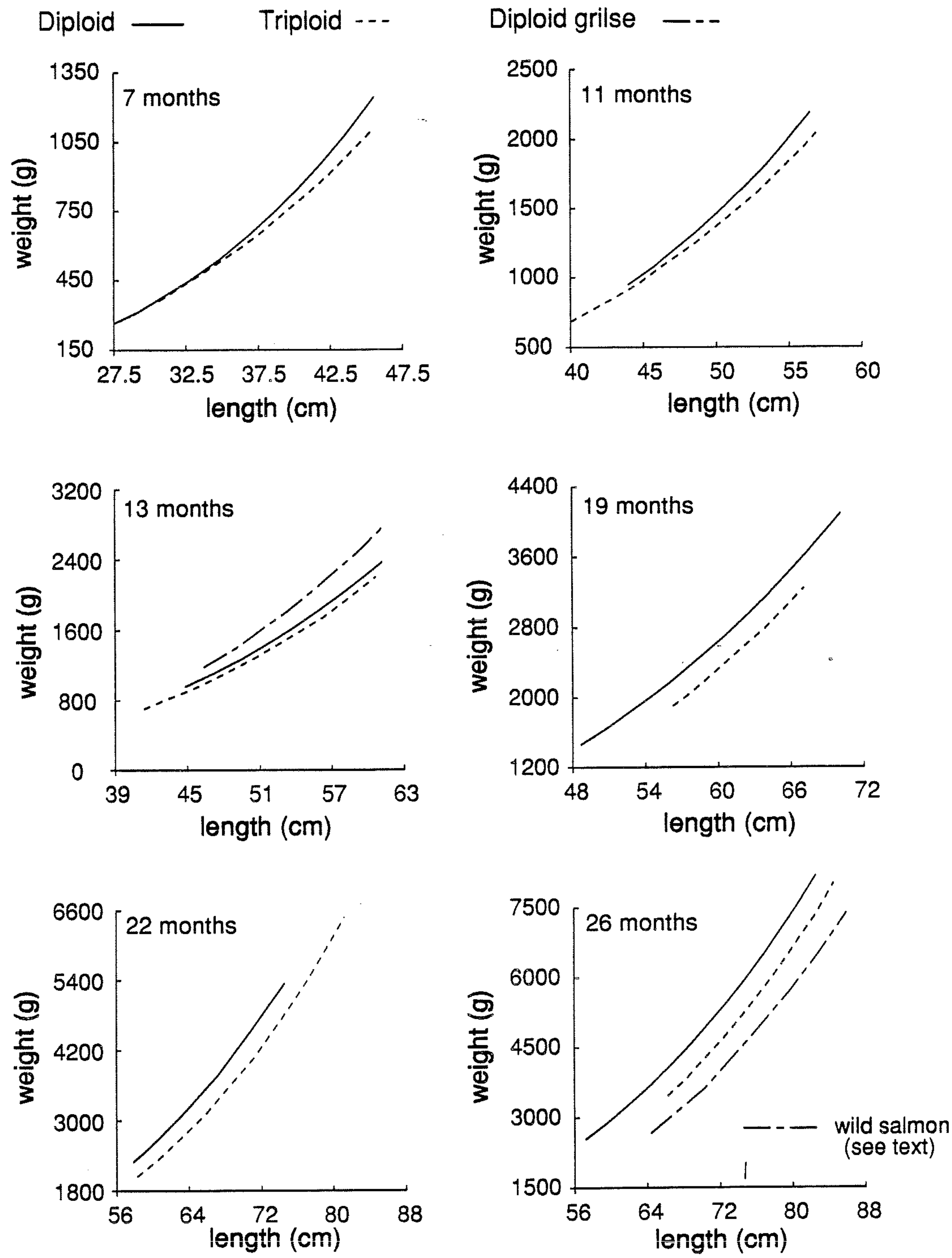


Figure 5 Farm B. Weight / length relationships at different times after transfer.



PARTICIPANT DISCUSSIONS

Rapporteur: Kevin Davidson

Q: Friars — Do you go right back to the egg stage when you calculate triploid yield?

A: Johnstone — Yes. The percentages given represent the number of animals which are both triploid and which survive to the point of first feeding expressed as a percentage relative to control survival values. Any grossly deformed animals at this time are counted as mortalities since they are unlikely to survive. In our hands, heat shocks produce more deformed survivors than appropriate pressure shocks.

Q: Friars — Is the time of development the same for triploid fish?

A: Johnstone — Triploid fish take 30 to 50 degree days longer to achieve first feeding. I had assumed that later first feeding would result in slower growth throughout development. Surprisingly, this does not always seem to be the case. Triploid fish may be able to 'catch up' in terms of growth.

Comment: Friars

The animal has the opportunity to make up time in terms of growth. The shocking may result in selection for healthier animals. I have noticed that shocking of eyed eggs seems to result in selecting for healthy fish.

Comment: Benfey

We have crossed triploid males with diploid females and ended up with an "average" ploidy of 2.5 N and very poor survival. These results are very similar to those found for grass carp where similar crosses resulted in poor survival of fry.

Comment: Johnstone

This type of cross results in a broad range of aneuploids which will ultimately die.

Comment: Benfey

In the experiment with grass carp, no triploid eggs were produced and there was broad variability in ploidy with a mean of approximately 1.5 N.

Q: Friars — It would seem to me that the crossing of tetraploids with diploids to give triploids would be the preferred situation, but I understand that there is a viability problem with tetraploids that is difficult to avoid. It seems that the French are having some success with tetraploidy. Is this true?

A: Johnstone — I agree that the use of tetraploid broodstock has attractions, however tetraploids may be difficult to produce and to maintain. I certainly find them difficult to produce since the 'window' for their creation is very narrow. Because of these difficulties, their production and maintenance might be considered to be more suited to governmental rather than industrial action.

Comment: Boulanger

We have had good success in getting tetraploid rainbow trout but have been unsuccessful when attempting this with speckled trout. Tetraploid males have very large sperm that are too big to enter the micropore.

Comment: Saunders

A Swedish scientist, Svardson, proposes that tetraploids occur naturally in wild populations.

A: Johnstone — Salmonids generally are considered to have undergone a tetraploidisation event in their evolutionary history. This has been followed by a movement towards the original diploid number by fusion of chromosomes.

Q: Saunders — Would this suggest that it should be easy to create the tetraploid condition?

A: Johnstone — I haven't found this for Atlantic salmon although the French have produced rainbow trout tetraploids. I have found tetraploid salmon difficult to produce, perhaps we actually are trying to produce octaploids.

Q: Glebe — Is triploidy applied to Norwegian or Scottish stocks?

A: Johnstone — I suspect that the triploid technique will have to be more generally applied. This is because there are both genetic and environmental aspects to the early maturity phenomenon. Thus, stocks which are genotypically predisposed to mature at low rates may not always perform according to type and may mature at quite high rates when grown under some conditions.

Q: Glebe — There are Scottish salmon being used in Chile. Is there a reduced number of grilse in these fish?

A: Johnstone — I don't know. Irish producers experience generally higher grilse rates than do Scottish farmers although some apparently use lower maturing Scottish and Norwegian strains. The real problem with early maturity is the unpredictability of the grilse fraction and their necessarily reduced marketing window. The use of triploids would avoid this problem and might enable farmers to put fish to sea cages at different times than at present and enable them to move away from the single input systems as practised at present. I discuss this option in my paper.

Q: Anderson — Your research seems to focus on improving the situation for your aquaculture industry. What is your view of this strategy for protecting wild stocks?

A: Johnstone — The original emphasis of the research was to develop techniques that would enable the industry to maintain and, possibly, improve its efficiency. More recently, the "near market strategy" has meant that, increasingly, this research and development has had to be passed on to the industry and this has now happened in Scotland. In the interim, the recognition of the potential of accidental releases to interact with wild stocks has come more to the fore and is perceived by some as an additional benefit of triploidisation. My results confirm the expectation that female triploids will be sterile. Increased triploid usage would therefore minimise any impact of releases.

Comment: Anderson

This supports my contention that science and technology is where we should be and this should be more emphasized.

Q: Sutterlin — What pressure treatments have you been using in your triploidy program and how effective are these treatments, both in terms of survival among alternative treatments and subsequent performance of treated fish both in aquaculture and in the wild?

A: Johnstone — I am not at liberty to divulge the experimental details of the protocols that we use since this is considered by my Directors as being commercially sensitive information.

Q: Pepper — You have noted that 1% of triploid females develop eggs/oocytes. Will these fish behave like normal spawners?

A: Johnstone — I don't know. Since triploid fish have different weight-length relationships to diploids, their success in attracting and mating with wild males is, perhaps, questionable.

STUDIES ON HORMONAL STERILIZATION AND MONOSEX FEMALE TECHNOLOGIES FOR SALMONIDS AT THE WEST VANCOUVER LABORATORY.

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ABSTRACT

Research on suppression of maturation of salmonids at the West Vancouver Laboratory and its predecessor, the Vancouver Laboratory, dates back to the early 1960s when studies were conducted on the surgical sterilization of maturing sockeye salmon Oncorhynchus nerka. In the mid 1970s, research was initiated on reproductive suppression of salmonids utilizing androgen administration during early development. Recent research has focused on attempts to eliminate dietary treatment and induce sterility by androgen therapy during the incubation phase alone. Studies on the induction of triploidy have compared temperature and pressure shocking procedures. However, our main emphasis has been on methods for the production of monosex female and monosex female triploid stocks. These include the two generation procedure which involves progeny testing, the use of gynogenesis followed by androgen treatment and currently masculinization by androgen treatment followed by use of a Y-specific DNA probe to determine the sexual genotype.

The feasibility of combining two different maturity control technologies to ensure 100% sterility will be addressed as will the potential for producing inherently sterile transgenic fish.

INTRODUCTION

The quest for sex control technologies in animal husbandry in general and aquaculture in particular is not a recent phenomenon. Thus, Watson (1755) demonstrated the feasibility of surgical sterilization in a number of species, including a salmonid, primarily as a means of improving flesh quality. More recently, Huxley (1938) expressed the desirability of developing means of controlling sex differentiation as follows: "It thus becomes of great interest to discover the mechanism by which sex is determined, and to find whether by any means we can bring it under our control". Apart from the development of surgical castration techniques for mammals, the development of sex control techniques has proceeded more rapidly in finfish than in higher vertebrates.

On Canada's Pacific coast there are basically three types of salmon: wild salmon, hatchery salmon and aquacultured salmon. All five species of Pacific salmon that are indigenous to British Columbia are the subject of enhancement efforts in hatcheries or spawning channels. In commercial aquaculture, coho was initially the major species, but was rapidly replaced by chinook as eggs for the latter species became more available. Current production figures place chinook as the primary species with Atlantic salmon in second place and growing in popularity. Coho is currently in third place. Cultivated strains of the rainbow trout are also grown commercially in both fresh and seawater. In addition, trials are under way on the commercial culture of sockeye salmon in seawater and Arctic char in freshwater. In this document we provide information on: the history of sex control in salmonid culture on the Pacific coast of Canada; the techniques which have been utilized, and current and future research and development activities.

DEVELOPMENT OF SEX CONTROL TECHNOLOGIES IN BRITISH COLUMBIA

At the West Vancouver Laboratory and its predecessor the Vancouver Laboratory of the Department of Fisheries and Oceans, research on sex control and technologies for suppression of maturation has shifted in focus over the last three decades in line with changes in species priorities and culture objectives.

Initial studies were conducted on the surgical sterilization of the sockeye salmon. These investigations were part of a program of studies which investigated the biological basis of ageing and death in Pacific salmon. Surgical sterilization of sexually maturing sockeye salmon was shown to prevent post-spawning death which normall occurs. When these fish were fed a post-operative diet containing frozen zooplankton they lost their secondary sexual characteristics and returned to the sea green coloration characteristic of sexually immature sockeye salmon (McBride et al. 1963).

In the mid-1970's, studies at the West Vancouver Laboratory were initiated on the hormonal control of sex differentiation in Pacific salmon (Goetz et al. 1979). These investigations preceded the development of the commercial aquaculture industry in British Columbia and were at that time focused on two enhancement objectives:

1) The production of all-female stocks to, a) maximize the landed value by increasing the proportion of female salmon and thus the yield of marketable roe and b) increase the proportion of females in hatchery escapements, thus increasing egg take or reducing the total escapement required for a given egg take.

2) The production of sterile stocks for stocking purposes with the objective of, a) producing trophy sized fish for the fishery and, b) producing fish for out-planting which would not interact reproductively with wild stocks.

This early phase of our research was focused on the production of sterile coho salmon as a number of coho stocks reach sexual maturity at a relatively small size, and on the production of monosex female coho and chinook stocks.

With the advent of commercial sea pen aquaculture in British Columbia, the emphasis has been in two areas:

1) Methods for the production of sterile coho salmon to eliminate sexual maturity, which occurs at 2 years from egg in zero age coho and thus provide a larger sized product at market and permit marketing during the winter after the normal spawning season.

2) The production of monosex female chinook salmon which provide the culturist with 3 years from egg in which to grow a market sized individual.

The combination of androgen induced suppression of gonad development with zero age coho smolt production proved to be problematic. The original sterilization procedure had been developed for the production of 1+ coho smolts in Federal hatcheries. In some commercial hatcheries, in particular those with low water temperatures, insufficient time elapsed between the completion of the androgen treatment and sea water transfer. Under these conditions fish were of sub-optimal size at sea water transfer and were not completely smolted, thus impairing sea water performance. Androgen sterilization still offers potential for the culture of 1+ coho smolts or for 0 age smolts grown under optimal conditions.

The production of monosex female chinook salmon has, on the other hand, been a major success. The economic advantage of growing monosex female chinook is such that a very high proportion of total chinook production in British Columbia is now monosex female. There is interest in extending monosex female culture technology in British Columbia to Atlantic, coho and sockeye salmon and in further investigation of reproductive suppression technologies both for culture purposes and for the prevention of reproductive interaction between farmed and wild stocks.

REPRODUCTIVE SUPPRESSION BY ANDROGEN TREATMENT

A number of studies have been conducted on androgen induced suppression of maturity of Pacific salmon and trout. In our first study, coho salmon were immersed as eyed eggs and alevins in 25-400 ug 17α-methyltestosterone/l and were fed 20 mg 17α-methyltestosterone/kg diet for three months from first feeding. These groups were 94-100% sterile, the 25 ug/l immersion group having the lower percentage of steriles. In a parallel group of coho that received the dietary treatment alone the percentage of steriles was only 52% (Goetz et al. 1979). In a subsequent study, coho were immersed twice at the eyed egg and alevin stages in 400 ug 17α-methyltestosterone/l and then fed a lower, 10 mg/kg, dose of 17α-methyltestosterone than in the earlier study. In this study the sterility rate was 94% when fish were examined at 3 yrs of age, i.e., the time when all but 3% of control fish and all but 6% of a direct estrogen feminized group matured (Hunter et al. 1982). These sterile coho salmon lived for 3-4 years in captivity beyond the normal time of death, i.e., to 6 or 7 years of age at which time they probably went through a natural ageing process unlike the rapid ageing process which normally occurs at 3 or occasionally 4 years of age, directly after sexual maturation and spawning. Under experimental study certificates issued by Health and Welfare Canada, coho salmon sterilized by androgen treatment have been released from two Federal salmon hatcheries into the coastal waters of British Columbia. Sterile fish were harvested by the commercial fishery in coastal waters at 2, 3, 4 and 5 years of age. Owing to the intensity of the fishery, 73.6% of the total harvested were captured in year class 3, approx. 24.7% in year class 4, and 1.3% in year class 5 (Solar et al. 1986). The only androgen treated fish which underwent the normal anadromous migration to the hatchery of origin were a relatively small percentage of incompletely sterilized fish. No reproductively deactivated fish returned to the hatchery of origin confirming for the first time on an experimental basis the hypothesis that sexual maturation is essential for the initiation of the anadromous migration. It was concluded that sterile fish remained in the marine environment until they were either captured or died of natural causes.

Recent studies have demonstrated that chinook salmon also can be rendered reproductively inactive by androgen treatment. This species also requires both immersion at the alevin stage plus dietary administration to achieve a high percentage of sterility. However, in chinook the effective dose of dietary androgen, up to 80 mg/kg, is higher than in coho (Solar et al. unpublished). Recently it has been reported that dietary 17α-methyltestosterone administered at > 20 mg/kg for 600 degree days from first feeding is extremely effective in producing non-maturing Atlantic salmon (Johnstone 1989). Further studies recently have been conducted on the sterilization of coho salmon to determine whether an immersion regime can be devised which results in a high level of sterility without the necessity for dietary androgen administration. Immersion of coho for 2 hrs on 10 occasions during the alevin stage, at a dosage of 10 mg 17α-methyltestosterone/l, resulted in the production of 51% sterile fish and 15% partially sterile fish. Immersion of alevins on a continuous basis for 30 days in 100 ug 17α-methyltestosterone/l resulted in a sterility rate of 43% (Piferrer and Donaldson 1988, unpublished in Piferrer and Donaldson, 1988; Piferrer 1990). These results indicate the potential, after further manipulation of appropriate variables such as dose, duration, timing and form of androgen, to induce a high percentage of sterility in salmonids by immersion during the incubation process.

REPRODUCTIVE SUPPRESSION BY INDUCTION OF FEMALE TRIPLOIDY

Studies on the production of triploid Pacific salmon recently have been reviewed (Benfey and Donaldson 1989) and a bibliography on triploid teleosts has been published (Benfey 1989). Our current emphasis is on the comparison of heat versus pressure shocks for different species (Guoxiong et al. 1989) and on the development of alternative methods for the production and quality control of monosex female milt. As other speakers at this workshop are addressing the issue of triploid salmonids, I will focus on the monosex female aspect.

Alternative procedures for the production of female triploid salmonids are shown in Fig. 1 (modified from Donaldson 1986). The recommended technique is the use of monosex female sperm to fertilize ova prior to shock treatment, although direct feminization techniques can be used if monosex sperm are unavailable. Methods for both the generation of monosex female sperm and for direct feminization in salmonids have been reviewed recently (Donaldson 1986; Donaldson and Benfey 1987; Piferrer and Donaldson 1988). Once genotypic monosex female embryos have been generated, a portion can be treated with androgen during early development to generate phenotypic males which, when mature, produce additional monosex female spermatozoa Fig. 2 (modified from Donaldson and Benfey 1987). If methods are refined for the generation of diploid spermatozoa from tetraploid fish then it would also be desirable to manipulate these to produce monosex female diploid sperm, i.e., the tetraploids would be genotypic females with a male phenotype.

Our current research efforts are directed into several aspects of monosex female and monosex female triploid production which are described in the following sections.

A) MASCULINIZATION

The development of simple effective masculinization techniques is essential to the production of monosex female sperm either by, 1) masculinization of monosex female embryos in a pre-existing monosex line (Fig. 2) or, 2) by masculinization of gynogenetic embryos or embryos of mixed sex for the generation of new monosex lines. By investigation of the timing of sex differentiation in Pacific salmon and by comparison of the effectiveness of aromatizable versus non-aromatizable androgens (Piferrer and Donaldson 1987; Piferrer and Donaldson 1991) and natural versus synthetic androgens (Piferrer 1990) we have been able to obtain 100% masculinization with a single two hour immersion treatment on or about the time of hatch in coho and chinook salmon.

B) GYNOGENESIS

Gynogenesis offers a means of generating genotypic female embryos which can then be masculinized with androgen (Piferrer et al. 1988). We currently have studies under way for the production of monosex female sperm by this procedure from both Pacific and Atlantic salmon.

C) Y SPECIFIC DNA PROBES

It has been recognized for some time that development of Y-specific DNA probes for salmonids would greatly assist in the generation of monosex female stocks. These probes would permit the sorting of genotypic female, phenotypic male salmon from groups of masculinized salmon of mixed sex. They would also provide a means of confirming the genotype of mature phenotypic male, genotypic female salmon prior to milt collection, thus guaranteeing the production of monosex female or monosex female triploid offspring. In addition, such probes would facilitate the early selection of high performance male and female salmon for broodstock purposes. Recently, there has been significant progress in the development of Y-specific DNA probes for chinook salmon (Devlin et al., in preparation; Vaisius, personal communication) and we are currently applying this technology to the production of monosex female chinook salmon milt from alternative genetic stocks and to the screening of existing masculinized genotypic female broodstock. The development of Y-specific DNA probes for Atlantic salmon would be of considerable value for the generation of new sources of monosex female Atlantic salmon milt from desirable genetic stocks.

D) DIRECT FEMINIZATION

For salmonid species or situations where appropriate sources of monosex female sperm are not available direct feminization during early development provides a viable alternative for the production of monosex female or monosex female triploid stocks for grow out purposes. The labile period for estrogen treatment has been investigated in coho salmon (Piferrer and Donaldson 1989) and in our recent studies with a synthetic estrogen we have produced 97% phenotypic females after a single 1 hr immersion and 100% females after a single 2 hr immersion treatment. Growth at five months was better in treated than in control salmon and survival was reduced somewhat to 85.7% and 89.5% in these two treatment groups compared to 99.0% in controls (Piferrer 1990). Clearance studies utilizing isotope labelled estradiol indicates that estrogens are cleared rapidly from alevins and even more rapidly from fry (Piferrer 1990).

FUTURE DIRECTIONS

There are several promising lines for further research and development on the production and utilization of sterile salmonids. First of all there are varying reports in the literature concerning the growth performance of triploid salmon. This variation could depend upon whether mixed male and female triploids are being cultured and whether triploid and diploid fish are grown in a single container. Clearly, the ultimate salmonid for culture would be one which is sterile, but grows at a rapid rate. We have shown in a series of studies that growth in salmonids can be accelerated by administration of recombinant somatotropins (Gill et al. 1985; Down et al. 1988; McLean et al. 1990). We have now been able to demonstrate that the growth rate of triploid salmon can also be accelerated utilizing recombinant somatotropin treatment (McLean et al. in preparation).

When maturity suppression procedures are implemented for the purpose of preventing reproductive interaction between escaped farm fish and wild fish it is essential that sterility be assured. For this purpose it may be appropriate to combine two forms of sterilization. Thus female triploids could be produced and the resultant embryos and alevins could be treated with a sterilizing dosage of androgen.

In the future it may be possible to produce transgenic fish which are inherently sterile. This could be achieved by linking a gene which generates a toxic gene product to the regulatory portion of a gonad specific gene in such a way that gonad development is blocked at the stage when the regulatory portion of the gonad specific gene is activated (Maclean and Penman 1990). Inhibition of gonadal development could also be achieved by producing transgenic salmon in which a specific enzyme regulated step in the endocrine control of reproductive development has been compromised. As the resultant transgenic fish derived from these procedures would be sterile, it would be necessary to maintain a special monosex or bisexual broodstock in which the gene construct inducing sterility is not expressed.

There are thus a number of avenues that could be pursued during further research on the production of sterile salmonids. There is no doubt that the further implementation and integration of monosex and reproductive suppression technologies into salmonid production systems will become increasingly important to the salmonid aquaculture industry.

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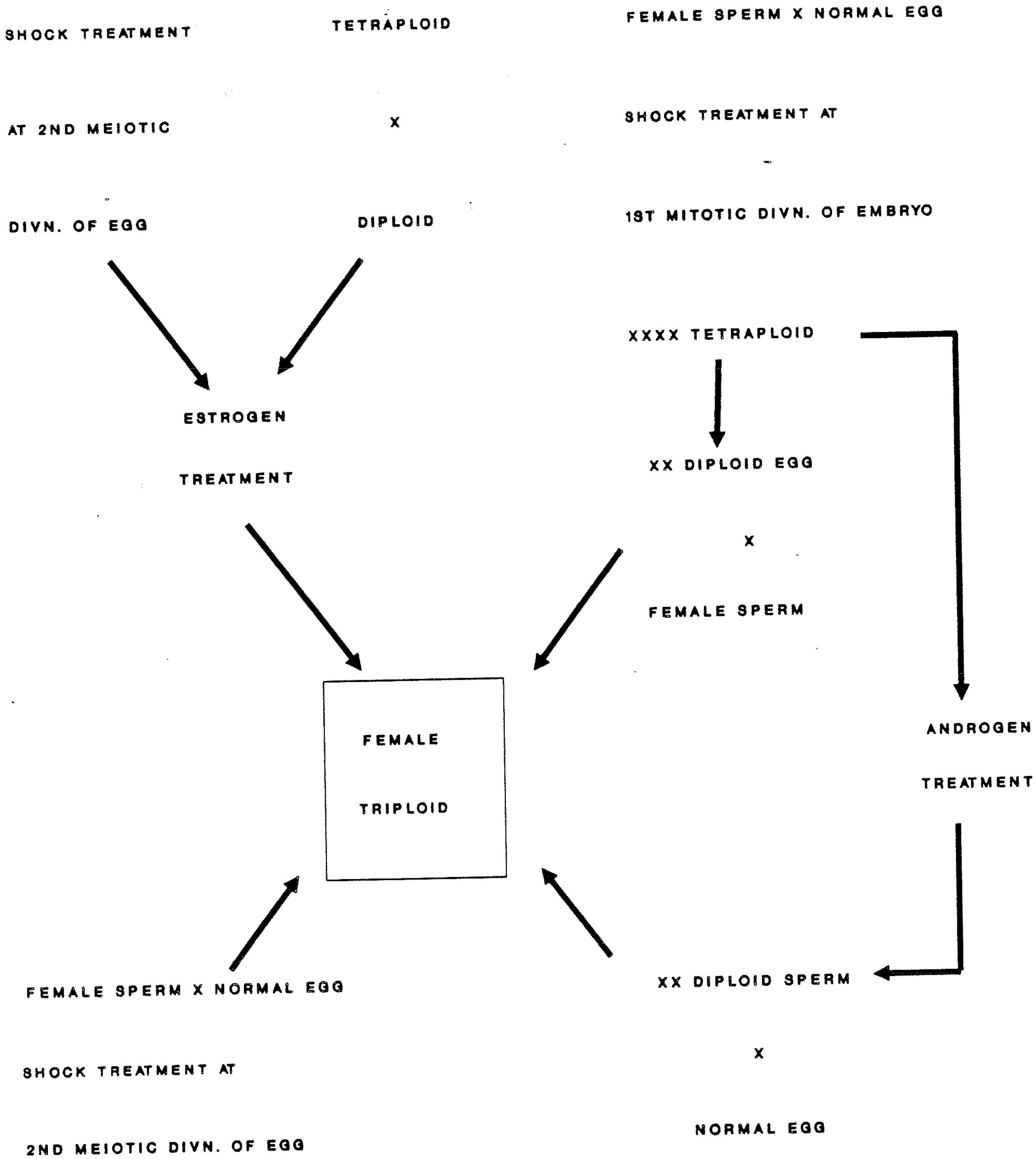

FIG. 1. ALTERNATIVE METHODS FOR THE PRODUCTION OF TRIPLOID FEMALE SALMONIDS (Modified from Donaldson, 1986)

PRODUCTION SYSTEM FOR GENOTYPIC FEMALE TRIPLOID SALMONIDS

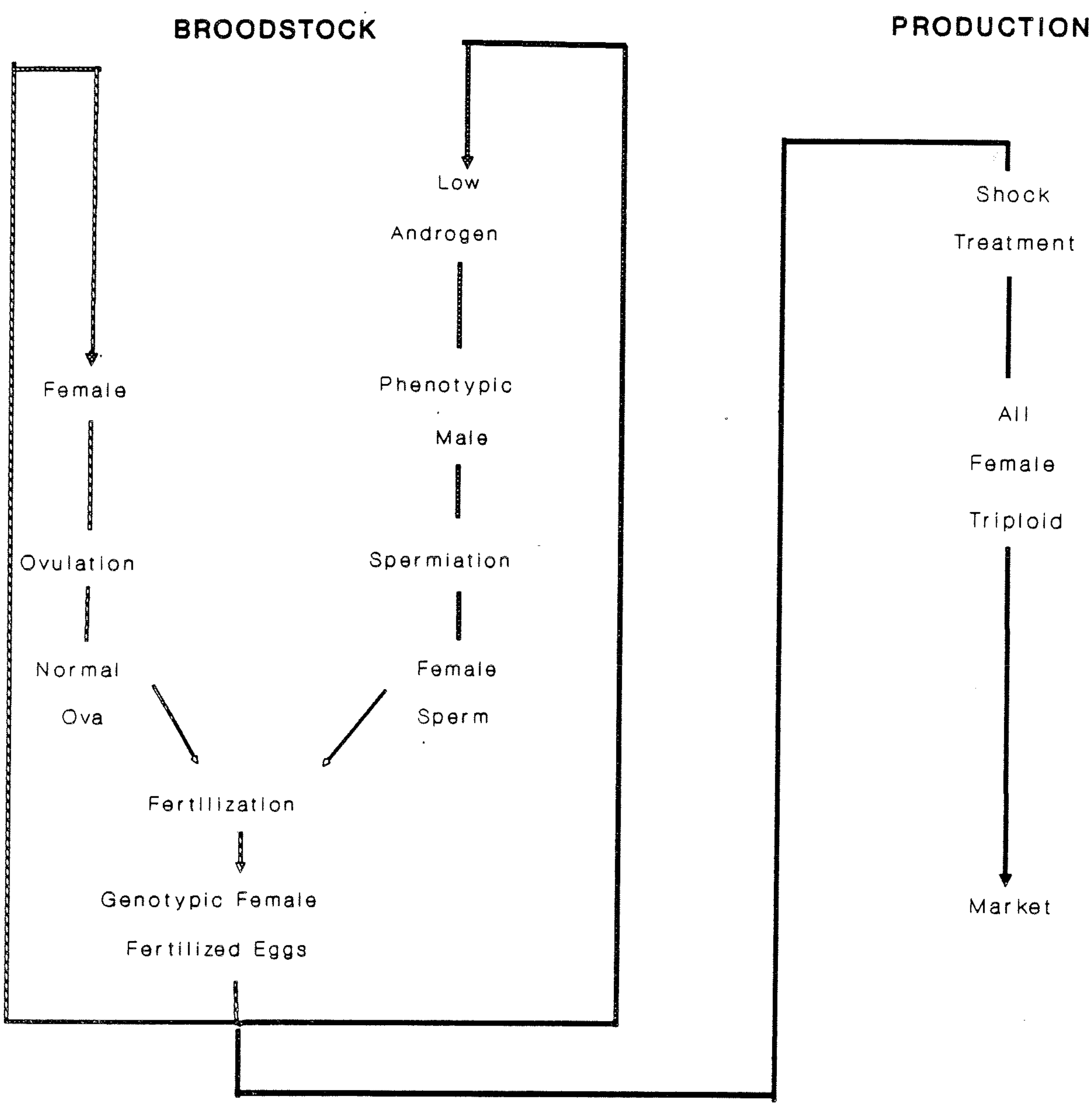

FIG. 2. A PRODUCTION SYSTEM FOR THE GENERATION OF TRIPLOID MONOSEX FEMALE SALMONIDS (Modified from Donaldson & Benfey, 1987)

PARTICIPANT DISCUSSIONS

Rapporteur: Brian Glebe

Q: Davidson — If triploids do not return to the river with maturing salmon, where do they go?

A: Donaldson — I expect they would remain in the ocean until they die of old age. Our Pacific salmon are hormonally treated to produce sterility. They are not triploids. The hormonally sterile salmon appear to remain at sea. I expect triploids would do the same.

Q: Davidson — Do sterile fish and non-sterile fish use the same marine environment?

A: Donaldson — Yes. They are caught in the same area as other salmon. However, commercial harvest only occurs in certain areas. No one is looking for sterile fish in non-traditional fishing areas.

Q: Davidson — Do sterile fish have any benefits for enhancement?

A: Donaldson — The presence of sterile fish may allow for greater escape of non-sterile salmon from the commercial harvest.

PRODUCTION OF NON-MATURING ATLANTIC SALMON IN TASMANIA

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ABSTRACT

Atlantic Salmon (Salmo salar) have been established in Australia for a number of generations and more recently have become the basis of a marine farming industry in Tasmania. Adaptation of the species to the new environment has included a shift in reproductive strategy, to become a wholly grilse stock. Favourable water temperatures allow these grilse to attain harvest size (3.5 kg) after just 15 months in the sea cages. This high growth rate benefits the industry but carries a penalty in curtailing the harvest period.

Thus, suppression of maturation is identified as a desirable goal. The rationale for selection of our preferred method is outlined. The techniques of sex-reversal and triploidisation used on the path to producing all-female triploid stock are discussed.

Performance of triploids for the three year classes generated to date is compared to that of diploids. Two physiological problems currently occurring in post-smolts are described.

In spite of transient problems, all-female triploidy appears to be the appropriate method for extending the harvest of Atlantic salmon in Tasmania.

INTRODUCTION

HISTORY OF SALMON IN TASMANIA

In 1865 the first formal release of Atlantic salmon (Salmo salar) fry was attempted in Tasmania. In spite of repeated introductions, the salmon failed to establish a breeding population. Subsequent introductions of other salmonids were successful. Interest in salmonids broadened from purely recreational fishing to some commercial interest with the establishment of a freshwater trout farm in Tasmania in 1964. The late seventies and early eighties saw a series of small scale trials with salt-water farming of rainbow trout (Oncorhynchus mykiss) by individuals rather than organisations. Much of this work probably duplicated the struggles being experienced by novices in other parts of the globe, but these efforts provided glimpses of a potentially bright future for the salt-water farming of salmonids in Tasmania.

The Tasmanian Fisheries Development Authority (T.F.D.A.) recognised the potential and, in 1981, began an investigation into this form of aquaculture. Support from local industry and endorsement by the Government resulted in more extensive investigations. By late 1983, an interim plan for the establishment of an industry had been formulated. It was envisaged that majority funding by government would provide a secure financial base to a "Salmon Enterprise Foundation", which could reliably provide stock and an advisory service during the formative years of the new industry.

It was proposed that the industry be based mainly on Atlantic salmon, with rainbow trout retaining a niche, but with less obvious potential for additional commercial development. A ban (since 1968) on importation of fresh salmonid products into Australia meant that the source of broodstock was restricted to a landlocked stock of Atlantic salmon in the State of New South Wales. These salmon were of Canadian origin, from the Cobequid hatchery (River Phillip stock) in Nova Scotia. They were known to be a large, genetically sound, late maturing population. The fish had been introduced into the Government hatchery at Gaden in New South Wales in three imports between 1963 and 1965.

STRUCTURE AND FUNCTION OF SALTAS

In February 1985, Salmon Enterprises of Tasmania (SALTAS) was established under the Salt-Water Salmonid Culture Act 1985 of the Tasmanian Parliament. The initial equity participation in Saltas was 51% with the Tasmanian Government and 49% with the Private Sector. The company was to operate a hatchery and smolt production unit at Wayatinah, a model sea-farm at Dover, and a research foundation to aid the developing industry.

The Parliamentary Act inaugurating Saltas also accorded Saltas an exclusive licence to operate the only Atlantic salmon hatchery in the State for the first ten years. The entitlement to purchase of smolts from each year's production was defined on a pro-rata basis according to equity shareholding in Saltas. The Government share of equity only attracts 1% of the total smolt production for broodstock and research purposes required by Saltas.

Concomitant with the exclusive smolt producer status, came a requirement embodied in the Articles of Association to provide a comprehensive Research and Development Programme. This programme was to be funded by the application of a minimum of 25% of the gross revenue to Saltas from smolt sales to the industry. Submissions to the programme are made by the industry, research branches of government agencies, and Saltas, but the programme itself is monitored, revised and recommended to the Board of Saltas by an R & D Steering Committee. This provides a forum for strategic planning on behalf of the whole industry, and represents an efficient mechanism for channelling available funds (currently somewhat in excess of Aust. $670,000 per annum) to the most relevant applied research topics.

SALMON CULTURE IN TASMANIA

The Gaden broodstock was isolated and put through an exhaustive disease testing programme for 18 months prior to stripping. At the end of July 1984, having passed all tests, the first 115,000 Atlantic salmon ova were shipped to Tasmania. After spending some 15 months in a specially created quarantine facility at the T.F.D.A.'s Research Laboratory, 36,000 of the best smolts were transferred to sea-water cages to become foundation stock for the industry. Subsequent imports from Gaden to Saltas' hatchery at Wayatinah in 1985 and 1986 (180,000 and 275,000 respectively) under similar protocol, supplied stock to the Tasmanian industry while the 1984 year class fish completed a cycle, to provide the first Tasmanian reared progeny in 1987.

From empirical observation of the seven year classes handled to date (1984 to 1990), a number of performance characteristics of this stock in the Tasmanian aquaculture environment are now evident. Broodstock are transported back to freshwater some two to three months prior to spawning. This provides the appropriate declining temperature regime (see Appendix 2 - Temperatures). The majority of mature females spawn between the first week of May and the first week of June (See Appendix 1b for seasonality in the Southern Hemisphere).

Artificially elevated temperatures of egg incubation allow eye-up by June/July, and fry first-feed in August/September. The fry are progressively moved outdoors, into 4 m^3 tanks, starting in October/November, and end up in the main 44 m^3 production tanks by December, at some 2-3g in weight. Summer growth is allowed to proceed to maximum feeding opportunity, but autumn and winter growth is deliberately restricted on the "large" size category of the fish. This is used as a means of reducing size variation of smolts, and as a means of restricting overall biomass of smolt distribution. All salmon parr are vaccinated against vibrio.

In Tasmania, the stock has consistently demonstrated a very high S1 rate of some 97%. The 2 to 3% of potential S2 smolts occurring in each year class are effectively removed by culling the bottom of the size range at the winter grading sequence. Salt water challenge and subsequent plasma-sodium tests are routinely carried out on smolts. An elevation from 150 millimoles per litre in freshwater to 190 millimoles per litre at 24 hours in salt water, is considered acceptable. The smolt window is believed to span from mid-September to end October, and smolts at transfer range between 60 and 100g in weight.

The million plus smolts produced by Saltas each year are distributed to the marine farms, pro-rata according to shareholding, and parsi-parsu with regard to physical and genetic characteristics. A number of the farms have access to brackish water sites for initial transfer. Others use a three to five day acclimatization regime, in on-shore tanks, prior to transferring the fish to full sea water (34 ppt).

Two diseases of significance do occur in the marine rearing phase. One is a paramoeba which infests the gills and causes erosion of the lamellae. The other is a marine flexibacter which causes skin lesions on the flanks of the fish. Both diseases show greater clinical expression in smaller fish and at higher temperatures.

The seawater temperatures around Tasmania are generally favourable for salmon growth (Appendix 2b). This is part of the reason why the stock reaches an average of 3.5 kg (live weight) by the middle of the first summer in the sea cages.

A feature of the stock's performance which is of great importance is the very high (95% plus) grilsing rate demonstrated by these fish in Tasmania. These grilse however, ranging from 3 to 5 kg, do not show any of the poorly marketable traits traditionally associated with grilse. The only serious drawback to the industry, of having such a high grilsing rate, is the restriction on the harvest period. The fish are not large enough to harvest before October and the onset of maturity renders the fish unpresentable to market by the following March.

As a matter of perspective, the Tasmanian Industry produced 1,773 tonnes of salmon in 1989/90 and is forecast to produce some 2,400 tonnes in 1990/91. Even at these modest production levels, there is clearly a benefit in extending the harvest period beyond six months each year.

PRODUCTION OF NON-MATURING FISH

RATIONALE FOR SELECTION OF FEMALE TRIPLOIDS

The positive correlation between growth and maturation rate is well documented. The genetic selection for rapid growth required by aquaculture thus entrains selection for early maturity. Where a situation arises, as in Tasmania, that virtually a whole year class attains maturity together, it poses a definite time limit to the harvest period, and coincidentally an upper limit to the size of fish which can be produced economically. One clear solution to the problem is to continue genetic selection for high growth rate, while suppressing maturation in part of the stock from each year class.

Saltas did an extensive review of the literature and in 1988, the Marine Research Division produced an internal report on the Production of Non-Maturing Fish (Foster and Percival 1988). Several methods other than "Triploidy" were considered, and rejected for a variety of reasons.

i) Autoimmune sterilisation - Injection was considered commercially impractical and administration with feed an unproven technique.

ii) Hormonal sterilisation - Scientific evidence notwithstanding, this technique was considered to be unacceptable because of the potential for adverse reaction in the market place.

iii) Chemical sterilisation - The variability in survival rates and percentage of mosaic embryos were considered unacceptable, as was the administration of Cytochalasin B and Colchicine to animals destined for market.

iv) Irradiation sterilisation - Considered attractive because of the complete suppression of gonad development, but rejected because of the logistical problems of using a gamma radiation source and the potential for inspiring consumer resistance.

With triploidy emerging as the most acceptable way to suppress maturation, the various methods of inducing triploidy by other than hydrostatic pressure were considered and rejected.

i) Tetraploid X Diploid Crosses - Rejected because the production and maintenance of fertile tetraploids was considered impractical.

ii) Chemical induction - Cytochalasin B was again rejected, and nitrous oxide treatment was regarded as being an unproven technique for the larger eggs of salmon.

iii) Temperature shock - Initially considered attractive because of the apparent ease of application, this method was also rejected because of the reported variability in results.

It is noteworthy that heat shock is now used on rainbows by the two trout hatcheries in Tasmania with acceptable results.

With hydrostatic pressure shock emerging as the technique of choice to produce triploidy, there remained the problem of dealing with the 50 percent of male triploids, concurrently generated in the treatment of a normal zygote supply. Clearly, a phenotypically all-female stock was required. After consideration, two of the three known methods of producing all-female stock were rejected.

i) Hormonal feminisation - Rejected because of the potential for adverse market reaction.

ii) Gynogenesis - Rejected because of logistical problems of using a radiation source, and concern that all genetic material would be derived from the female only.

The hormonal masculinization of genotypically female fry, to provide all-female sperm at maturation, was considered the most appropriate path towards obtaining a supply of "all-female" zygotes. By exposing such zygotes to an appropriate hydrostatic pressure shock, it was expected to generate an all-female, triploid stock, which would not develop any secondary sexual characteristics, and which had not directly been exposed to any chemical treatment.

This rationale is summarised in Appendix 3. The sequence of events used to produce female triploids is represented schematically in Diagram 1.

SEQUENCE OF PRODUCTION OF TRIPLOIDS 1988 - 1991

In 1988, Saltas conducted a series of ranging trials, to define optimum pressure regimes for the induction of triploidy under Tasmanian conditions. Only normal sperm (50% XX, 50% XY) was available in that year. Hence, the test batch of fry retained for rearing was mixed-sex triploids. Some 2,000 of these were sent to sea cages as smolts in 1989.

As a means of keeping options open, a small number of fry had been treated with androgen in 1986, and again in 1987. Thus the 1986 year class of masculinized females was available to provide all-female sperm, to generate an all-female stock for triploidy in 1989. A first commercial-scale run of this stock was produced in this year, and some 48,000 of these were sent to sea cages as smolts in 1990.

By 1990, encouraging results from the previous year classes prompted Saltas to undertake full scale production of female triploids, with an intention of supplying up to 30% of the total smolt requirement for 1991 as female triploids. This sequence of triploid production by year-class is represented schematically by the growth curves in Figure 1.

MECHANICS OF SEX-REVERSAL

Based on the contemporary literature of 1986, two dosage regimes, of 3.0 mg/kg feed and 1.0 mg/kg feed, of 17 alpha methyltestosterone were applied to two batches of fry in each of 1986 and 1987. Some obviously masculinized females were available as sperm donors in 1989 and 1990, but poor tagging technique and interim fish losses rendered a comparison of the two regimes inconclusive. As of the 1989 year class, a dietary concentration of 3.0 mg/kg has been fed as starter feed, and continued for 900 degree days. The first results of this regime will be evident in the 1991 broodstock.

The reported absence of sperm ducts in mature, masculinized, female salmon is believed by Saltas to be an unreliable feature for differentiating these fish from normal functional males. Treated fish that have freely expressed milt, have been shown upon dissection to have clearly lobulated testes, and improperly developed ducts have been observed in normal males. A strict procedure is therefore adopted at spawning, before a sperm donor is accepted as a masculinized female. Each presumptive donor (i.e., treated fish showing all male secondary sexual characteristics) is squeezed, and accepted only if no milt is expressed. If upon dissection the testes appear normal, the fish is again rejected. Only if the testes are lobulated is the fish accepted. The presence of only one lobulated testis, and/or the presence of a vestigial ovary, increases confidence that the donor is indeed a masculinized female. If the testes appear ripe, then they are macerated in an extender solution, and held under oxygen at 4°C until required later in the day. Each sample is tested for sperm motility just prior to use.

This procedure for selecting donors is clearly tedious and wasteful of fish (under-ripe testes are often found) but in the absence of better knowledge, it is nevertheless adopted. It is expected that confidence will increase as subsequent year classes are identified as being genuinely 100% genotypic females. The nominal all-female population generated by Saltas in 1989 has been tested to be 93% female. This figure is barely acceptable for triploidy, and not acceptable for sex-reversal. Further work in this area is required, and the advent of a rapid, non-destructive test for sex-determination is awaited.

MECHANICS OF TRIPLOIDISATION

Data from the contemporary literature, and advice from the pre-eminent workers in the field, guided Saltas' initial attempts at triploidisation in 1988. A pressure vessel was built, and a series of ranging trials were undertaken to define optimum regimes for local conditions. The pressure vessel, of two litre capacity, was modelled on the equipment developed by Dr. R. Johnstone and his group at D.A.F.S. in Aberdeen.

The trials applied four different pressure shocks to eggs at each of three different periods post-fertilisation, and for each of three different durations. The treatment outline and mean results are shown in Figure 2. A detailed report will be published in the near future (Foster et al. in preparation).

Once fertilised, eggs were held at a constant temperature of 10°C (±0.2) prior to and during each pressure run. Build-up to treatment pressure was maintained at 90 seconds. Duration of treatment was timed from when full pressure was reached, to when pressure was entirely released. The last 60 seconds within each treatment duration was used to release pressure.

Eggs were incubated in discrete cells, but in otherwise standard conditions at 8 to 10°C. Mortalities were routinely recorded but the survival figure finally used was "survival to swim-up stage", normalised to the control eggs.

Assessment of triploidy was carried out on swim-up fry, using erythrocyte nuclear dimensions from fixed and stained blood smears. Various manipulations of basic lengths and widths of the nuclei were considered as a measure to determine triploidy. Nuclear length was finally adopted as the standard. The formidable task of measuring two dimensions, on each of 27,000 erythrocyte nuclei, was unavoidable at the time, but Saltas has since learned the value of using Flow Cytometry.

Unfortunately, analysis of the results was weakened by high variation among the fish used, and the non-orthogonal design of the experiment. However, there was clear indication that a satisfactory yield could be obtained with a pressure regime within the experimental range.

For the 1989 spawning season, a taller pressure vessel, of six litre capacity, was built. Pressurisation characteristics were retained, but a functional change was made in the locking mechanism, from a screw cap to a plug, to speed up recycling time (this system is now capable of cycling 22 litres of eggs per hour). Some 30 litres of eggs were put through a regime of five minutes duration, at 9500 psi, at 30 minutes post fertilisation (300° minutes). Assessment by Flow Cytometry in early 1990 showed 100% triploidy in the surviving fry.

In 1990, the treatment was repeated on some 90 litres of eggs, considered to be a production run. Triploid yield (to eyed egg stage) was 85%, and subsequent assessment by Flow Cytometry showed surviving fry to be 100% triploid.

PERFORMANCE OF NON-MATURING FISH

FRESHWATER GROWTH AND SURVIVAL

The triploid yields (to eye-up stage), of the experimental batches retained for ongrowing in 1988 and 1989, were approximately 52% and 44% respectively. There is strong evidence however that these low yields were caused by other factors, independent of the triploidy regime. The equivalent figure for the 1990 YC was 85% and it is expected to be similar in 1991.

Growth and survival figures from eye-up stage, for each of the year classes, are graphed in Figures 3, 4, and 5. Data for the triploid population is compared to the data for the larger diploid population for each year class. Thus the data for the diploids may not be used as a control in the scientific sense, but rather as a reference line as to what was the norm for the farm in that year.

For the 1988 YC mixed-sex triploids, it is not clear as to why survival from eyed eggs to first feeding was so low in contrast with the two subsequent year classes (which were all-female triploids). The obvious conclusion that the males died is not borne out by a recent sampling of these fish (49% males). The improved survival in subsequent years, with larger populations involved, indicates that female triploids (at least) offer survival rates comparable to a normal diploid population.

Growth of the 1988 YC from the middle of summer (February) onwards reflects the lower stocking density experienced by the small number of this particular population. The 1989 YC was kept under more realistic stocking densities encountered in commercial production (up to 36 kg/m^3). This stock was also exposed to the grading culls normally performed on production stock in aquaculture. The latest of the truncated data available on the 1990 YC shows the average size of the triploid population to be smaller than the diploids. It is not known at this stage if this inversion of relative average size is significant.

While the performance of triploid parr relative to diploid parr under equally intensive culture remains an open question, one difference in early feeding has been observed consistently and is noteworthy. For the first two to three months of feeding, triploid fry are significantly slower in accepting feed, growing, and using the full water column in tanks. Even after 60 days from first feeding, it is easy to dismiss a large percentage of fry as runts. Triploid fry require more bottom surface area than diploids and take longer to reach 1g. After this stage, their growth rate catches up and perhaps exceeds that of diploids.

No significant differences in disease susceptibility between diploids and triploids have been observed over the three year classes that have been produced. Samples of smolts from each of the year classes were exposed to seawater challenge and subsequent plasma sodium tests. No significant differences were recorded between diploids and triploids.

From Saltas' experience to date, it is believed that in general the triploids are capable of offering at least a fair performance in terms of growth and survival during their freshwater phase. They also appear to smoltify normally.

SEAWATER GROWTH AND SURVIVAL

The 2,000 mixed-sex triploids sent to sea cages in 1989 were marked, and merged with diploids during assorted periods of their sea water growth. Thus, stocking densities they were held at were similar to those of the diploids they have been compared with. Performance data is graphed in Figures 6a and 6b. The sharp drop in numbers in January 1990 represents a die-off of 10% of the stock, all of which were severely emaciated fish (pinheads), that had managed to survive for the three months, apparently without acclimatising to sea water. The significance of this loss was not recognised at the time. The survivors all showed signs of being healthy smolts and continued to grow. Such pinheads also have been seen in diploid stocks but at much lower incidence.

The 48,000 all-female triploids, sent to sea cages in October 1990, were spread between Saltas' Marine operation and two commercial sea-farms. The 12,000 Saltas triploids were again held in conditions similar to the comparable diploids, and it is data from these fish which are graphed in Figures 7a and 7b. The loss of pinheads is again evident but it had started earlier and has been more severe. The latest report (mid-February, 1991) indicates that the mortality is easing off. It is estimated that losses due to pinheads by the end of February will be approximately 30%. The two other sites are reporting a similar pattern but with losses as at the end of February expected to be 25% and 40% (weighted average for three sites equals 35% losses). Industry figures for diploids show only a marginal increase of pinhead incidence in diploids for this year class (2.6% in 1990 compared to 2.1% for same period in 1989). It is therefore, assumed that the higher incidence of pinheading in triploids is a function of the triploid state.

Another phenomenon which has manifested itself in the 1990 triploids at the post-smolt stage is a lower jaw deformity. From radiological examination, it is seen that the dentary bone of the lower jaw and the lingual plate bone are both curved downward, at mid-length. The upper jaw remains normal. This syndrome is identical to deformities reported (in diploids) from Scotland (Bruno 1990). An undetermined percentage of the pinheads have the jaw deformity, but this also has been observed in post-smolts of healthy appearance. The total incidence amongst Saltas' triploids is estimated at 13%. Figures of 2% and 15% are reported at the other two sites. Such deformities have been reported in diploids in previous years, but only as isolated specimens.

For both the syndromes of pinheading and jaw deformity, a preliminary screening of parameters such as batch history, husbandry technique, and site specific environment have failed to show any obvious correlations. Investigation of this (very current) problem is still at the data gathering stage.

It is of some consolation that the unaffected triploids, of both smolt intakes, have shown encouraging seawater growth. From Figure 6a, it can be seen that the pre-maturation growth of the diploids is contrasted by the slower increase in size of the triploids. This test batch of triploids will be retained through to the next harvest season, to provide performance data during their second season in seawater. Over the cumulative 18 months that triploids have shared seawater sites with diploids, there have been no significant differences in pathogenic disease susceptibility.

SUMMARY

The biological feasibility of propagating and farming Atlantic Salmon in Tasmania is established. In adapting to Australian and then Tasmanian conditions over the 25 generations since its import from Nova-Scotia, the stock has altered its reproductive strategy to become an entirely grilse stock. This feature, together with the favourable temperature regimes, allows the industry to harvest stock after just 14 months in the sea, technically as grilse, but at an average weight of 3.5 kg. The disadvantage of such a grilse based industry is the short season of the harvest period. The suppression of maturation is therefore a desirable goal.

The rationale for selecting a particular method to suppress maturation was influenced by market considerations. The potential for adverse consumer reaction, to a product irradiated or chemically treated at any stage, precluded the use of radiation, hormones, or chemicals directly on the stock destined for market.

Sex-reversal of broodstock, and triploidy per-se, have been successful. From the three year classes handled to date, some differences between the early growth of diploids and triploids have been observed. In general, freshwater performance of triploids seems similar to that of diploids, and therefore commercially acceptable.

From the two year classes which have been sent to sea cages, it appears that a significant percentage of triploid smolts fail to adapt to seawater. Unaffected fish continue to grow satisfactorily and show no special susceptibility to diseases or environmental degradation. A jaw deformity, apparently site specific in its severity

and not sibling-related, has also appeared in the 1990 smolts, after some three months in the sea. The epidemiology of both these syndromes is still unclear.

In spite of these last two problems, which are considered to be transient, the Triploid Project has progressed satisfactorily. Predictions are never certain, but there appears to be a viable niche for all-female, triploid Atlantic salmon stock within the Tasmanian salmon farming industry.

ACKNOWLEDGEMENTS

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As well, I wish to acknowledge the instrumental role, played by Craig Foster and Steve Percival of Saltas' Marine Research Division, in initiating the project and I particularly thank the staff of Saltas for the sustained effort required to implement and maintain such a long term project.

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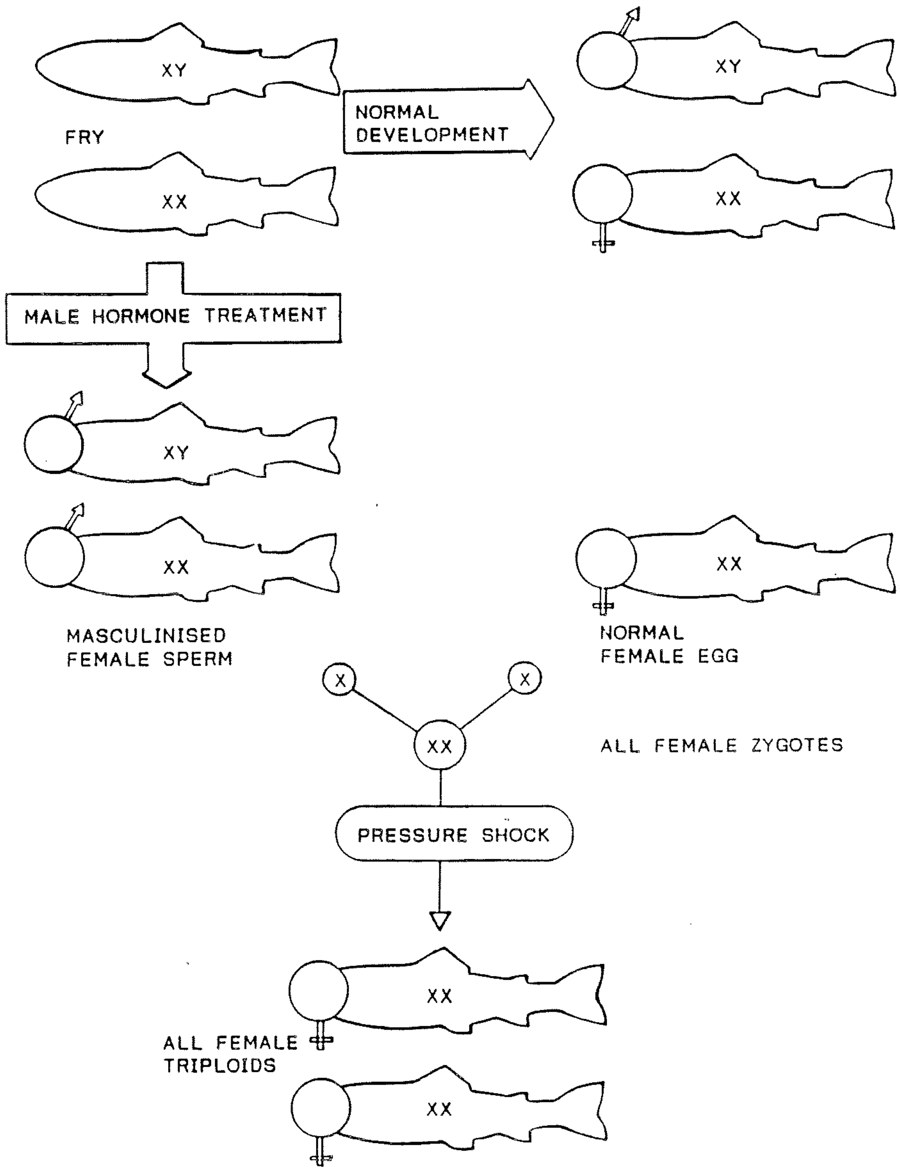
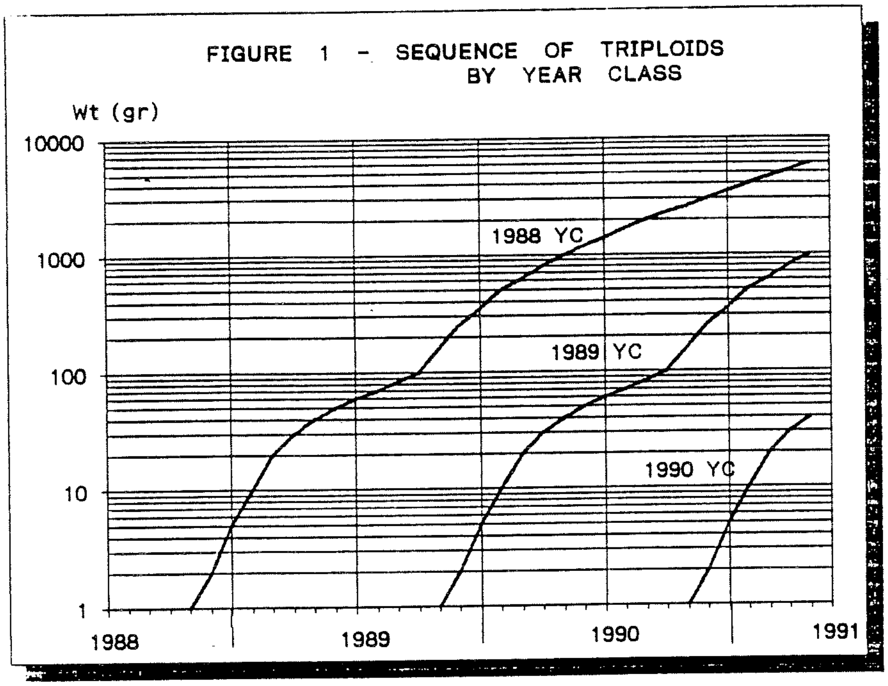
DIAGRAM 1 - SEQUENCE OF EVENTS USED TO PRODUCE FEMALE TRIPLOIDS





NOTE :- 1988 Year Class - approximately. 2,000 mixed-sex Triploids sent to sea.

1989 Year Class - approximately 48,000 female Triploids sent to sea.

1990 Year Class - approximately 300,000 female Triploids to be sent to sea.

FIGURE 2 - TRIPLOIDY TRIALS 1988

a) NORMALISED SURVIVAL (%)

MINS PF	8500 PSI 3m	8500 PSI 5m	8500 PSI 7m	9000 PSI 3m	9000 PSI 5m	9000 PSI 7m	9500 PSI 3m	9500 PSI 5m	9500 PSI 7m	10000 PSI 3m	10000 PSI 5m	10000 PSI 7m
25	12	33	42	30	54	63	36	81	67	54	72	96
30	36	66	86	47	79	67	76	98	71	46	85	89
35	87	66	68	45	95	50	72	75	93	76	61	47

TREATMENT - PRESSURE / MINUTES

b) TRIPLOID PERCENTAGE (%)

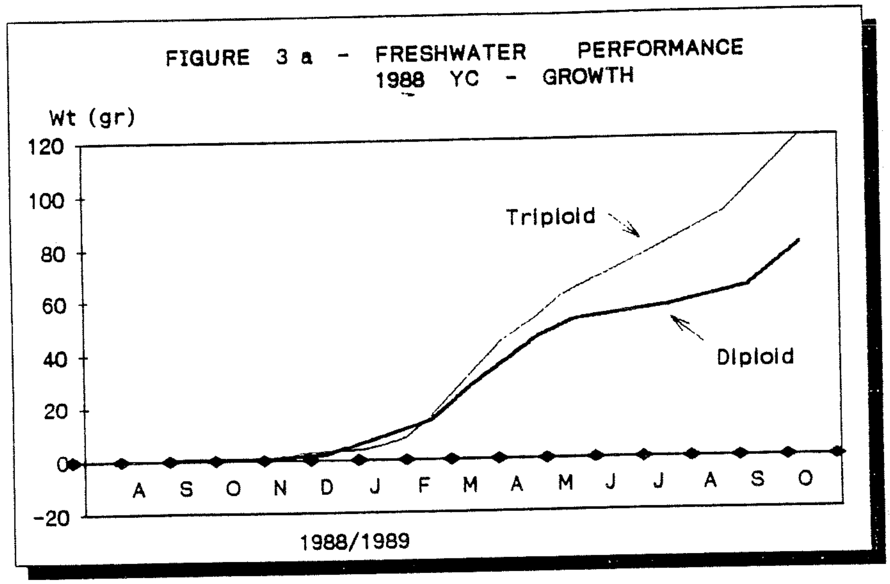
MINS PF	8500 PSI 3m	8500 PSI 5m	8500 PSI 7m	9000 PSI 3m	9000 PSI 5m	9000 PSI 7m	9500 PSI 3m	9500 PSI 5m	9500 PSI 7m	10000 PSI 3m	10000 PSI 5m	10000 PSI 7m
25	38	69	92	97	93	97	90	99	98	100	99	100
30	79	99	98	84	100	100	99	100	100	99	100	98
35	96	96	97	89	100	99	98	100	99	99	100	100

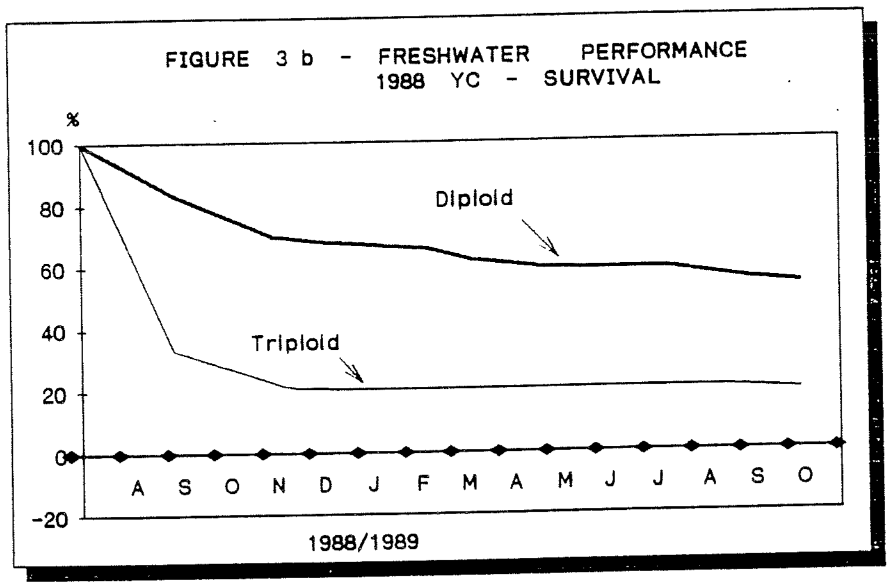
TREATMENT - PRESSURE / MINUTES

c) TRIPLOID YIELD (%)

MINS PF	8500 PSI 3m	8500 PSI 5m	8500 PSI 7m	9000 PSI 3m	9000 PSI 5m	9000 PSI 7m	9500 PSI 3m	9500 PSI 5m	9500 PSI 7m	10000 PSI 3m	10000 PSI 5m	10000 PSI 7m
25	4	27	39	29	48	62	34	80	65	54	71	**96**
30	27	65	86	42	79	67	76	**98**	71	45	85	87
35	84	64	66	38	**95**	49	70	75	92	75	61	47

TREATMENT - PRESSURE / MINUTES





Note :- "Triploids" - mixed-sex Triploids, ex 12,000 eyed eggs.
"Diploids" - mixed-sex Diploids, ex 2,080,000 eyed eggs.

FIGURE 4 a - FRESHWATER PERFORMANCE
1989 YC - GROWTH

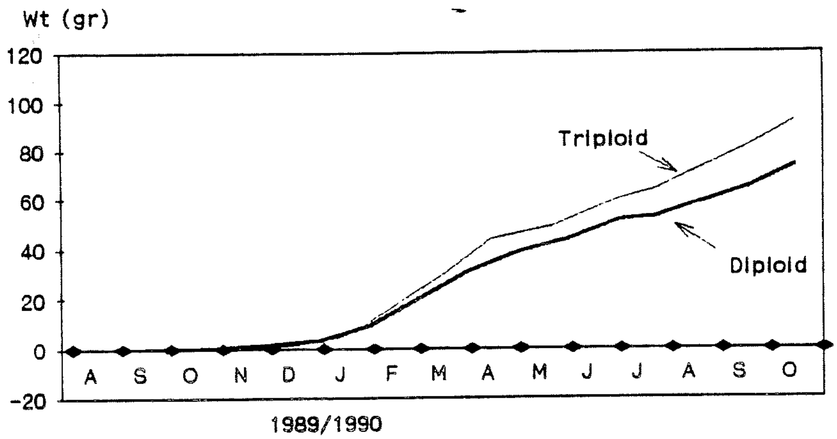
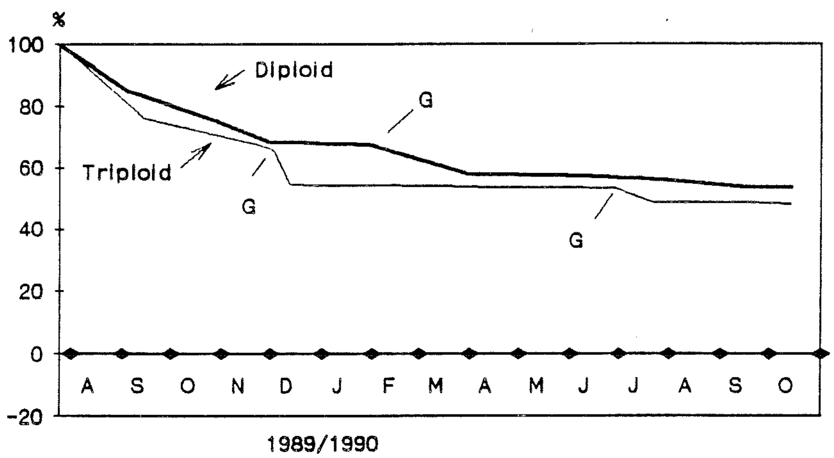
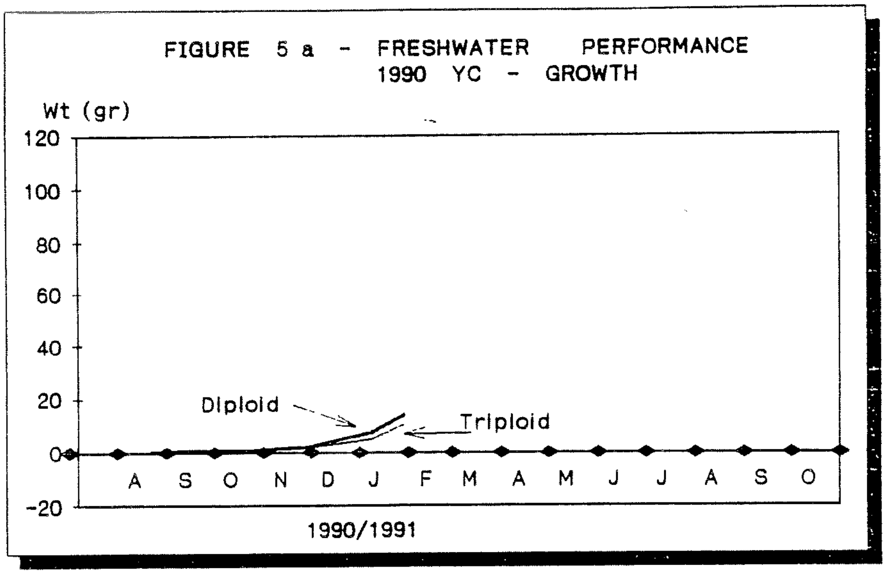
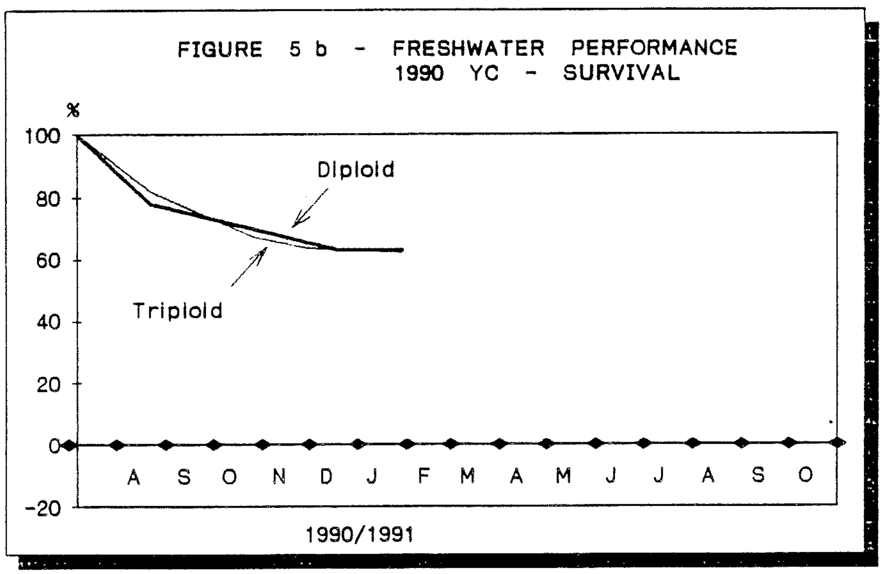


FIGURE 4 b - FRESHWATER PREFORMANCE
1989 YC - SURVIVAL

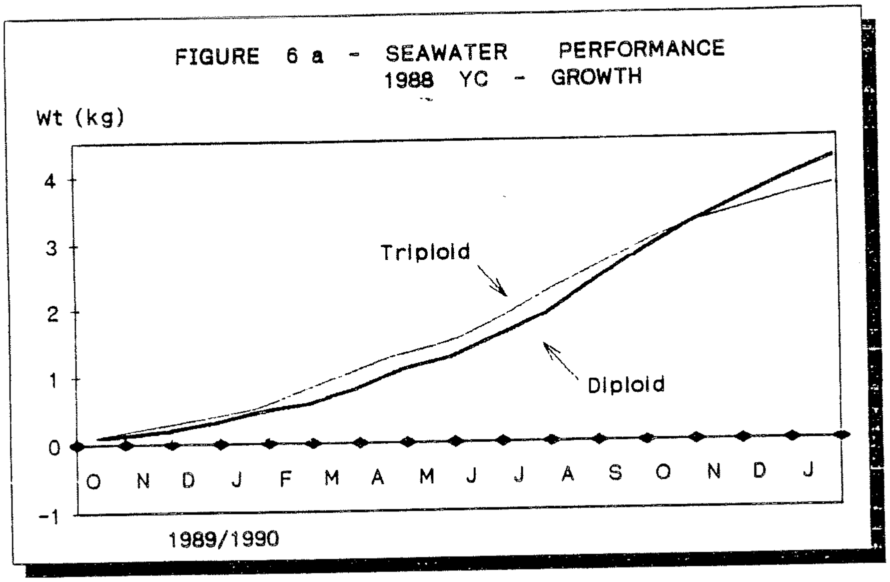


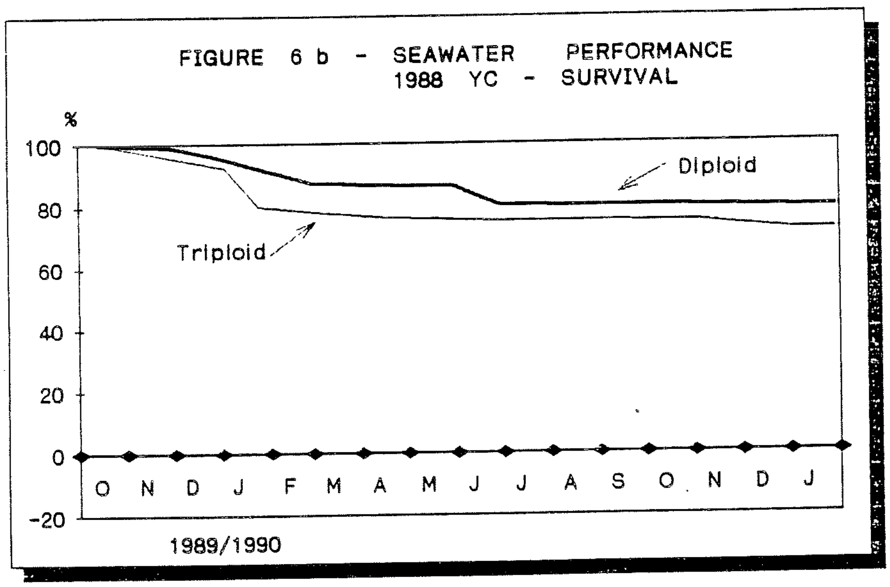
NOTE :- "Triploids" - female Triploids only, ex 100,000 eyed eggs.
"Diploids" - mixed-sex Diploids, ex 2,000,000 eyed eggs.
"G" - grading culls.





NOTE :- "Triploids" - female Triploids only, ex 550,000 eyed eggs.
"Diploids" - mixed-sex Diploids, ex 1,570,000 eyed eggs.





NOTE :- "Triploids" - mixed-sex Triploids, sample = 1,970 fish.
"Diploids" - mixed-sex Diploids, sample = 2,470 fish.

FIGURE 7 a - SEAWATER PERFORMANCE
1989 YC - GROWTH

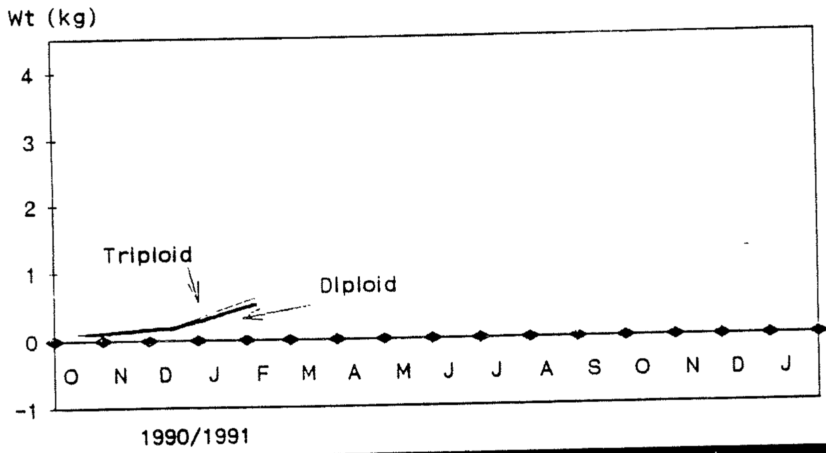
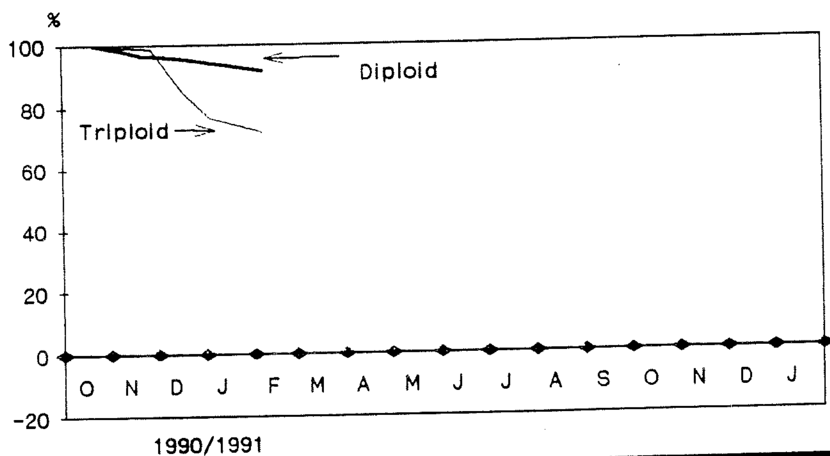
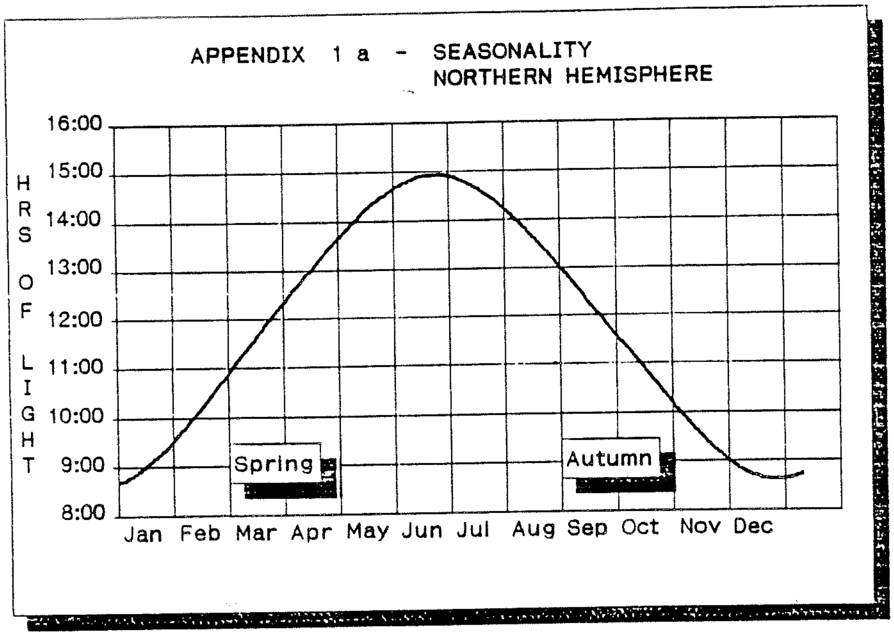
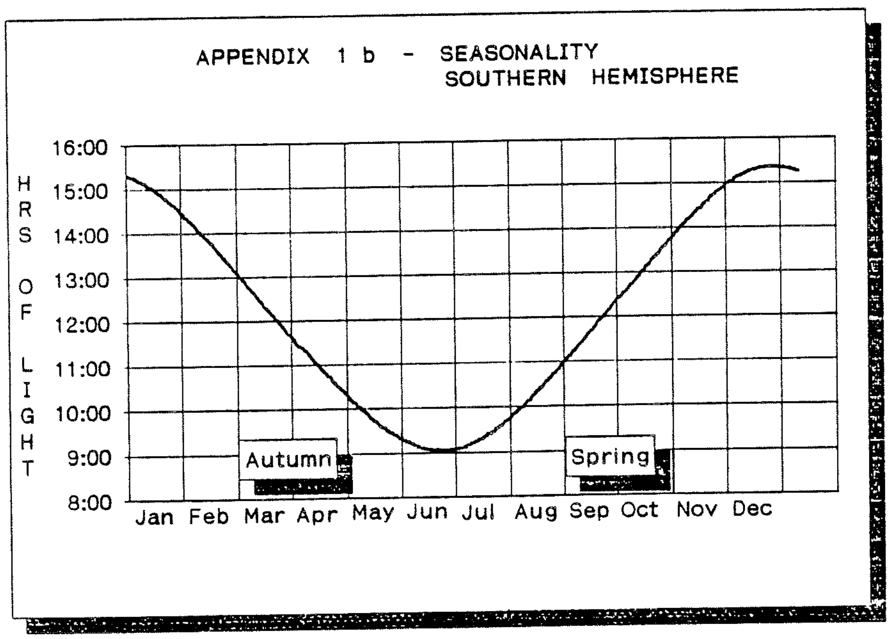


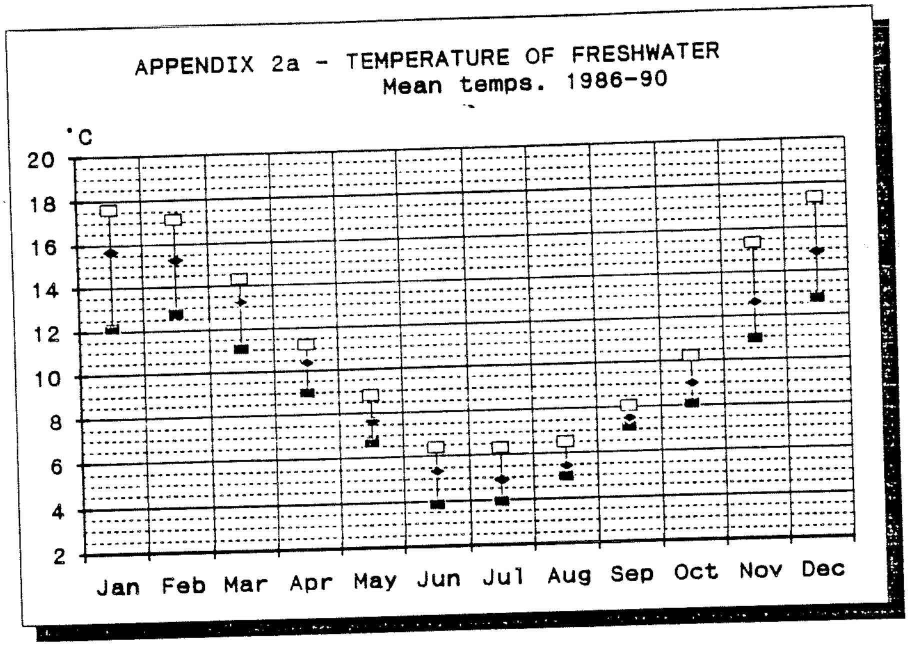
FIGURE 7 b - SEAWATER PERFORMANCE
1989 YC - SURVIVAL

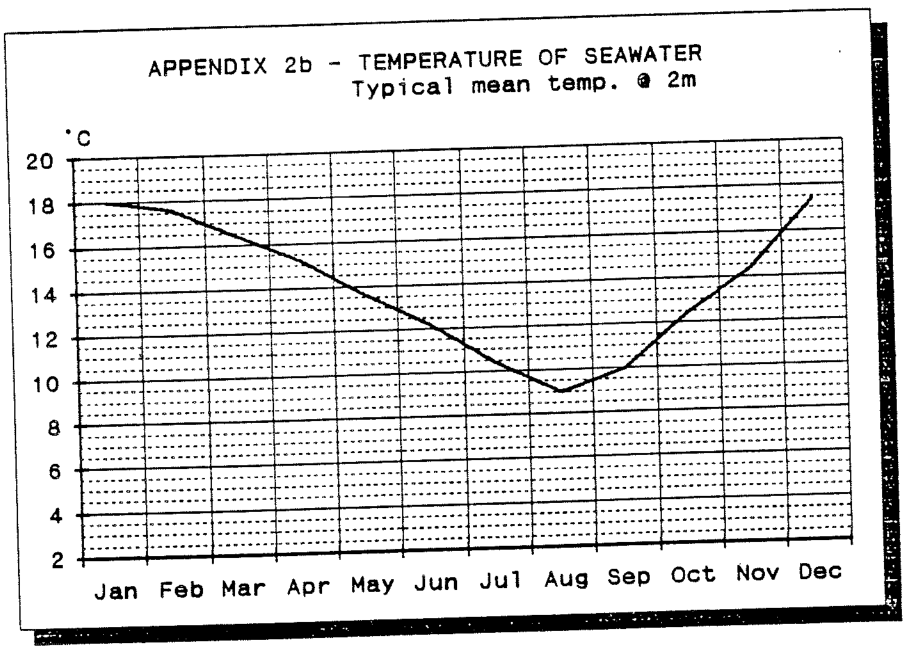


NOTE :- "Triploids" - female Triploids only, sample = 11,730 fish.
"Diploids" - mixed-sex Diploids, sample = 14,150 fish.


APPENDIX 1 a - SEASONALITY
NORTHERN HEMISPHERE
HRS OF LIGHT
16:00
15:00
14:00
13:00
12:00
11:00
10:00
9:00
8:00
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
Spring
Autumn


APPENDIX 1 b - SEASONALITY
SOUTHERN HEMISPHERE
HRS OF LIGHT
16:00
15:00
14:00
13:00
12:00
11:00
10:00
9:00
8:00
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
Autumn
Spring


APPENDIX 2a - TEMPERATURE OF FRESHWATER
Mean temps. 1986-90
°C
20
18
16
14
12
10
8
6
4
2
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec


APPENDIX 2b - TEMPERATURE OF SEAWATER
Typical mean temp. @ 2m
°C
20
18
16
14
12
10
8
6
4
2
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec

APPENDIX 3 - SUMMARY OF RATIONALE FOR SELECTING PRESSURE SHOCK ON FEMALE ZYGOTES

Autoimmune sterilisation	- NO -	impractical & unproven
Hormonal sterilisation	- NO -	adverse market perception
Chemical sterilisation	- NO -	variable results & adverse market perception
Irradiation sterilisation	- NO -	difficult logistics & adverse market perception
Triploidy	- YES -	BUT males still develop secondary sexual characteristics

Tetraploid X Diploid cross	- NO -	cumbersome technique
Chemical induction	- NO -	unproven technique & adverse market perception
Temperature shock	- NO -	variable results
Hydrostatic shock	- YES -	BUT need to generate all-female stock

Hormonal feminisation	- NO -	adverse market perception
Gynogenesis	- NO -	logistics & potential inbreeding
Masculinised female X Normal female cross	- YES -	All-female (zygotes) without chemical exposure

Therefore provide hydrostatic shock to all-female zygotes to give all-female triploids

PARTICIPANT DISCUSSIONS

Rapporteur: Randy Penney

Q: Friars — Are eggs taken from fish returning to rivers or from a wild fishery?

A: P.J. — All the Tasmanian salmon industry is based entirely on sea cage culture. There is no wild fishery.

Q: Donaldson — Is SALTAS involved with hatchery production only. How is it related to the cage owners?

A: P.J. — SALTAS is partially owned by the cage farmers. Smolt produced from the hatchery are made available to each grower in direct proportion to their ownership in SALTAS.

SALTAS also has a Marine Research Division that carries out research on the marine phase of the operations, provides an extension service, and holds broodstock.

Q: Pepper — Are broodfish stripped on the cage sites?

A: P.J. — No. Broodfish are returned to freshwater holding prior to stripping.

Q: Benfey — Are the pressure treatments you referred to for induction of triploidy done at 10°C?

A: P.J. — Yes. The additional details of pressure and timing are provided in Figure 2 of my report.

Q: Johnstone — Is there a problem with using even higher pressures, i.e., have you found any problems with higher pressures?

A: P.J. — We have not noticed any particular problems with the higher pressures.

Q: Boulanger — The sensor used to monitor pressure, where is it located?

A: P.J. — On the outlet side of the pressure pump.

Q: Boulanger — Where you position the sensor has a bearing on the lag time to get an accurate reading as pressure is being increased. Is it possible your times are inaccurate?

A: P.J. — Pressure is built up over 90 sec. before treatment time is considered to have started. Under these specific conditions, our times are accurate.

Q: Johnstone — Of those male triploids which are in your sea cages now, have you noticed any changes as they approach the normal time of maturation?

A: P.J. — No noticeable changes yet.

Q: Donaldson — Is there a size difference emerging between male and female triploids?

A: P.J. — Yes, the males seem larger.

<u>Comment: Stevenson</u>

With respect to the jaw deformities you mentioned, we have seen similar problems in rainbow trout. We attributed this to stress. It occurs in about 1% of the population.

Comment: Friars

There seems to be a strong family component to this deformity problem but it isn't entirely genetic. There appears to be some association with a history of other things, i.e., fungal treatments, etc.

Q: Friars — How many sex reversed female were used for the broodstock?

A: P.J. — About 20.

Q: Pepper — What is your feeling on where you are going with your triploidy program? If I understand you correctly, you are not concerned about your grilse as such since these grilse exceed 3 kg in 14 months in the sea cages. Your interest in triploidy is simply to circumvent the likelihood of all of your fish coming into market condition at the same time and therefore, having to be marketed in an unacceptably short period of time. Do you have a good grasp at this time on the relative proportions of diploid vs. triploid fish you consider desirable in your operation? Where is Tasmania headed with development of triploidy?

A: P.J. — The chronological progression we have followed was something in the order of 2,000 eggs in 1988, 50,000 in 1989, and 300,000 in 1990. The number of triploid fish in commercial production is increasing steadily. This progression reflects our ability and confidence to produce sufficient triploids to make it all worth while. Meanwhile, the requirement of the Tasmanian industry has remained static at one million smolts per year. What we have been doing is increasing the percentage of triploid smolts. The idea is to provide the industry with perhaps 30% of its requirements as triploids. At this point in time, I have no hesitation in saying that triploidy seems to be the way to go. Any difficulties we have encountered along the way we consider transient. We intend to proceed with our triploidy program and to deal with circumstances as they develop.

Comment: Anderson

We had similar deformity problems some years ago. They had nothing to do with triploidy. You should not assume the problems you have observed are necessarily related to the triploidy program.

Comment: Boulanger

We have noticed similar problems with trout at hatching. Maybe this is the same problem.

A: P.J. — I don't think it is the same problem. It isn't of the kind associated with teratogenic effects. The fish are normal in appearance until they are released as smolt.

Comment: Johnstone

You must be careful not to confuse correlation with causation. The occurrence of pinheads and jaw deformities may not be due to triploidy. Jaw deformities have occurred in Scotland in diploid stocks. David Bruno from our laboratory has documented one such occurrence in Veterinary Record. There is also a condition in diploid stocks in Scotland called "failed smolt syndrome" that looks, superficially, to be similar to your "pin head" condition. I have attributed the condition we see to a failure to come onto feed in the immediate post transfer period and believe that social/husbandry interactions are most important in its generation.

Comment: Donaldson

In hormonal sterilization studies, we have seen a spinal deformity condition we refer to as the "Volkswagen effect". We cannot identify the cause.

Comment: Glebe

It is possible such effects could be the result of stress such as can result from salmonid aggression under high cage density.

THE PHYSIOLOGY OF TRIPLOID SALMONIDS IN RELATION TO AQUACULTURE

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INTRODUCTION

Triploids have three sets of chromosomes in every somatic cell rather than the normal (diploid) number of two sets. The goal of this paper is to describe the physiological changes associated with induced triploidy in salmonids (i.e., fish of the family Salmonidae), especially as it relates to their suitability for aquaculture. These physiological changes can all be related to two basic differences between triploids and diploids, namely differences in cell size and number, and impaired gametogenesis in the triploids.

Triploidy has been induced in most species of salmonids (Benfey 1989), generally with the aim of determining the suitability of this genetic manipulation for suppressing reproduction of fish used in commercial aquaculture. Although the artificial induction of triploidy is a relatively new technique in aquaculture, it is important to realize that polyploidy has played a major role in the evolution of the salmonids (Schultz 1980; Allendorf and Thorgaard 1984; Johnson et al. 1987), and that "spontaneous" triploids are frequently encountered among these fishes (e.g., Cuellar and Uyeno 1972; Thorgaard and Gall 1979; Ueda et al. 1983). Furthermore, there are a few naturally-occurring triploid species of fish (none are salmonids) which have evolved atypical modes of reproduction to overcome the problems associated with chromosome movement during meiosis in triploid cells, and therefore are fertile (Schultz 1980; Purdom 1984).

EFFECTS OF CHANGES IN CELL SIZE AND NUMBER

Nuclear and cellular size are increased in triploids to accommodate the increased amount of nuclear DNA while maintaining a normal ratio of nuclear to cytoplasmic volume. Cell numbers, on the other hand, are reduced in triploids to maintain a normal organ and body size. These changes have been best described for erythrocytes (erythrocyte size is commonly used to identify triploid fish), but also apply to leucocytes and to the cells and nuclei of the brain, retina, cartilage, kidney, liver, and epithelium in fish (Swarup 1959a; Lou and Purdom 1984; Small and Benfey 1987; Aliah et al. 1990; Strussmann and Takashima 1990).

As cell volume increases, the ratio of surface area to volume must decrease; the same is true of cell nuclei. Thus, with increased cell size and decreased cell number in a given tissue or organ that remains the same overall size, the total cell surface area of that tissue or organ must decrease, as must the total nuclear surface area. Such a reduction in surface area in the absence of any other changes should lead to a reduced ability of triploid cells and their nuclei to take up or secrete any molecules that normally pass across their surfaces (hormones, nutrients, metabolites, ions, etc.), and thus impair the ability of the various tissues and organs to perform their normal functions. However, there is an advantage to decreasing the surface area to volume ratio of cells, namely that less energy is required to maintain their internal composition (Szarski 1976).

Increases in triploid erythrocyte size are balanced by decreases in erythrocyte number, to the extent that haematocrits are the same for diploid and triploid Atlantic salmon, Salmo salar (Benfey and Sutterlin 1984a; Graham et al. 1985), coho salmon, Oncorhynchus kisutch (Small and Randall, 1989) and various cyprinids (Barker et al. 1983; Sezaki et al 1983; Ueno 1984). However, triploid Atlantic salmon and coho salmon have a lower blood haemoglobin content than diploids (Benfey and Sutterlin 1984a; Graham et al. 1985; Small and Randall 1989), which is not the case with triploid cyprinids (Barker et al. 1983; Sezaki et al. 1983). Triploid Atlantic salmon also have lower blood pH and lower haemoglobin-oxygen loading ratios than diploids, and thus have a reduced blood oxygen carrying capacity (Graham et al. 1985).

In spite of these haematological differences, juvenile triploid Atlantic salmon are no different from diploids in their rates of oxygen consumption relative to body weight (Benfey and Sutterlin 1984b); the same is true of triploid three-spined stickleback, Gasterosteus aculeatus, (Swarup 1959b). Similarly, Oliva-Teles and Kaushik (1987a, 1987b, 1990a, 1990b) found little or no difference in oxygen consumption rates between triploid and diploid rainbow trout, O. mykiss, at various stages of development. In studies of sustained swimming ability, Small and Randall (1989) found no difference in critical swimming velocity between triploid and diploid coho salmon. However, Virtanen et al. (1990) found that triploid rainbow trout have a lower aerobic swimming capacity than diploids, as indicated by a rapid increase in plasma lactate and depletion of liver glycogen during forced swimming. The results of this latter study must be viewed with caution since the triploids had a lower haematocrit than the diploids at the start of the experiment for no apparent reason.

Studies of other physiological processes also indicate remarkable similarities between triploids and diploids. Triploid rainbow trout have the same metabolic rates as diploids at various stages of development (Fauconneau et al. 1986, 1989; Oliva-Teles and Kaushik 1987a, 1987b, 1990a, 1990b); the same is true of non-salmonid species (Wiley and Wike 1986; Henken et al. 1987). Triploid salmonids also have the same osmoregulatory abilities in seawater as diploids (Lincoln and Bye 1984; Johnson et al. 1986; Quillet et al. 1987; Quillet and Gaignon 1990). Triploid and diploid rainbow trout have the same ability to fix dietary canthaxanthin, except that there is a shift to ovarian deposition in diploid females as they mature (Choubert and Blanc 1985, 1989). Triploid and diploid coho salmon synthesize and secrete gonadotropin and vitellogenin at the same rates when injected with estrogen (Benfey et al. 1989a).

Leary et al. (1985) suggested that developmental rates must be the same for triploids and diploids (as is the case with stickleback; Swarup 1959a), but studies with triploid rainbow trout indicate that they hatch earlier than diploids (Chevassus et al. 1984; Happe et al. 1988; Quillet et al. 1988), and that tetraploids hatch earlier still (Quillet et al. 1988). These differences in developmental rate may be due to increased heterozygosity (Allendorf and Leary 1984; Leary et al. 1985) rather than, or in addition to, changes in cell size and number (Quillet et al. 1988). Leary et al. (1985) also pointed out the remarkable morphological similarity between triploid and diploid rainbow trout; the same is true for non-salmonid fish (Beck and Biggers 1983; Cassani et al. 1984; Bonar et al. 1988). There is only a single reported morphological abnormality associated with triploidy in fish, this being lower jaw deformities in triploid Atlantic salmon (Sutterlin et al. 1987; Jungalwalla, this workshop). Okada (1985) observed that triploid rainbow trout occasionally have a spleen separated into two parts.

Depending upon the particular study, triploids have been shown to grow faster, slower, or at the same rate as diploids. The question of what effect triploidy has on growth rates is confused by the interrelationship between growth rates and the onset of sexual maturation, and by possible behavioural differences between triploids and diploids. At the onset of sexual maturation, energy is diverted from somatic growth to gametogenesis, and thus different growth rates between triploids and diploids should be expected in adult fish. In rainbow trout, growth rates decline with the onset of sexual maturation in diploids of both sexes and in triploid males, whereas triploid females continue to grow well (Chevassus et al. 1984; Lincoln and Bye 1984, 1987). In this case, triploid females do not exhibit superior growth rates, but simply continue to grow at a constant rate whereas growth rates decline in the maturing fish. Some studies have found that triploids do not grow well in competition with diploids but do grow as well as diploids when reared separately (Cassani and Caton 1986; Lincoln and Bye 1987). However, others have not found such an effect (Henken et al. 1987).

Decreased cell numbers in the brain and sensory systems could have a profound effect upon the ability of triploids to recognize and respond to changes in their surroundings. Fankhauser (1941) suggested that the visual acuity of triploids should be reduced by about a third, and showed that triploid salamanders learn less quickly than their diploid siblings how to negotiate a simple maze (Fankhauser et al. 1955). More recently, it has been shown that triploid fish are less sensitive to sound and light than diploids (Aliah et al. 1990). Such behavioural and sensory effects may be of more direct concern in aquaculture than the physiological effects of changes in cell size and number, and may help explain the decreased aggressiveness and fitness frequently attributed to triploid salmonids, especially when reared under sub-optimal conditions (Johnson et al. 1986; Boulanger 1987; Quillet et al. 1987; Quillet and Gaignon 1990).

EFFECTS OF IMPAIRED GAMETOGENESIS

Normal gametogenesis is disrupted in triploids due to the inability of three homologous chromosomes to pair correctly prior to crossing-over, early in meiosis. As a result, triploids are generally sterile. However, because of the enormous difference in the number of pre-meiotic germ cells produced in male and female salmonids, there is a pronounced difference between the sexes in the effect of triploidy on sexual maturation; triploid males develop large testes, whereas triploid ovaries remain miniscule. A consequence of this is that triploid males have fully active testicular steroidogenic cells and pituitary gonadotrophs, and therefore go through all the physiological changes normally associated with sexual maturation, whereas triploid females produce no detectable levels of ovarian sex steroids, gonadotropin, or vitellogenin, and retain the appearance of immature fish throughout their lives (Lincoln and Scott 1984; Sumpter et al. 1984; Nakamura et al. 1987; Benfey et al. 1989b). A further result is that triploid females have a higher lipid content and lower water content in their flesh than triploid males or diploids of either sex at maturity (Chevassus et al. 1984; Lincoln and Bye 1987), and also have large fat deposits in their viscera (Chevassus et al. 1984; Lincoln and Scott 1984; Johnson et al. 1986).

In aquaculture, where the goal of delayed maturation or suppressed maturation is to avoid the deleterious physiological and behavioural effects of sexual maturation, all-female stocks of triploids are generally used. The production of all-female triploids is a simple extension of inducing triploidy, requiring the use of sperm from sex-reversed, genotypic females to fertilize normal eggs prior to triploidy induction (Lincoln and Scott 1983; Okada 1985; various authors, this workshop).

A small percentage of germ cells do progress beyond the initial meiotic block in triploid salmonids of both sexes. Triploid male rainbow trout produce small numbers of fully functional aneuploid spermatozoa (Lincoln and Scott 1984; Okada 1985; Benfey et al. 1986), which are capable of activating development in normal eggs, but all the progeny are aneuploid and die prior to feeding (Lincoln and Scott 1984; Okada 1985; Ueda et al. 1987). Triploid female salmonids typically produce a small number of pre-vitellogenic oocytes, and fully mature (but apparently aneuploid) oocytes have been obtained from triploid Atlantic salmon (Johnstone, this workshop). Similar observations have been made for triploids of non-salmonid species (Lincoln 1981a, 1981b; Wolters et al. 1982; Suzuki et al. 1985; Ueno 1985; Penman et al. 1987; Pandian and Varadaraj 1988).

Recent studies with triploid grass carp, Ctenopharyngodon idella, which are used in the USA for biological weed control (Allen and Wattendorf 1987), have given slightly different results. Triploid male grass carp also produce small numbers of fully functional spermatozoa which are generally aneuploid (Allen et al. 1986; Van Eenennaam et al. 1990), but occasional viable diploid and triploid progeny are obtained when triploid males are crossed with either diploid or triploid females (Goudie 1988; Van Eenennaam et al. 1990). Fully mature, ovulated eggs have been obtained from triploid females and crosses between triploid females and either diploid and triploid males also yield occasional viable diploid and triploid progeny (Goudie 1988).

CONCLUSIONS

With the exception of the study by Virtanen et al. (1990), it would appear that haematological differences between triploids and diploids are either not extreme enough to cause differences in whole animal physiology, or that triploids are in some way able to compensate for inefficiencies in blood oxygen carrying capacity. In fact, triploid fish are remarkably normal in all regards aside from their sterility. However, triploid salmonids are frequently said to perform poorly in aquaculture, especially when reared under sub-optimal conditions (Johnson et al. 1986; Boulanger 1987; Quillet et al. 1987, Quillet and Gaignon 1990) or when kept in mixed groups with diploids (Lincoln and Bye 1987).

Although no physiological explanation can be given at present to support this observation, more research is required to exclude it. Potential physiological disadvantages may be balanced by the increased genetic heterozygosity resulting from induced triploidy (Allendorf and Leary 1984; Leary et al. 1985). Apparent differences between triploids and diploids may be due to behavioural and sensory problems rather than due to direct physiological effects.

What has become clear is that triploid female salmonids retain the characteristics of immature fish throughout their lives, and may therefore serve a useful function in aquaculture to eliminate problems associated with sexual maturation, such as decreased flesh quality and increased susceptibility to disease. In addition, the risk of unwanted spawning by genetically-altered fish can probably be eliminated by the use of all-female triploids, although recent observations with triploid grass carp suggest that this may not be completely effective (Goudie 1988). What remains to be determined clearly is whether induced triploidy can be used to meet these objectives without compromising the economic viability of commercial aquaculture.

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PARTICIPANT DISCUSSIONS

Rapporteur: Gil Farmer

Q: Davidson — Do triploids take longer to reach the first feeding stage?

A: Johnstone — Yes, in strictly equivalent groups of triploids and diploids, the triploids appear to be some 30-50 degree days later in coming on to first feed. However, this does not mean that the growth and performance of triploids post-first-feeding will be inferior to that of diploids.

Q: Davidson — Have differences in the auto-immune system of diploids and triploids been observed?

A: Benfey — Not much work has been carried out on this subject. One study does suggest that triploids demonstrate more disease resistance than diploids (reference Ron Roberts). Triploids may react differently to vaccines since they have a smaller total cell surface area than diploids. Because of this, the uptake of vaccines by triploids during immersion may not be as rapid. The general impression of the aquaculture industry is that triploids are immuno-competent.

Q: Saunders — How do you explain the production of peptide in triploids by the pituitary?

A: Benfey — Although it has not been examined, the structure of the pituitary gland must be similar in triploids and diploids. Hormones secreted by the testes stimulate synthesis and secretion of gonadotropin in the pituitary of triploid males; triploid ovaries do not produce high enough concentrations of the appropriate hormones to fulfil this role in triploid females.

Q: Anderson — If the oxygen carrying capacity of triploids is less than that of diploids, why do triploids perform well when their swimming performance is measured in a chamber?

A: Benfey — I cannot explain this. It may be possible that some other physiological mechanism allows compensation.

TRIPLOIDY AND SEX-REVERSAL IN RELATION TO SELECTION IN THE SALMON GENETICS RESEARCH PROGRAM

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ABSTRACT

The Salmon Genetics Research Program has been involved with the development of strains suitable to aquaculture. Grilsification, as represented by sexual maturation after one sea winter, is undesirable in sea-cage culture because of inferior market quality and growth impediment. This paper gives details on studies incorporating triploidy with all-female progeny and selection effects in relation to the grilsification problem.

IMPLICATIONS OF GRILSIFICATION IN THE CAGE-CULTURE INDUSTRY

Domestication and genetic improvement in the Atlantic salmon represent relatively new endeavours, when one considers the long-term efforts that have been directed toward many agricultural species. The high reproductive rate and the extent of the phenotypic and genetic variation in this species make it a very promising candidate for improvement through selection (Gjedrem 1983; Gjerde 1986). Improved growth rate to market size is a trait with high potential for the enhancement of economic efficiency. However, the genetic and environmental interrelationships of this trait with grilsification complicate the selection process.

Grilsification, defined as sexual maturation after one sea winter, is an undesirable trait in aquaculture because flesh quality deteriorates when nutrients and energy are directed toward reproductive rather than carcass tissues. Also, secondary sex characteristics, such as dark colour, that accompany the sexual maturation process diminish retail value. Fish that eventually become grilse grow faster in the early stages (Anon. 1987; Skilbrei 1989). However, in the Bay of Fundy industry, the size attained before the onset of sexual maturity is usually about 3.0 kg or less. Smaller fish command lower prices per kg when they compete with those that reach 4.0 to 4.5 kg after two sea winters and before sexual maturation. Despite the fact that larger pre-grilse are being grown in Tasmania than in the Bay of Fundy (Jungalwalla, pers. comm.), the industry perceives that the avoidance of grilse in the Northern Hemisphere is a requirement of efficient operations at this time.

STRAIN EFFECTS ON GRILSIFICATION

In the wild, the ratio of grilse:larger salmon was higher in the Big Salmon River (BSR) than in the Saint John River (SJR). Samples of these stocks reared in common hatchery and sea-cage environments again yielded higher proportions of grilse in the BSR than the SJR stocks, even though the general frequency of grilse was greatly reduced (Saunders et al. 1983). The SJR stock has become virtually the only genetic resource used in the Bay of Fundy cage-culture industry. The SGRP is involved as a primary breeder in the development of four strains from the SJR stock, one in each of four year-classes entering a four-year generation cycle in consecutive years.

The synthetic strain used experimentally, named the Biotechnology Strain, is comprised of approximate percentages of BSR (55), Magaguadavic (23), SJR (15), Rocky Brook (6), Dennis (1) genes. The incidence of grilse has ranged from 48.0 to 73.6 percent in samples of these stocks reared in sea cages. The high proportions of grilse have rendered these stocks undesirable for industry. However, the diversity in the gene pools makes this synthetic strain attractive for long-term development, particularly if means of eliminating early sexual maturation can be found.

MASS SELECTION FOR GRILSE FORK LENGTH

The Biotechnology Strain was divided into a control line and a line mass-selected for grilse length in 1985. The realized heritability for that trait was 0.27. Several correlated responses for increased growth and smoltification rate were observed.

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The proportion of grilse was 70.5 in the control and 65.8 in the select line contemporaries of the Biotechnology strain. Hence, selection for improved growth and decreased grilsification rates appears feasible (O'Flynn 1990).

By about 18 months post-fertilization (S1), high smoltification rate and large smolt size are associated with fast growth during the first few months in sea water. However, these positive relationships become very weak when the seawater performance is measured nearer market time (Friars, et al 1988; O'Flynn 1990). Consequently, selection on both freshwater and seawater traits is necessary to yield improved productivity for both the smolt producer and the cage grower.

Index selection takes into account the genetic and environmental variation as well as the relationships between several traits. The economic merit of each trait is also considered where, for example, the price of a smolt at $3.50 and the worth of a market fish at $40.00 requires greater selection emphasis on market size than on percentage S1 smolt. This approach also allows the combination of information from traits measured on relatives with that indicated by an individual's own performance. Genetic gain should then reflect maximum profits.

Two year-classes of the stock being developed for industry from stocks derived from the SJR by the SGRP have been selected on the basis of indices. The index used on the second of these strains penalized families that produce grilse. In the total population, 11.67 percent grilse were encountered and the family scores ranged from zero to 65 percent grilse. In addition to selection for improved growth and smoltification, overall economic merit discounts grilsification.

The Biotechnology Strain, previously described as having as high as 73.6 percent grilse, also has been subjected to a second generation of selection. The first generation of selection was for grilse fork length and an index was involved in the second. However, that index did not penalize families for the production of grilse. Instead, this strain is being used in connection with other means of reducing the incidence of grilse, as described below.

TRIPLOIDY AND ALL-FEMALE PROGENY IN RELATION TO THE GRILSIFICATION PROBLEM

Males usually show a much higher incidence of grilsification when compared to female counterparts. For instance, in one population of SJR fish, where the grilse represented 5.8 percent, 0.8 percent were females and 5.0 percent were males. The production of all-female progeny should therefore tend to alleviate the grilse problem. Furthermore, the hooked lower jaws of males tend to make them undesirable to the market in the early stages of grilsification. Consequently, all-female populations would effectively reduce the incidence of grilse in the SJR stock.

Fish with three sets of chromosomes (triploids) in contrast to the normal two (diploids) are functionally sterile. While triploid males still tend to produce undesirable secondary sex characteristics, triploid females do not give any appearance of sexual maturation. Grilsification should be circumvented in all-female, triploid populations - a combination being explored in the SGRP.

STUDY OF REDUCED GRILSIFICATION IN THE BIOTECHNOLOGY STRAIN

The inclusion of three parts per million of methyl-testosterone in the diet of first-feeding fry, from the select line of the Biotechnology Strain in 1986, successfully reversed females inducing them to produce sperm in 1989. At spawning time these individuals looked mature because secondary sex characteristics were fully developed, but they did not yield sperm through routine stripping techniques. Subsequent postmortem examination of these individuals revealed mature though abnormally shaped testes and under-developed sperm ducts. Maceration of the testes and screening through fine-mesh nylon yielded milt with viable sperm.

The splitting of eggs from individual females and the use of sperm from individual fish on multiple lots of eggs allowed the production of four mating types. Subsequent splitting of the eggs from each mating allowed a heat shock to be applied to a sample from each female (Table 1). The eight types of progeny from single parents formed a set. Five such sets of matings were made, but the eggs from one female had to be discarded because of bacterial kidney disease organisms in the ovarian fluid. This left 36 groups for study.

Visual examination of the gonads from 11-month-old untreated progeny of these matings confirmed that all five males that were presumed to be reversed females had, in fact, been genotypic females (i.e., ten females found out of a random sample of ten progeny from each of these five males).

Matings involving reversed-female milt were carried out in both a control and select line, and both with and without heat shock of the fertilized eggs (Table 1). The heat shock was only partially successful in inducing triploidy. The desired heat shock was 10 minutes at 28°C, applied 20 minutes after fertilization, followed by incubation at 9.5-10°C. In fact, the temperature of the water bath gradually declined as the treatments progressed (based on an electronic thermometer - from 27.5°C to 26.5°C; based on a mercury thermometer - from 28.0°C to 27.5°C). Ploidy level of the progeny from these treatments was determined at 11 months of age by flow cytometry (a random sample of ten fish from each group). The results are presented in Table 2.

The production of all-female triploids is a two-phase program, requiring firstly the development of broodstock having genotypic females as the source of milt, and secondly the efficient induction of a high rate of triploidy. It is apparent that only the first phase has been achieved so far in the SGRP. The heat shock treatment used in 1989 did not yield the high rates of triploids required for the proper evaluation of triploids throughout the entire production cycle. Since both the success of the heat shock treatment and the heat shock temperature itself declined as the experiment progressed, the poor results are likely due to the use of a sub-optimum heat shock. However, it also appears that lower rates of triploidy were obtained from eggs fertilized with the milt from sex-reversed females.

PRODUCTION OF TRIPLOIDS IN SAINT JOHN RIVER FISH

Although the Biotechnology Strain is a good candidate for the study of triploidy and all-female progeny because of its high grilsification rate, it is not currently used in the cage industry. Consequently, studies on triploidy were commenced in the 1990 spawning year-class of SJR fish. About 300 eggs from each of twenty single-pair matings, with sex-reversed females, were subjected to pressure shocks of 9500 psi (=65,500 kPa or 647 atm) for five minutes. A portable Carver lab press with a 50 ml pressure cell was used. Twelve of the twenty shocks were applied at twenty minutes after fertilization, with the remaining shocks applied at various times between eighteen and twenty-six minutes after fertilization. The pre-shock incubation temperature was 9.2°C - 9.5°C. The progeny from one of the females were discarded because of the presence of Bacterial Kidney Disease organisms in the ovarian fluid. The remaining progeny will be compared to full sibs produced without shock.

Additionally, plans are being made for the production of all-female progeny in conjunction with triploidy in SJR fish. Although grilsification is not usually a serious problem for cage culturists using SJR fish, the proportion appears to vary between groups and has been reported to be as high as 40 percent at one Nova Scotia site (Farmer, G.J., pers. comm.). Also, concerns about cage escapees entering rivers and mating with wild stocks has heightened the cage-culture industry's interest in sterile stocks for the future.

ACKNOWLEDGEMENTS

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Table 1. Layout of each of the five sets of matings in the sex-reversal, triploidy experiment.

Egg donor (line of female)[1]	Egg lot (Heat Shock)[2]	Sperm donor: Reversed female	Sperm donor: Control line male	Sperm donor: Select line
Control	No	x	x	
	Yes	x	x	
Select	No	x		x
	Yes	x		x

1 Select line subjected to two generations of selection in contrast to no selection in the control.
2 Heat shock intended to produce triploids in contrast to diploids where no shock was applied.

Table 2. Percentages of triploids in the eighteen heat shocked groups of eggs.

Type of progeny	Type of dam	Set 1	Set 2	Set 3	Set 4	Set 5
Mixed sex	Control	50	10	40	0	-
	Select	70	40	10	30	10
All female	Control	30	22	0	0	-
	Select	70	0	0	10	10

PARTICIPANT DISCUSSIONS

Rapporteur:	Vern Pepper
Q: Donaldson	Have you checked your smolts yet to see if they are monosex fish?
A: Friars	No. At this point we need to look at all of our families. There may be some coming through that will be different in their performance than others. This will be done.
Q: Donaldson	Have you been able to pick out males that have ducts?
A: Friars	We have been successful in sorting out the sex reversed sperm donors. We make this statement on the basis that 10 out of the sample of 10 of each of their progeny were confirmed to be all-female. We now have data on sex-reversal vs. non-sex-reversal but our data on the triploids is pretty sparse.

Comment: Johnstone

At first maturity for precocious parr, lack of ducts gives a good indication of female genotype. Among grilse, lack of ducts is not such a good indicator. With salmon, this is an even worse indicator. In my experience, in salmon the best indicator of female genotype is hermaphroditism. When I use methyltestosterone, I get much more sterility than reversal.

Q: Benfey	A question for Ray Johnstone. You were using 600 days. Why did you go from 3 ppm to 1 ppm methyltestosterone?
A: Johnstone	3 ppm MT gives high levels of sterility and most animals are not usable even by salmon age. We hoped that lowered dietary levels would give increased yields of reversed fish and 1 ppm is now our preferred dietary dose.
Q: Donaldson	Did you reverse any of your reversed fish back into males?
A: Friars	Yes, but we have only five effective sires. Methyl-testosterone also has been fed to mixed sex groups from the Biotechnology strain. This particular strain has a high grilsification rate.

Comment: Donaldson

A Y-specific probe will be useful for determining sexual genotype. We have developed this technology for chinook. It is not yet available for Atlantic salmon.

Comment: Johnstone

Ed Donaldson is the expert with sex-reversal using reduced concentrations of hormones. This could be useful to the SGRP.

Comment: Friars

Sex-reversal by immersion in reduced concentrations of hormones could save the 60 day feeding period.

Comment: Boulanger

There has been good incidence of sex-reversal with rainbow trout using immersion at 5 day intervals at concentrations of 600 mg/l methyltestosterone.

Comment: Johnstone

Our work has shown that one immersion shortly after hatch at 400 ug/l MT is ineffective in inducing reversal. However, 10 immersions administered between hatch and first feeding at the same concentration achieved very high levels of sterility. Presumably the optimal immersion regime lies between these two extremes.

SOME OBSERVATIONS ON THE COMMERCIAL USE OF TRIPLOID RAINBOW TROUT AND ATLANTIC SALMON IN NEWFOUNDLAND, CANADA

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ABSTRACT

Government regulations require that only non-reproductive rainbow trout be commercially cultured throughout most of the Province of Newfoundland. We describe commercial marine rearing trials using 77,000 female, triploid rainbow trout that were reared to the size of 1.5 to 3.5 kg and marketed. No differences in the hatchery pre-smolt growth rates were noted between female diploids and triploids. After 17 months in sea pens, early maturing diploids were largest, late maturing female diploids intermediate and sterile female triploids smallest. Early maturing, diploid females exhibited slightly lower gutting losses with lower visceral fat compensating for the enlarged ovary. For the particular stock of trout under consideration, the use of all-female triploids is recommended if fish are to be harvested at weights exceeding 2 kg; other possible strategies are considered as are recommendations preventing components of the male genotype from entering the Province. Because of an unacceptably high incidence of embryonic losses and subsequent lower jaw abnormalities observed in triploid Atlantic salmon produced by heat shock, the industry is advised to adopt these techniques at a cautious rate.

INTRODUCTION

Federal Fisheries regulations prevent the use of reproductively capable rainbow trout (Oncorhynchus mykiss) for commercial marine farming throughout most of the Province of Newfoundland and Labrador. Despite the availability of proven salmon stocks in the maritime provinces, the use of non-indigenous, reproductively capable Atlantic salmon (Salmo salar) for farming in this Province is also "discouraged". Because of the exceedingly high grilse composition of local salmon stocks (100% for both sexes in both wild and their farmed counterparts), the prospects of developing a commercial salmonid farming industry in the province appeared to be severely limited if not completely impossible.

In 1985, consultants for the industry recognized the implications of such regulatory constraints. Accordingly, the industry began adopting and testing methods to produce sterile trout and salmon in order to eliminate sexual maturity, both as a negative economic trait and as a perceived detrimental factor impacting wild salmon populations.

We report at this workshop some preliminary commercial experience with mixed sex, triploid Atlantic salmon and with all-female triploid rainbow trout cultured in Newfoundland.

MATERIALS & METHODS

RAINBOW TROUT

In January 1985 at Rainbow Springs Hatchery, Ontario, 12,000 swim-up fry were fed feed containing 17-alpha methyl-testosterone (3mg/kg of feed) for 90 days. A sample of 100 of the 8,000 surviving fish was examined a year later, all were found to be male with a complete absence of female or sterile fish. Genotypic males and sex-reversed males (those lacking sperm ducts and having lobulated testes) first matured sexually in the fall of 1987. Homogametic milt has been obtained from this sex reversed population for four successive years with the presence of a hermaphroditic gonad being the diagnostic factor in the later years. Homogametic milt from a masculinized all-female population became available in 1991. In the fall of 1988, approximately 100,000 eggs from mature females were fertilized with homogametic milt at Rainbow Springs Hatchery, subsequently heat shocked (28°C for 10 min., 40 min. post fertilization), incubated to the eyed stage and shipped to the Bay D'Espoir Salmon Hatchery, Newfoundland. The resulting trout were cultured for seven months at the hatchery and 77,000, 58 gram smolts were stocked in fish pens situated at a brackish water, commercial grow-out site in Roti Bay, Newfoundland (48°N; 56°W). The size distribution of diploid and triploid smolts was determined prior to smolt stocking. The trout were reared in fish pens for a period of 16 months, whereupon approximately 30% of the larger fish (above 2 kg) from each pen were graded out, processed and marketed. The remaining smaller grade of trout was reared an additional 40 days and then harvested. Random samples of both grades of fish were obtained at the processing plant and measurements of ploidy, gonad development, and meat/viscera yield were conducted.

ATLANTIC SALMON

Attempts were made in 1986 and 1987 at Bay D'Espoir hatchery to sex-reverse salmon fry derived from gametes taken from local grilse stocks. In both years, these experimental groups of fish were lost or inadvertently mixed with other groups and no homogametic milt became available. To evaluate the performance, particularly of triploid females, eggs from anadromous grilse parents and landlocked salmon were heat shocked using the protocols of Benfey and Sutterlin (1984). Approximately 10,000 of these fish, consisting of mixed-sex triploids, were stocked as one year smolts in fish pens at Roti Bay and reared for 14 months. The pre-smolt aspects of this study describing the survival rates of triploids and their morphological abnormalities are described elsewhere (Sutterlin et al. 1987). Some of these data will be presented at this workshop, but will not be included in this working paper.

RESULTS AND DISCUSSION

RAINBOW TROUT

Gonad examinations of a sample of 100 rainbow trout smolts undertaken prior to stocking revealed that all fish were female as anticipated and that 91% of the smolts were triploid with 9% being diploid. The size distribution of the ploidy types (Figure 1) indicates that the diploid fish were distributed proportionately throughout the general population. This suggests that pre-smolt growth and smolt size are similar and would not likely become a factor to confound the subsequent assessment of their relative marine performance. These results are consistent with previous unpublished studies (Stevenson, presented at this workshop) in which the relative pre-smolt growth of triploids does not appear to be depressed by the presence of up to 20% diploid fish. At proportions approaching 50%, however, there is some evidence that the triploids become disadvantaged. An examination of the large grade (30% total in the cage) of fish from different fish pens, harvested after 16 months of marine culture (Figure 2A), revealed the presence of the following three types of trout and their abundance:

8% Early maturing, ovulating female diploids.
23% Late maturing diploid females (destined to mature next year).
69% Sterile, female triploids.

After an additional 40 days of culture, samples of the remaining smaller grade (70% of total of trout in the fish pens) consisted of 3% early maturing, 6% late maturing and 91% triploids (Figure 2B).

In the early harvest, it was apparent to plant workers that the early maturing fish (recognized by their dark coloration) represented many of the very large fish. Because of their dark skin and pale flesh colour, these fish were not marketed but were set aside for local consumption. The larger size of early maturing fish is confirmed in Figure 2 and Table 1. The late maturing diploids and triploids could not be distinguished externally as both were bright fish; the flesh colour was also identical.

A comparison of the round weight of the late maturing diploids and triploids, processed on November 9, indicates only a marginally significant difference. However, had we sampled the entire population, including the smaller grade of trout that remained in the pens, the triploids would have been considerably smaller than the non-maturing diploids (Figure 2). The apposed skewness of the size distribution of the triploids on the two sampling dates also supports our contention that the grading process, while effectively removing nearly all non-triploid fish, resulted in the splitting of the triploid population.

While diploid fish represented only 9% of the trout smolts originally stocked (Figure 1), they accounted for 16% of the fish harvested. Mortality from smolt to harvest averaged less than 10% as reported by the growers, and the exclusive loss of triploid fish cannot by itself explain this discrepancy.

The weights of the various organs expressed as a percent of body weight (Table 1, Figure 3) reveal that early maturing fish have a larger ovary and larger liver but smaller residue (caeca, fat bodies, stomach and intestine) than late maturing fish and triploids. The reduced size of the residue is largely due to the reduction in size of the fat bodies in early maturing fish. Early maturing fish have a lower percentage dressed weight (gutting losses are higher) than either of the other two groups. No other differences are considered significant ($P > 0.05$). No maturing oocytes were observed in over the 1,000 triploid fish examined.

Based on measurements of 10 fish from each group, Lincoln and Scott (1984) did not observe a difference between the round weight of maturing diploid female trout and triploid females. However, they did note the larger liver, smaller gut weight (residue) and lower dressed-out percentage of maturing diploids. Chevassus et al (1984) also noted the reduced size of the viscera in maturing diploids compared to triploids. Lincoln and Bye (1984) report that, if mixed groups of female diploid and triploid trout are cultured together, the early growth rates of the diploid trout during the early maturation phase is superior to triploids; our results are similar. They suggest that the triploids are perhaps disadvantaged in competing for food.

Atlantic Salmon:

Upwards of 95% triploidy was induced in landlocked, anadromous and hybrid (landlocked female x anadromous male) Atlantic salmon using heat shocks (5 min. at 32°C, 20 min. after fertilization and incubation at 10°C). On average 23% of the treated eggs died within 24 hours of fertilization. Most of the subsequent mortality occurred prior to hatching.

Two types of morphological abnormalities were apparent in underyearling parr derived from control and heat shocked groups. Short gill covers were present in both diploid and triploid groups, and were not associated with the heat shock. Protruding lower jaws, however, were found almost exclusively in triploid fish, (irrespective of sex) but were not present in all triploid fish within any particular treated group. No diploid fish originating from previously heat shocked eggs had protruding jaws, so the predisposition to this abnormality seems to depend on the triploid condition and not the heat shock.

After 14 months of sea culture, all diploid males, all triploid males, and all diploid females were in the early stages of becoming sexually mature as grilse. Although the condition of short gill covers largely corrected itself, the protruding lower jaw syndrome, much to the displeasure of the farmers, persisted in a frequency of between 10-30% in triploids of both sexes; these fish were deemed not marketable.

Based on our analysis, our recommendations to the growers and the government are as follows:

1. A mixed sex line of either diploids or triploids would not be suitable for producing fish above 1 kg as most males of this strain of trout would likely mature during the second summer at sea.

2. With this strain of trout, there appears to be no particular production advantage in using female triploids over an all-female line if fish no larger than 2 kg are to be produced and all fish are harvested prior to October. For the production of larger fish and for extending the marketing period, maturity rates in diploid females of 28% are not an acceptable trade-off for their better growth rates compared to the triploid females. A mixed strategy wherein female diploids and triploids are held in different cages might be considered.

3. The gutting losses of non-maturing diploid and triploid trout are high (17%) compared to 11% for 3 kg salmon. This is due largely to the high visceral fat, and a lower fat level in the diet should be examined.

4. Government regulations requiring certain growers to use sterile triploid trout in the production of small trout could place them at a competitive disadvantage over growers in other provinces not subjected to such constraints.

During the 1990 harvest of about 70,000 trout, there were reports of two males (both maturing) encountered at the processing plant; their ploidy was never determined. These males most likely entered along with the imported all-female groups of eggs as a result of cross contamination during incubation with groups of eggs of mixed sex rather than incorrect selection of sex-reversed females as sperm donors. The occurrence of these males at such a low level (10^{-5}) is nearly impossible to detect during screening prior to smolt stocking. The inadvertent escape of such males, along with diploid females (heat shock treatment is not 100% effective), could result in the development of self maintaining trout populations. Complete elimination of genotypic males should be stressed. In other words no Y chromosomes should be allowed into the Province. This can best be accomplished by restricting brood stock development entirely to a limited number of genotypic females of both sexes at a Newfoundland hatchery rather than importing female eggs from mainland hatcheries that are also producing fish of mixed sex. The cost of importing all-female, triploid eggs, several times each year into the Province of Newfoundland & Labrador, is expensive. There also is no possibility for further genetic selection. Fisheries and Oceans should consider allowing a brood stock line of normal females and sex-reversed females to be developed and kept at a Newfoundland hatchery. In the interim, mainland hatcheries supplying eggs for export to restricted zones such as Newfoundland should explore pressure shock to enhance triploid content. These facilities should maintain a separate room for incubating eggs, remote from the normal production incubators. Also the use of milt from masculinized, all-female populations should be encouraged.

6. The higher embryonic mortality of heat shocked triploid salmon should not be a problem for the industry, providing excess eggs are available. Alternatively, pressure shocking, which has been reported to be less damaging, should be evaluated. The morphological abnormalities we observed in triploid salmon do not seem to have been reported elsewhere as a result of heat or pressure shocks; the condition could be unique to our stocks or the methods we used and may be of no concern to the industry. Although we feel that the techniques for producing female triploid rainbow trout are commercially applicable at this time, the existing literature, and unpublished experience in Canada prior to this workshop, would indicate that a transfer of comparable technology to produce an"environmentally friendly" salmon proceed at a systematic but cautious rate.

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Group	BW.	Ovary	Liver	Kidney	Residue	Gutted.
Late Maturing n=34						
2n	M 2626.6	0.120	1.49	0.87	14.47	83.03
	SD 286.4	0.062	0.21	0.29	1.72	1.74
Early Maturing n=31						
2n	M 3002.2	8.83	2.16	0.81	8.54	79.65
	SD 370.5	2.05	0.42	0.21	2.99	3.40
Triploids n=23						
3n	M 2539.9	-	1.59	0.83	16.55	82.43
	SD 208.7	-	0.26	0.22	1.86	2.17

Table 1. Organ contributions to gutting losses in diploid and triploid trout

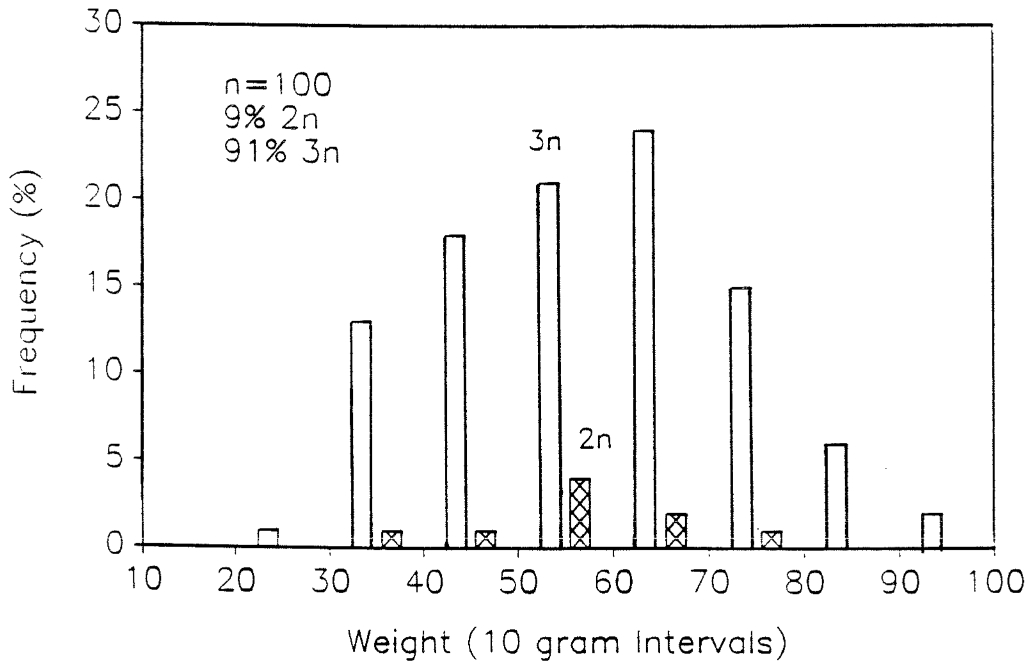

Figure 1. Size distribution of diploid and triploid trout smolts.

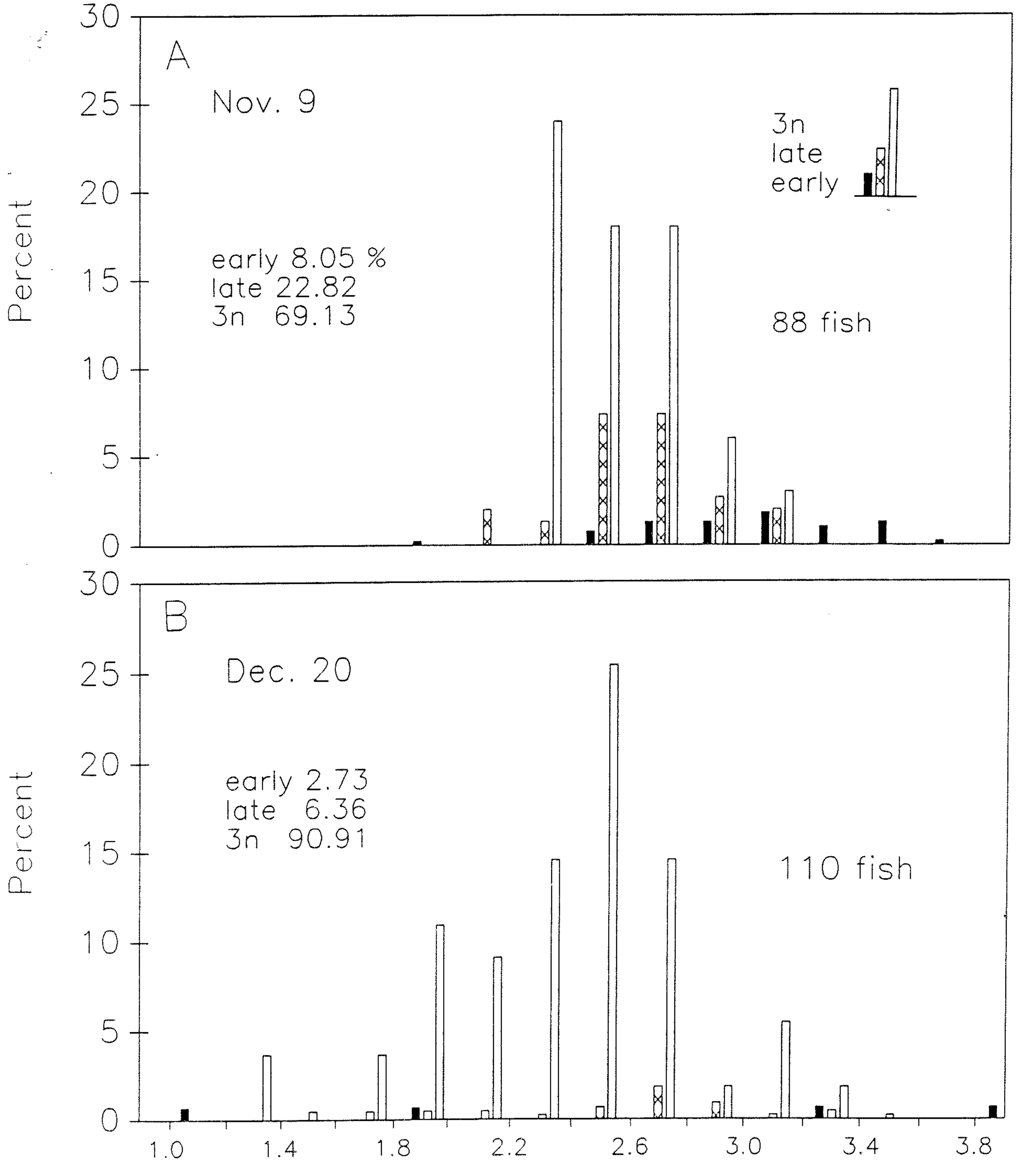

Figure 2. Weight of two grades of trout at harvest

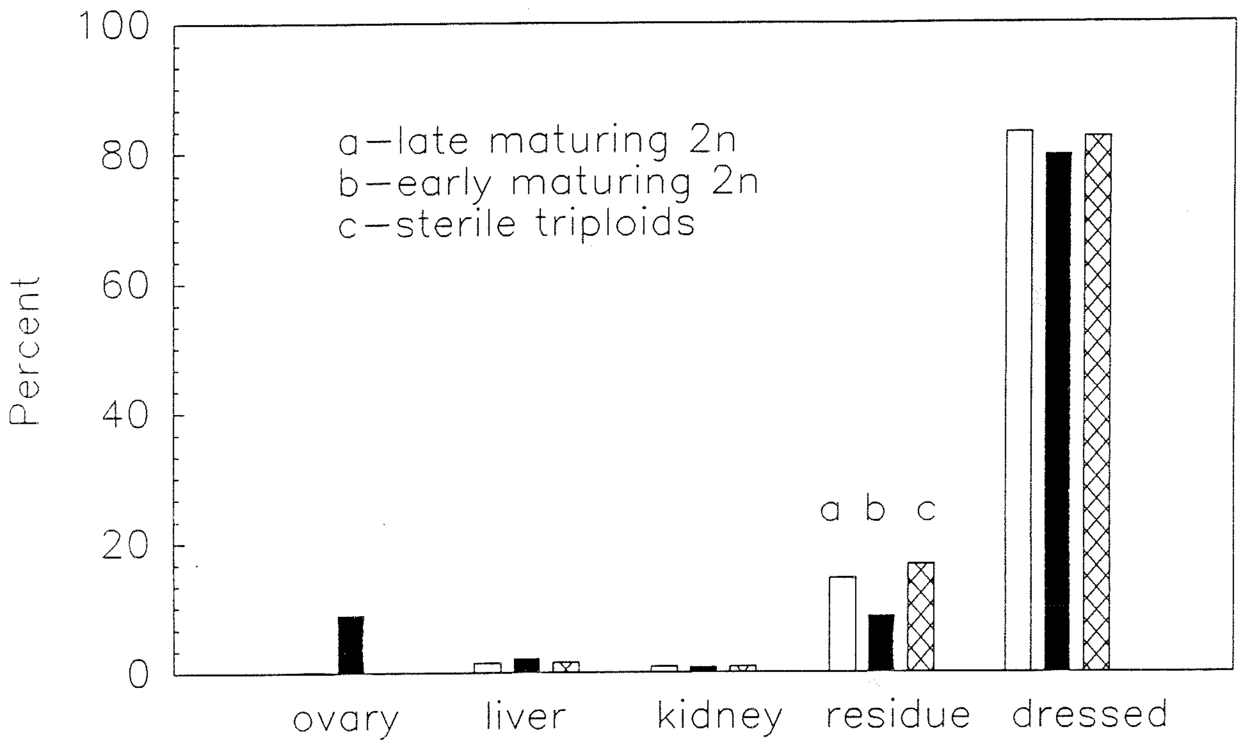

Figure 3. Composition of female diploids and triploids at harvest.

PARTICIPANT DISCUSSIONS

Rapporteur: J.A. Ritter

Q: Donaldson — Regarding the rainbow trout growth data, was the data examined for differences by sex?

A: Sutterlin — The data presented were for an all-female line.

Q: Davidson — The higher fat levels in rainbow triploids is not evident in Atlantic salmon, according to Ray Johnstone. Could the difference in fat levels noted between triploids and diploids be compensated for through nutrition, i.e., could/should special diets be developed for triploids?

Q: Friars — Could the difference in fat levels be the result of domestication?

A: Sutterlin — Higher fat levels in market-size triploids is evident also in the juvenile stages.

Comment: Saunders

The occurrence of mature salmon post-smolts in the Newfoundland industry is unusual and troublesome. Is it possible that some of the smolts going into the cages underwent some form of smolting sometime prior to spring transfer to the cages, e.g., during the previous fall?

A: Sutterlin — Possibly. It was noted that the smolts were quite large when transferred to the cages. Newfoundland salmon stocks (that produced this result) may be quite different than other North American and European stocks.

SEX-REVERSAL AND INDUCTION OF TRIPLOIDY IN ATLANTIC SALMON: AN INDUSTRY PERSPECTIVE

Eugene B. Henderson
New Brunswick Salmon Growers' Association

ABSTRACT

The salmonid aquaculture industry is concerned that they may be pressured to use non-maturing stocks in their operations. It is very important that the performance of such stocks be measured and that the implications for a commercial operation are clearly understood before serious consideration is given to such a move.

NON-MATURING SALMON IN COMMERCIAL AQUACULTURE

From an industry point of view it could be said that there is no pressing interest in reproductively incapable salmon. Nevertheless, looking to the future we recognize that there may be an increase in the concern expressed by many scientists that escapees from sea cages will mate with wild salmon and cause genetic drift and have a detrimental affect on wild stocks.

Being realistic, we recognize that the pressure may mount to have all cage reared salmon rendered non-reproductive to reduce the potential for impact on wild stocks from accidental releases. To my knowledge no commercial trials have been conducted to measure the performance of non-maturing Atlantic salmon in sea cages.

Such trials are imperative if we are to expect an industry to use a reproductively inactive stock. Not being an expert in this field, I can only assume that selective breeding will continue and sterilization will take place through treatment of eggs or early juvenile stages. I presume and hope that this workshop will clarify such technical points that are confusing to many of us. A benefit of non-maturing salmon, from an industry point of view, would be to solve the grilsing problem. Although grilsing is not a major problem in the Saint John River stock of salmon grown in sea cages in New Brunswick, we do have a low but variable number of grilse each year. The average, as best one can assess it, is only about five percent but I have recorded as high as twenty percent in two year smolts.

I am sure from the above that you will agree the industry has a poor understanding of the implications of having to use non-reproductive salmon in commercial operations. I feel sure that at the end of the day the salmonid aquaculture industry will have a better understanding of the implications of using triploids in commercial operations. I also believe that a great deal of research will be required before the full implications are understood. An outline of research requirements should be included in the proceedings of this workshop.

ACKNOWLEDGEMENTS

I compliment those who had the foresight to arrange and sponsor this workshop.

PARTICIPANT DISCUSSIONS

Rapporteur: John Ritter

Comment: Henderson

It is important that research be done and results evaluated before industry is pressured to rear sterile fish. Bear in mind, that this is perceived as a non-problem by the industry and producers are content with the performance of the fish now being used.

Q: Farmer — What are grilsing rates in the Saint John River fish?

A: Henderson — S1 between 2-12%. Almost all males vary from farm to farm and from year to year.

S2 between 20-30% depending on overall size and type of feed. Some maturity may go undetected. This rate could be higher if larger fish matured earlier and were not graded out.

Q: Donaldson — Is it the attempt of the N.B. industry to produce an "organic salmon". No hormone, drugs, pigment, or vaccines and what are the costs to do this?

A: Henderson — This seems a realistic goal for the industry. The biggest problem in drug use was in controlling vibriosis but this is getting under control. It is difficult to know the real costs.

Q: Sutterlin — In promoting an "environmentally friendly" salmon, would not terms such as genetically manipulated, and triploid cause the consumer some concern. While these techniques to overcome seed production are common practices in agriculture, certainly no one advertises peas or bananas as such, except in a seed catalogue.

A: Henderson — If concern arises, common education is best approach.

Comment: Johnstone

I agree, and I think it is sensible and necessary to accentuate the positive. To everybody, we should say that triploids are not unnatural animals but that they do occur, albeit rarely, in the wild. It was this occurrence, after all, and the observed apparent sterility that attracted the attention of researchers and farmers alike, and which led to research into efficient means for their production. To the farmers, we should say that the sterility aspect of triploids is likely to be of benefit by making rearing and marketing strategies more predictable. This in turn should make producers more efficient. We must avoid saying that we have all the answers or that there will not be problems that will need to be addressed in future research but, even at this preliminary stage, the prospects are, I think, sufficiently encouraging to justify further evaluation. To stock managers and to those concerned with the potential for alteration of the genetic structure of existing stocks that might follow from accidental releases, we should say that the use of triploids should minimise these concerns. Finally, we might say to consumers that triploids might ensure uniformity of product quality and supply. The seasonal deterioration that takes place in diploid maturers and which conditions their marketing window, will not be a feature of triploid production. I believe, at this moment, that the likely benefits of triploids would seem to outweigh their costs.

Comment: Jungalwalla

Use terms such as non-reproductive as opposed to triploid or sterile.

Q: Benfey — Is there a problem with public response to methyltestosterone use in broodstock, which we know is a non-problem?

A: Henderson — There is a problem with the notion of hormone treatment. The public has been sensitized to such concepts. Keep in mind that perception is often reality to the public.

Q: Donaldson — Even with direct suppression of maturation, steroid levels are lower in aquaculture salmonids than in wild fish; with levels in dairy product being much higher.

Could maturity suppression not be of considerable use in producing very large salmon?

A: Henderson — There is limited demand for salmon over 15 lbs. The exception being for centrepieces for banquets or for smoking. Sizes of 8-10 lbs. suit steaking or restaurant trade best.

Comment: Farmer

Location of farms and type of environment influence grilsing. In Nova Scotia, an evaluation of three different stocks including St. John River, have given higher grilsing rates than in N.B. (20-30%) with high proportions of female grilse from S1 smolts. Also, there is a higher maturity rate among post smolts from S2.

Comment: Saunders

Many of these differences could be due to how we interpret the physical manifestation of what we call smolts and how the fish actually measure time. Also smolt status, measured by salinity tolerance, does not always coincide with subsequent good growth.

Comment: Benfey

Perhaps biggest difference between Aquaculture and Agriculture is the comparisons consumers make with wild and cultured aquaculture products. Ancestral forms of live stock seem to have been forgotten about. Many people catch their own salmon and trout and therefore have some notion of what these animals look like when they are being prepared for the table. There are not many people today who butcher their own livestock.

Comment: Saunders

Those who advocate that wild salmon are the best, are more inclined to select cultured fish when subjected to blind taste testing.

Comment: Henderson

Our salmon aquaculture operations provide a great opportunity for modifying feed to customize salmon for different products types including the health promoting Omega-3-FA.

MATURITY SUPPRESSION IN RAINBOW TROUT FROM THE PRODUCER'S PERSPECTIVE

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ABSTRACT

Rainbow Springs Hatchery has been experimenting with all-female and triploid lines of rainbow trout since 1985. Growth rate of the triploids is similar to that of mixed sex diploids up to approximately 230 to 280 g. Thereafter, triploids seem to grow slightly faster. The process for inducing triploidy seems to reduce the time to hatching. There is also: greater mortality among smaller eggs following heat shock than for larger eggs; a much higher mortality rate among triploid eggs than among diploids; and, an increased incidence of deformities among triploid fry. Triploid fish seem to grow slightly slower than all-female trout. Our manipulations of sex and chromosome number occasionally are accompanied by a low incidence of hermaphroditism. These hermaphrodites have produced viable eggs and sperm. While results with all-female and triploid lines of rainbow trout have been encouraging at Rainbow Springs, there is still a considerable amount of research that needs to be done. Some of these needs are identified in this paper.

INTRODUCTION

Successful application of reproductive sterility in rainbow trout farming is dependant on many things. Factors that contribute to the success or failure of such activities are:

- interest and determination of the operators and/or farmers;
- the facilities, equipment and conditions we have to work with;
- the goals we set for ourselves;
- the results we get; and,
- the knowledge, basic materials and information that is available to us.

In this presentation I plan to touch on each of these topics as we at Rainbow Springs Hatchery have confronted them.

We started our business at Rainbow Springs with a few tanks on a small parcel of land. We had only 180 litres of tank volume to work with. We had a few fish and a lot of nerve. Our trout farm was to be a hobby.

Our interest in animal genetics goes back to our early training on a dairy farm. Using a few rainbow trout for broodstock, together with some research and the basic knowledge we had from the dairy farm, we have built our small enterprise in the trout industry to what is today.

THE FACILITIES

We have outside holding tanks in which we keep our growers and our broodstock. I must point out here that our spawning is done outside in the open air without the benefit of housing. Inside our low barn we have four concrete tanks that hold our broodstock under controlled photoperiod. This barn has automatic light controls which reverse photoperiod from summer to winter. Therefore, together with the unmanipulated group outside, we have broodstock that produce eggs and milt all year round. Our ambient photoperiod broodstock provide gametes from August until March or April depending on the year. Our photoperiod manipulated trout start spawning in February and provide our egg requirements until mid-August.

The tall barn is our hatchery and early fry rearing area. Here we house 45 tanks for fry and fingerlings together with 11 incubators. We take advantage of the extra height in this barn by stacking our tanks three and four high. We have to pump all our water anyway so feel the extra height that we pump is compensated for by the extra use of the water. This winter we are trying to develop a small area where we can heat a minimal amount of water so we can speed the development of both eggs and fry depending on our sales demand. Here too is where we do our experimenting and triploidy.

METHODS

We have been trying to develop rainbow trout that:

1. are fast growing;
2. are strong;
3. have good conformity (i.e., low size variability; attractive shape and appearance);
4. are late maturing;
5. can be controlled physiologically to produce eggs throughout the year; and,
6. are disease resistant.

In the process of trying to develop these characteristics, we were fortunate in contacting a scientist from Newfoundland who was interested in procuring a strain of rainbow trout with the performance characteristics we wished to develop.

Over the years of purchasing trout from various hatcheries, including our own, and experimenting both in Newfoundland and at our trout farm, this individual apparently decided that our trout had many characteristics that were required in Atlantic Canada to compete with Atlantic salmon farms. In the course of our conversations, we discussed the concept of producing all-female, triploid trout. After considerable research, encouragement, and arm twisting to develop the sex-reversed female (i.e., XX sperm), triploid rainbow trout in Ontario, we finally decide to proceed.

To do this we needed :

- financial assistance for the extra equipment and expertise;
- extra tanks for these additional groups of fish; and,
- time to do the extra work, to keep the records and learn the new techniques.

We pursued these needs with a proposal to government for funding support ("Development of sources of homogametic milt and the production of all female, sterile, triploid rainbow trout at Ontario private hatcheries"). Thanks to the support of several agencies, we were able to receive the essential and much appreciated help.

There were two essential elements we pursued to achieve our goals. We had to:

a. develop the sex-reversed female line (i.e., to provide the XX sperm); and,
b. do several experiments to determine the most appropriate procedure to induce triploidy in rainbow trout eggs at our farm.

SEX-REVERSAL

In pursuit of the sex-reversed female sperm, we used four groups of fry, three of which were fed male hormone. These fry were all the same year class, but at different stages of development, varying from not-yet-swimming to start-feeding. These different stages of fry occupy different locations within the tank. The most advanced fry are found towards the water surface while the least developed fry stay on the bottom of our tanks. Fry that are found between these two extremes typically are of an intermediate stage of development. Accordingly, we applied our hormone treatments to different age groups as defined by their position in the tank. The three groups we used were as follows:

Group 1 fry were skimmed from the top of the tank;
Group 2 fry were taken from the middle of the tank;
Group 3 fry were from the bottom of the tank.

A fourth group, that was sampled randomly from within the fry tank, received normal commercial feed without any hormone. This was our control group.

Realizing that our trout were slow maturing, and wanting to get some early results before the typical three years required to complete such experiments, we decided to try to accelerate the onset of sexual maturity of some of our sex-reversed parr by implanting a cocoa butter hormone pellet into the body cavity. The procedure we used was based on what had been done in French experiments (Magri et al. 1985). The French results revealed that 50% of the groups injected matured sexually one year earlier than normal.

PRODUCTION OF NON-MATURING SALMONIDS

The procedure for inducing triploidy is very familiar to the participants in this workshop. Hence, I will not go into it in detail here except to say that we have enlarged our capabilities to induce triploidy on a commercial scale. We now are using laundry tubs for immersion of the eggs. The one tub used for heat shocking eggs is insulated with styrofoam. We use thermostatically controlled heaters that maintain water temperature of the egg immersion bath to within 1° C of the desired temperature. We use a stop watch for each tank, to assure precise timing of exposure of eggs to elevated temperature. Monitoring is done with a certified thermometer. Our incubator trays serve as holding trays during the heat shock treatment, thereby avoiding at least one additional handling of the eggs. We find this very convenient. Once the heat shock process is completed, the eggs do not need to be further disturbed but rather, can be placed directly into the incubator.

We have been maintaining lines of all-female rainbow trout at our hatchery from which we extract a relatively small number of fish for sex-reversal. We have found this advantageous in that, once we had sorted out and destroyed our diploid XY males from our initial work, sex-reversal of our all-female fish results in all XX males. We therefore do not have to be concerned about residual XY males that would otherwise "contaminate" our resulting sex reversed population. I will refer to these XX "males", produced from all-female lines, as second generation males.

Concurrent with our experimentation on sex-reversal and heat shock, we have encountered several instances of hermaphroditism. Within the gonads of these hermaphrodites, eggs and sperm most often develop at different times in the same fish. We do not usually encounter trout in which both eggs and milt are ripe at the same time.

Out of curiosity, we undertook an evaluation of the gametes of these hermaphrodites by attempting self fertilization of some eggs. We had two "sex reversed" females that had both milt and ripe eggs. Both looked viable to the naked eye and under the microscope. I will call one A and the other B. We crossed A's eggs with milt from other males, and with its own milt. As well, we crossed A's milt with a blend of eggs from 12 other females. We crossed B's eggs and milt in the same manner. These crosses are represented as follows:

B eggs crossed with other males;
A eggs crossed with other males;
B eggs crossed with its own milt;
A eggs crossed with its own milt;
B milt crossed with eggs of 12 other females; and,
A milt crossed with eggs of 12 other females.

RESULTS

Our experiments with hormone treatment of fry to provide XX sperm resulted in the following observations:

1. When examined at a size of 10 cm, all fish were female and very few were sterile. There was no apparent difference among the four groups in response to the hormone treatment;
2. mortalities were insignificant in all groups; and,

3. fish fed with hormone supplemented diets were 14% longer and 28% greater in size by water displacement than the control group (i.e., the hormone treated fish subsequently grew faster).

Unfortunately, results of our attempts to accelerate maturation in our sex-reversed groups were very disappointing. Instead of accelerating maturity, the hormone implants resulted in sterility and reduced testis development. The lower jaw of these injected fish was very enlarged, even at a fish length of only 15 - 20 cm.

It is not normally our intent to grow many trout beyond a 10 cm limit. However, for logistics reasons, we occasionally get left with "extras" that we did not manage to sell elsewhere. We have found from these trout that the growth rate of the triploids is similar to that of diploids up to approximately 230 to 280 g. Thereafter, triploids seem to grow slightly faster. Our heat shock process for inducing triploidy also seems to reduce the time to hatching. However, the difference in hatching time is small and may be due to elevated temperature during the heat shock process. Diploids seem to require an extra day to reach the eyed stage of development.

We also have observed the following differences between diploid and triploid lines of our rainbow trout:

- there is greater mortality among smaller eggs following heat shock than for larger eggs. The condition of the egg at stripping seems to effect the survival rate;
- there is a much higher mortality rate among triploid eggs than among diploids, both up to the eyed stage and thereafter until hatching;
- there is an increased incidence of deformities among triploid fry;
- triploid fish seem to grow slightly slower than all-female trout; and,
- when we have leftover trout after our normal commercial sales, we do not have a problem with early maturation if our lines are all female.

Our experiments with gametes from the two hermaphrodites produced the following results:

B eggs crossed with other males had no survival;
A eggs crossed with other males had good fertility;
B eggs crossed with self had no survival;
A eggs crossed with self had good fertility;
B milt crossed with 12 other females had good fertility in one case and poor in another; and,
A milt crossed with 12 other females had poor fertility in both cases.

DISCUSSION

We found that the stage of development, as represented by the three treatment groups in our hormone experiments, did not seem to influence our sex-reversal results. All of the three treatment groups performed well relative to the control group. In a hatchery environment, this greatly simplifies the sex-reversal procedure in that we are able to treat all of our fry in a relatively short time frame.

CASE STUDY

I tried to get information from our customers regarding mortalities, growth rates, deformities, etc. Unfortunately, fish farmers seem to be poor at record keeping (myself included). A common remark among our customers is, " My gut feeling is...".

I did get some interesting data. One farmer purchased two batches of fingerlings from our hatchery. One group consisted of diploids while the other group consisted of all-female triploids from the same day's egg take. The intent of this case study was to do a comparative evaluation at their facility. Their figures reinforce my conclusions regarding survival and growth rates. Triploids had a survival of 66.5% while the diploids had a survival of 75.5% up to the 10 g size. Growth rate was 2.74% per day for triploids and 2.09% per day for diploids. As of the most recent sampling of these groups, triploids were an average of 29% heavier than diploids. During the summer months, when surface water temperature exceeded 20°C, the triploids refused to feed at the surface and preferred to feed close to the thermocline. The diploid trout continued surface feeding throughout the summer. This study is proceeding with comparative performance evaluations throughout the grow out period.

In our experience, our manipulations of sex and chromosome number occasionally are accompanied by a low incidence of hermaphroditism. I interpret from our experiments with hermaphrodite gametes that B's eggs were not good, but the milt was viable. A's eggs were good, but the milt was only fair. Am I correct? If so what might be the reasons? Is there a point where the eggs and milt might both be viable at the same time or will they react with each other, one activating the other? Also, is it possible that these trout have two seasons, one for the egg and one for the milt? If so, is it possible to distinguish when each are ripe? My tentative conclusion is that gametes of these hermaphrodites develop within the same fish on different schedules. These hermaphrodites may produce eggs at one time and milt perhaps six months later.

QUESTIONS FROM A TROUT FARM.

There are many questions that have yet to be answered if reproductively sterile rainbow trout are to become popular among trout farmers. Some of these questions may not be directly related to sterility and triploidy but to production and delivery of good eggs and seed stocks. Without the initial seed stocks (i.e., quality eggs), any further steps with this technology are likely to be much less meaningful to the fish farmer.

1. Our customers ask, "What is your percentage triploidy?". I would like a simple, economical method of distinguishing a triploid from a diploid at the egg stage or even sack fry stage so that, not only will we know that we are doing our job correctly but also, we can provide our customers with an honest answer instead of "I think" or "I hope".

2. We need a foolproof method of distinguishing a sex reversed (i.e., XX sperm) female from an XY male to be certain that the result of our hormone treatments is an all-female line.

3. Does a second generation XX male have a vent? If so, how can we distinguish it from a regular XY male? Trout do have a habit of jumping from tank to tank. Unfortunately, the private sector does not always have the luxury of isolation tanks. I have seen trout jump from one tank to another, even when the tanks are about one metre apart.

4. We need an economical method of cryopreserving milt so any left over XX milt is not wasted (i.e., thrown out) and so we can have a reserve supply in the off season. Once the male is killed to extract the milt, that genotype is gone forever.

5. What are the nutritional requirements of broodstock? If we relate them to mammals, we deduce that there are quite different requirements for a pregnant animal. A dairy cow for example needs a growing ration during growth, dairy concentrate while milking, and dry and fitting ration between lactations, etc. My logic says that trout should be treated similarly. Here we have dietary needs for early rearing (not only for growers but for potential brood), near spawning, just after spawning, and between spawnings.

6. Is pigmentation in brood feed beneficial or not?

7. What are the effects of air climates and weather on spawning time? Spawning time seems to vary from year to year even when water temperature does not vary.

8. Eggs from photoperiod manipulations are not as strong as those from ambient photoperiods. Why? How can we improve them?

9. Egg size varies from female to female. Is this a genetic phenomenon? Is it nutritionally related? Is it just the norm for that particular fish? Is it that a female that gives small eggs will always give small eggs and should be culled?

10. Is the variation of fertility from female to female related to the ripeness of the egg at egg take or is this too genetic?

11. What are the effects of testosterone and other hormones on older trout down stream from the treated tanks?

12. Is there a proper procedure to transport trout eggs? We seem to have varying results using the same method from one time to the next. Why?

13. Sometimes the eggs seem soft. Why? This is evident in both regular and triploid trout.

14. What are the effects of various anaesthetics?

As is evident, the questions that need answers are endless. With sufficient support from the research community, I foresee in the not too distant future that more answers will become more readily available to the trout farmer.

I hope I have conveyed to you some of our thoughts and that we can work toward a better communication between the researcher and the commercial grower. The conditions under which the grower must work are much different from those in the lab so we require much more field research on many topics.

CONCLUSION

We have come full circle. To accomplish reproductive sterility in rainbow trout we need:

- interest of both farmer and researcher;
- facilities from the lab brought to the field;
- goals that are set for the benefit of everyone; and,
- knowledge and information with which we can work together to accomplish all of the above.

ACKNOWLEDGMENTS

I would like to thank the Department of Fisheries and Oceans for the opportunity to come to this workshop and to make this presentation on our work with rainbow trout at the Rainbow Springs Hatchery. I have been looking forward to learning many new ideas to take home and implement into our enterprise in Thamesford, Ontario. I give Dr. Arnold Sutterlin a great deal of credit for sowing that seed of interest in me that led to our experimenting with production of all-female rainbow trout and with production of non-maturing rainbow trout by heat shock. Also, I wish to thank Coldwater Fisheries for providing data on their experience with triploid rainbow trout performance.

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PARTICIPANT DISCUSSIONS

Rapporteur: Brian Meaney

Q: Friars — Was data from cage sites in marine or fresh water?

A: Stevenson — Freshwater cages.

Q: Friars — So the data indicate that the triploids are showing better growth at the 2 to 3 lbs. stage?

A: Stevenson — Yes. They are now about 1.5 years old.

Q: Friars — So the points raised regarding behaviour of these triploids may take a while to show themselves?

A: Stevenson — Ontario is behind the Maritimes in accepting all-female triploids. There are only two growers in Ontario to whom I have sold triploids. Their concern is with how much it will cost them for triploids vs diploids and what economic gain they can expect.

Q: Friars — On your question of egg size, are these females of a common age?

A: Stevenson — No. We have mature 15 lb. five year old fish which produce tiny eggs. In contrast, we have brood 3 years old and 8 lbs with very large eggs.

Comment: Friars

In two rounds of selection work on Atlantic salmon on the basis of growth, we have noticed that our selected line (longer fork length) produced both larger eggs and more eggs. This seems very related to size, and apparently reflects down through a number of performance traits in those eggs. I suggest you look for any common similarities in connection with both size and age in these observations.

Comment: Johnstone

In Scotland, farmed grilse produce smaller eggs than do salmon and survival rates following their fertilization are poorer. This is not the situation in wild grilse eggs which are similar in size to those of salmon and which, in my hands, perform excellently.

While I don't have as much experience with trout, I suggest that stripping females in consecutive years may put an immense strain on fish in culture. Individual females may, depending on the rapidity with which they came back on to active feeding after being stripped, decide either to produce many small eggs or fewer larger eggs and this may be part of the reason for your observed variability in egg size.

Comment: Anderson

In nature (based on Big Salmon River research) grilse can produce large eggs.

Comment: Johnstone

Quality of feed is better for wild salmon than farmed which may be the reason. Studies in Scotland have shown that growth rates in wild grilse are higher than farmed presumably because of the better feeding in the wild.

Comment: Anderson

This sounds odd to me. Our commercial salmon feeds are formulated to provide essential dietary ingredients and therefore, should be at least as good as natural diets.

Q: Glebe — Does the yield of triploids vary considering that you spawn your fish "out of season".

A: Stevenson — My feeling is that the stronger the egg the better it is able to withstand the "stress" of the heat shock. The eggs that we get from the photoperiod-manipulated broodstock do not appear to be as strong as the eggs from our ambient photoperiod broodstock. Therefore, when we heat shock these eggs from the photoperiod controlled brood fish, we get a higher mortality rate because of the apparently poorer quality of the egg. The yield of the triploid remains the same (i.e., > 90%) for the end consumer. We take the greater loss at the eyed stage because of the mortalities which we remove from the lot before shipment.

Comment: Sutterlin

We have received five or six shipments at varying times and yield has been the same (> 90%).

Q: Donaldson — So are you saying that once shipped to the farm, the performance is the same?

A: Stevenson — Yes.

Q: Glebe — So what is your rate of mortality during spawning in the off-season?

A: Stevenson — About 20% during the typical spawning season and 25-30% during the off-season for the triploids.

Q: P.J. — You say you are using ground water. Can I assume its temperature is constant year-round at say 8°C?

A: Stevenson — Generally it is 8°C year-round. However, this year, when we had no ground cover, it got down to 6°C.

Q: P.J. — So when you say you produce out of season, does the temperature vary and therefore the first feeding opportunity?

A: Stevenson — No, I think it may have more to do with photoperiod at the time of first feeding.

Comment: Donaldson

We have synchronized temperature and photoperiod for first feeding of chinook salmon which have been spawned out of season. That you are finding your eggs are less viable is not surprising, considering that the natural temperature and photoperiod is out of sync with off season spawning.

Q: Pepper — What criteria do you use to determine when you strip your eggs.

A: Stevenson — We spawn every Tuesday and Wednesday.

Comment: Pepper

Then it could have been that these eggs were overripe and this may account for the "soft eggs" you observe.

A: Stevenson — These eggs are fertile, alive, and eye up. However, they just don't ship well. If it was a case of over ripe, then why does that whole day's stripping result in soft eggs rather than just a few individual over ripe females?

Comment: Donaldson

Regarding your question of the use of pigmented feed for broodstock and its impact on egg quality, no published studies have shown that pigment has any effect on survival.

Comment: Johnstone

I agree. There are some studies that suggest that additional vitamin C supplementation can improve egg quality. If you can obtain specialized broodstock diets, I would recommend their use.

Comment: Gallant

I have read in the literature that pigmentation in the egg may act as a chemo attractant for the sperm thus enhancing fertilization.

Q: Saunders — How do you distinguish between 2N and 3N in eyed eggs?

A: Sutterlin — The data has shown that pressure treatment at the prescribed time after fertilization, together with proper pressure for the correct duration, will consistently produce 90% plus 3N individuals.

Q: Anderson — How do you know this?

A: Sutterlin — You have to wait until the fish are 5-6 inches in length, then sample the population and look at the gonads for oocytes under the microscope. If none are present they are 3N, if preset, 2N.

Comment: Johnstone

It seems to me necessary to say at this point that the appropriate techniques and strategies for maturity control might vary between species. Thus, we have heard at this workshop that heat shocks appear to give better results for rainbow trout and possibly also for Arctic char than for salmon and brook trout where pressure is preferred. The desire to produce steriles at all will also vary between species being clearly of most benefit in the earliest maturing species or where early maturity prevents animals from being grown to the larger sizes. All-female stocks alone are a relatively complete answer to the maturity problem experienced in rainbow trout culture in the UK since most animals are sold at 200-550g and maturity below this size is largely confined to males. This is less true in Atlantic salmon culture in Scotland where the poorer perception of grilse stems from their generally small size (ca 2 kg) as much as from their narrowed market window. Large grilse are not so disadvantageous. All-female stocks will, at best, be only a partial solution to the problem of early maturity in salmon culture. This is because at normal maturity levels, some grilse are females and at high grilse levels, most females mature. Therefore, sterile stocks have their attractions.

PERFORMANCE COMPARISON OF ALL-FEMALE, DIPLOID AND TRIPLOID BROOK TROUT

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ABSTRACT

Triploidisation by hydrostatic pressure has proven more effective than with heat shock in terms of: success (over 95%); reliability; speed of execution (500 000 eggs/hour); survival rate of the individuals; and, quality during the growing period (low rate of aneuploids). Triploidy inhibits the reproductive development of female gonads only. Males grow sexually in a normal fashion, even though their sperm is not viable. When the triploidy method is used, it is essential to use all-female stock in order to avoid growth and behaviour problems related to the sexual maturation of males. All-female, triploid brook trout growth continued unabated for three consecutive years of observation. In this interval, diploid females lost weight during spawning. Improved growth of the all-female triploids, relative to the diploids, was readily apparent at 500 g. Triploid weight may be double the diploid weight after one year. Food conversion has always been better for the triploids, regardless of age. The average gutting loss was 22% for the triploid females compared to 32% for the maturing diploid females. The difference is due mostly to the gonads.

INTRODUCTION

Sexual maturation is one of the major problems in intensive production of salmonids intended for the marketplace. Early sexual maturation, although desirable for the producer of eggs, carries many physiological and behavioural drawbacks that are responsible for significant losses in the production cycle. These drawbacks appear as soon as male and female brook trout (Salvelinus fontinalis) reach 200 g. For the rainbow trout (Oncorhynchus mykiss), these problems appear when the males reach 300 g and females exceed 400 g. A few years ago, consumer preference was for a portion of less than 300 g. Today, the tendency (75% of demand) is for a trout fillet weighting between 150 and 175 g. This requires a trout of at least 600 g. Furthermore, the consumer is more critical of the quality and of the purchase price for this product.

These new trends in the marketplace mean that producers of table trout will have to maintain their livestock over a longer period of time, thus making it impossible for them to sell their fish before sexual maturation. Furthermore, the marketing of brook trout in the provinces of Quebec and New Brunswick is regulated under very restrictive rules. Although a consumer's favourite, brook trout present many drawbacks related to their very early maturation. In our opinion, this may cause serious limitations to its marketing if these maturity problems are not corrected.

PRODUCTION OF ALL-FEMALE TROUT

Production of "all-female" rainbow trout, using the sex-reversal technique (i.e., with the help of the masculinizing hormone, methyl-testosterone), is a well-known technique today. This technique has been mastered by many producers. On the other hand, this technique does not seem to generate the same interest or yield the same degree of success as with other salmonid species, especially the brook trout. We at Pisciculture des Alleghanys Inc. have been able to produce viable, all-female rainbow trout eggs, on a commercial basis, since 1989. In contrast, four years of research has been required to produce our first sex-reversed brook trout males. We shall be in a position for commercial production of all-female brook trout eggs in the autumn of 1991. Unfortunately, the all-female production strategy by itself only partially addresses the rearing and marketing constraints related to sexual maturity for rainbow trout and has little advantage for brook trout.

PRODUCTION OF NON-MATURING TROUT

The technology commonly used to induce reproductive sterility in salmonids is that of triploidisation. This is induced either by heat-shock or hydrostatic pressure. Over a period of two years of research, we evaluated the effectiveness of these alternatives for producing triploid rainbow trout and brook trout. We examined these techniques both at the experimental and commercial levels. As a result, we have chosen the hydrostatic pressure method. Although more difficult at the technical level (applications of pressure of over 500 atmospheres), this method has proven more appropriate in terms of: success (over 95%); reliability; speed of execution (500 000 eggs/hour); survival rate of the individuals; and, quality during the growing period (low rate of aneuploids). Triploidy inhibits reproductive development of female gonads only. Males grow sexually in a normal fashion, even though their sperm is not viable. When the triploidy method is used, it is essential to use all-female stock in order to avoid growth and behaviour problems related to the sexual maturation of males.

METHODS AND RESULTS

PERFORMANCE OF TRIPLOID BROOK TROUT

A previous study on rainbow trout (Lincoln 1986) indicated that there were no growth or behaviour differences between diploid and triploid males. We set out to evaluate this for ourselves in 1988, before we had mastered the all-female technology for brook trout. We set up one group of diploid and one group of triploid, all-female brook trout in order to evaluate the performance of the triploids.

From 1988 to 1989, both lots were maintained in similar conditions of density, temperature, and feeding. The average weight of the fish at the beginning of the study was 100 g. Many parameters were studied. In this presentation, we shall discuss only the main parameters. Table 1 provides the temperature, weight and feed conversion data for the given period.

Figure 1 shows the comparative growth of both groups based on their average weight and the cumulative number of day degrees (10°C). The all-female, triploid growth was continuous for the three years of observation. Diploid females lost weight during spawning (1988-1989). Weight loss took place from the beginning of September to the end of December. Improved growth of the all-female triploids, relative to the diploids, was readily apparent at 500 g. Food conversion (Tables 2 and 3) has always been better for the triploids regardless of age.

Table 4 depicts the average percentage gutting loss and percentage of water in the muscle for female, diploid brook trout during the spawning period. The average gutting loss was 22% for the triploid females compared to 32% for the maturing diploid females. The difference is due mostly to the gonads. Gonad maturation becomes significant at the beginning of August and continues to detract from somatic growth until the end of December. Frequent autopsies were performed on the triploid females during these three years of observation. We were unable to find any development of female gonads in these triploid fish. Reduced production costs, varying between $0.22 and $0.40/kg, have been obtained because of improved food conversion and lower gutting losses among triploid trout.

The percentage of water in the muscle (Table 4) has been quantified, mostly during the spawning period. The percentage always has been lower by approximately 2% in the triploid females compared to the diploid females (73.2% for triploids vs 75.3% for diploids). There were pronounced flesh quality differences as follows:

1) A firm and consistent texture of the triploid muscle for a period of 12 months vs an elastic and flaccid texture, spreading over a period of 5 months for the diploids;

2) A shelf-life and pigmentation which increases inversely to the musculature moisture content;

3) During the five month period of diploid gonad maturity, fillets of the triploids remain intact during processing, whereas those of the diploids do not.

4) Losses due to gutting are 30% lower among the triploids during the spawning period (Table 4).

DISCUSSION

Problems related to the marketing of the brook trout come mostly from early maturity of this species. Maturity appears in a high percentage of Age 1+ brook trout and Age 2+ rainbow trout. This results in significant losses in growth and quality of the flesh and confines the marketing period to the interval from January to August. Market demand is low during this interval.

Early maturity brings to the producer a lot of economic and management problems. The consequences of these problems increase with trout size. As shown in Table 4, all-female, triploid brook trout allow the producer to solve most of these problems. The comparative growth results for diploid and triploid rainbow trout, obtained by Lincoln (1987), are similar to those of Pisciculture des Alleghanys for the brook trout.

Even when one takes into consideration the additional cost of triploid eggs, there is a net gain in production efficiency from triploid stock. Reduced production costs, varying between $0.22 and $0.40 per kilo, can be obtained because of improved food conversion and lower gutting losses among triploid trout. The saving is even more significant if we take into account an average production period for a 1600 gram trout of 4500 degree days for triploid females compared to 8200 for diploid females. Furthermore, the flesh quality of the triploids could command a better price on the market, or at least guarantee a product of high and consistent quality the year-round.

Triploidy and all-female production technology could also be beneficial for Arctic char. Lately, this species has generated a wide interest among Canadian trout producers. Opinions of the scientists and breeders who are working with this particular species, suggest that Arctic char could meet the same growth impediments due to the early sexual maturity encountered with brook trout. Also, the disparity in growth potential between maturing and non-maturing individuals exists to the same degree for both species. With brook trout, this disparity is strictly bound to sexual maturation phenomena, that appear in the very first months of growth. Our own observations show that 15 to 20% of males and 10% of females already have developed gonads when they reach 75 g (6 months) and will be fully matured in the first fall.

We think it reasonable that the same maturity problems with Arctic char will be tied to the same physiological causes observed with the brook trout since both species are taxonomically similar to the each other. Similarities between these two species have encouraged us to develop the same technologies with the Arctic char.

A potential application of triploidy that has not been taken advantage of yet in Canada is the possibility to use triploidy to control or destroy undesirable wild fish populations. Mass introduction of triploid male rainbow trout, into natural spawning areas, could be used to destroy or control undesirable populations of this species since eggs fertilized with such sperm all die within a short time. Triploid males develop sexually but are incapable of fertilizing eggs. This factor could seriously disrupt natural spawning populations. This trait could be used for population control and therefore could permit enhanced production of species of economic or social benefit but, if mismanaged, could represent certain danger for native species.

Rainbow trout production is prohibited in large areas of Quebec and New Brunswick since they are not native to these areas. If reproductively viable rainbow trout were to escape from hatcheries in these areas, it is likely these escapees could establish self sustaining populations and therefore, potentially threaten wild stocks. An alternative and more constructive regulatory mechanism, that required the use of all-female, triploid hatchery stocks, would represent a desirable compromise to foster development while still protecting natural fish stocks in these areas. We believe that accidental contamination of the natural surroundings with all-female, triploid individuals, would result only in a short-term, point impact and that such an environmental perturbation would be limited to predation and competition and most probably would be insignificant. Triploid females have not demonstrated territorial behaviour and would be unable to reproduce.

CONCLUSION

These results demonstrate that all-female, triploid production of brook trout is a dependable and profitable means to assure market quality and saleability of this species. With brook trout, it is evident that techniques must be employed to assure that hatchery stocks are all-female and triploid. In contrast, we have found that our markets for rainbow trout can be satisfied with just all-female hatchery stock and that triploidy is not necessary for the size of the rainbow trout product that we are marketing. By autumn, 1991, our enterprise (Pisciculture des Alleghanys Inc.), should be ready to provide all-female, triploid brook trout on a regular commercial basis.

Technology for the production of non-maturing salmonids is relatively new and has not yet been exploited adequately by salmonid farmers. It is our opinion that such chromosome manipulation, if used properly with intelligence and reserve, could bring answers to many problems that hamper commercial producers as well as fauna managers.

ACKNOWLEDGMENTS

We wish to thank the National Research Council for the monetary assistance they have granted for this project. Without this aid, it would have been impossible to complete this project in such a short period of time.

We also want to thank, for his technical support, Dr Robert Peloquin, veterinarian for the Ministry of Agriculture, Fisheries and Food, in Rock Forest.

REFERENCES

Lincoln R.F. and Victor J. Bye. 1986. Growth rates of diploid and triploid rainbow trout (Salmo gairdneri) over the spawning season. Reproductive Physiology of Fish 1987:134.

TABLE 1 : COMPARATIVE DATA OF ALL-FEMALES TRIPLOID AND DIPLOID BROOK TROUT (<u>SALVELINUS FONTINALIS</u>) GROWTH FROM 1988 TO 1990

DATE	PERIOD # DAY	PERIOD AVG. TEMP. (0 C)	DEGREE.DAY PERIOD	DEGREE.DAY CUMMUL.	TRIPLOID FEMALES WEIGHT AVG. (Kg)	TRIPLOID FEMALES WEIGHT TOTAL (Kg)	TRIPLOID FEMALES TROUT #	TRIPLOID FEMALES CONV. RATIO	DIPLOID FEMALES WEIGHT AVG. (Kg)	DIPLOID FEMALES WEIGHT TOTAL (Kg)	DIPLOID FEMALES TROUT #	DIPLOID FEMALES CONV. RATIO
25-Feb-88				2000	0.099	128.18	1298	1.20	0.122	100.90	825	1.40
29-Jun-88	125	3.7	463	2463	0.145	208.00	1430	1.42	0.210	125.00	595	1.40
20-Jul-88	21	11.0	231	2694	0.166	237.00	1431	1.20	0.218	129.70	595	1.80
15-Sep-88	57	11.0	627	3321	0.238	146.80	617	1.40	0.314	186.80	595	1.20
18-Oct-88	33	8.5	281	3601	0.263	189.30	721	1.75	0.292	182.70	626	-0.11
07-Nov-88	20	6.5	130	3731	0.290	209.00	720	2.25	0.289	182.20	630	-0.06
21-Nov-88	14	5.0	70	3801	0.291	214.00	736	2.53	0.295	183.20	621	5.28
13-Dec-88	36	4.0	144	3945	0.309	225.00	729	1.21	0.312	192.00	615	1.20
19-Jan-89	37	3.0	111	4056	0.327	238.20	728	1.85	0.346	210.00	607	1.17
10-Mar-89	50	1.8	88	4144	0.363	264.00	728	1.36	0.378	228.00	603	1.85
18-Apr-89	39	1.7	64	4208	0.384	278.2	724	1.83	0.400	237.3	593	2.56
13-Jun-89	56	5.7	319	4527	0.469	342.2	729	1.18	0.486	289.3	595	1.45
20-Jul-89	37	11.1	411	4938	0.571	171.4	300	2.90	0.592	336	568	1.73
17-Aug-89	28	12.7	354	5292	0.706	173.6	246	2.00	0.642	352.7	549	4.41
19-Sep-89	33	11.9	393	5685	0.795	194	244	2.07	0.669	350	523	3.25
06-Dec-89	78	7.2	558	6242	1.011	238.6	236	2.73	0.599	299	499	-0.91
30-Jan-90	55	3.2	173	6416	1.141	270.4	237	2.11	0.677	341.8	505	1.53
26-Feb-90	27	2.0	54	6470	1.200	275.9	230	1.62	0.679	339.5	500	7.60
29-Mar-90	31	2.0	62	6532	1.300	296.4	228	1.18	0.727	357	491	1.56

TABLE 2: CONVERSION RATIO OF ALL-FEMALE, DIPLOID AND TRIPLOID BROOK TROUT AT DIFFERENT WEIGHTS.

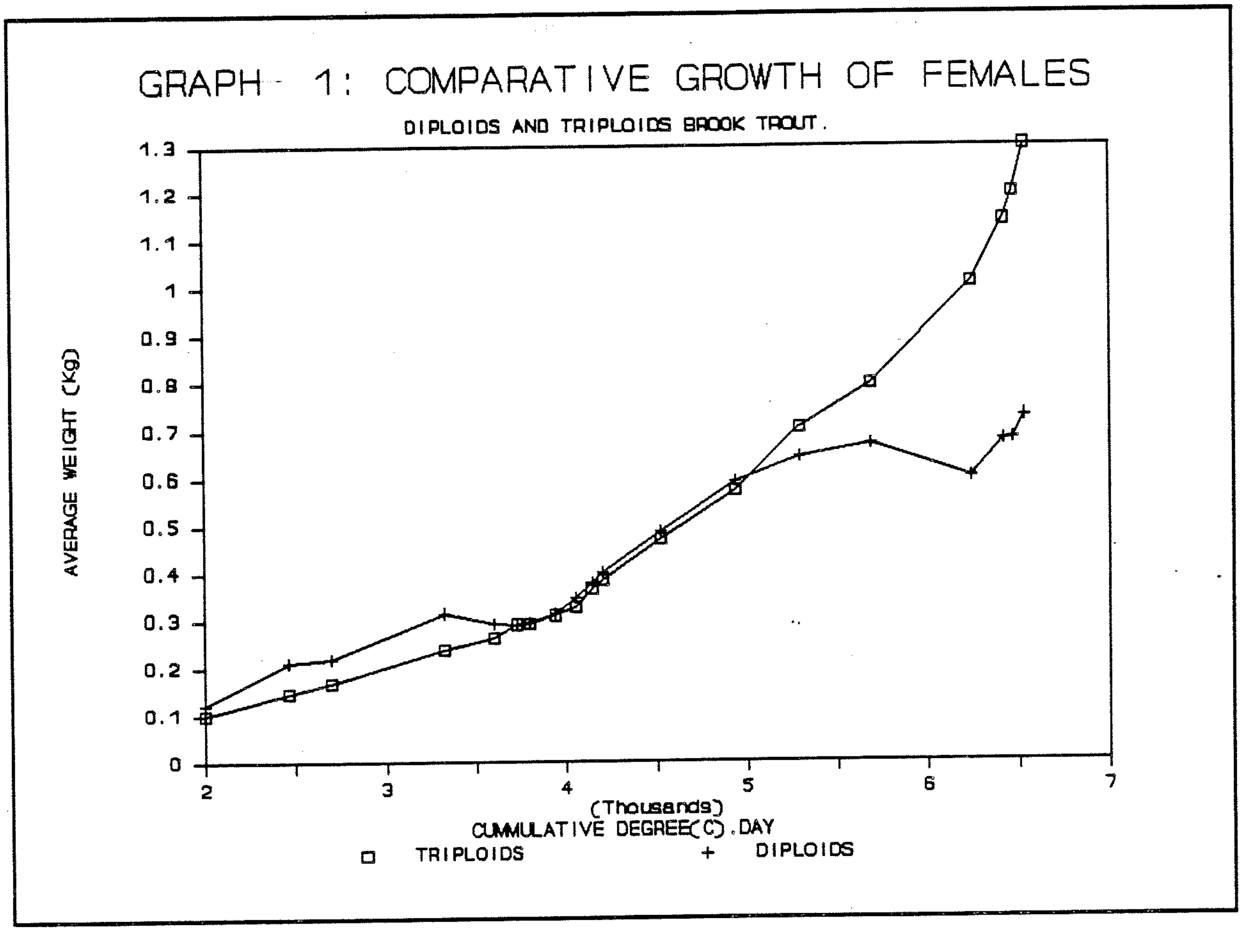
	CONVERSION RATIO	
WEIGHT (g)	TRIPLOID	DIPLOID
460	1.41	1.62
700	1.74	2.03
1300	1.87	2.20

TABLE 3: WEIGHT OF VISCERA AND GONADS AND FLESH MOISTURE CONTENT OF TRIPLOID AND DIPLOID BROOK TROUT.

WEIGHT						FLESH MOISTURE
BODY (g)	GUT (g)	GUT % B.W.	GONADS (g)	GONADS % GUT		%
TRIPLOIDS						
1545	305	19.7	0	0		73.92
1760	390	22.2	0	0		72.57
1525	345	22.6	0	0		72.59
1900	445	23.4	0	0		74.11
1215	280	23.0	0	0		72.81
	X:	22.2			X:	73.20
	STD:	1.3			STD:	0.67
DIPLOIDS						
1830	630	34.4	365	58.0		73.28
1490	495	33.2	295	60.0		75.11
1745	350	20.1	70	20.0		72.64
1310	450	34.4	265	59.0		75.82
625	250	40.0	140	56.0		79.6
	X:	32.4	X:	50.4	X:	75.29
	STD:	6.6	STD:	15.2	STD:	2.45

TABLE 4: COST PARAMETERS OF ALL-FEMALE, TRIPLOID AND DIPLOIDS BROOK TROUT ON 1000 Kg BASIS

PARAMETERS	TRIPLOID		DIPLOID		COST DIFFERENCE 460 g	COST DIFFERENCE 1,600 g
PRODUCTION (Kg)	1,000	1,000	1,000	1,000		
AVG. WEIGHT (g)	460	1,600	460	1,600		
DEGREE/DAY (C)	4,500	6,700	4,500	8,200		
CONVERSION RATIO	1.41	1.90	1.62	2.20		
FEED						
Kg	1410	1900	1620	2200		
$ ($0.85/Kg)	$1,199	$1,615	$1,377	$1,870	$179	$255
GUTTING LOSSES	Kg	$ ($4.00/Kg)	Kg	$ ($4.00/Kg)		
AUGUST-DEC. (5)	92	$368	134	$536		
JAN.-JUL. (7)	128	$512	128	$512		
TOTAL	220	$880	262	$1,048	$168	$168
MUSCLE MOISTURE (%)	73.2		75.3			
EGG COST	$182	$48	$61	$16	($122)	($32)
SAVINGS FOR ONE TON OF PRODUCTION :					$225.00	$391.00
ONE Kg :					$0.22	$0.39


GRAPH 1: COMPARATIVE GROWTH OF FEMALES
DIPLOIDS AND TRIPLOIDS BROOK TROUT.
AVERAGE WEIGHT (Kg)
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
2
3
4
5
6
7
(Thousands)
CUMMULATIVE DEGREE(C).DAY
TRIPLOIDS
DIPLOIDS

PARTICIPANT DISCUSSIONS

Rapporteur: Vern Pepper

Q: Donaldson — Do I understand that you are producing tetraploids in rainbow trout now?

A: Boulanger — We have found the window in which to apply the pressure shock. This is very easy with rainbow trout. It is not like triploidy where we have an egg loss near 60%. The eggs that survive perform normally. We have just produced female tetraploids. These are too small yet to produce eggs. They are 120 g now and will mature next year. We then will try mating these tetraploids with female milt to produce a triploid. The present female tetraploids seem to be growing normally. There is considerable loss of eggs with pressure shock. The first mitotic division takes place at 3,200° minutes. We have tried pressure shock at 60% to 120% of time to first division. The best result within this interval, that is the time of treatment that results in the lowest egg losses, is at first mitotic division.

Q: Friars — I understand that you are using 4N females in the production of 3N fish. Do you use 4N males?

A: Boulanger — No. We cannot use 4N males to continue the 4N lines. It has been reported that 4N sperm have such big heads that they cannot enter the micropore and therefore cannot fertilize eggs.

Q: Benfey — Do I understand that you have not been successful in hormonal masculinization of brook trout?

A: Boulanger — We were successful last year. This is the first year we have had sex reversed brook trout. It took us only two years to figure this out for rainbow trout. It has taken five years to do this successfully for brook trout.

Q: Anderson — You indicated that 3N trout, both brook and rainbows, have better food conversion. This is the first time I have heard this confirmed.

A: Boulanger — It is not a big difference but it is definitely there.

Q: Anderson — Does this happen in other species such as Atlantic salmon?

<u>Comment: Johnstone</u>

During the reproductive cycle in Atlantic salmon, maturing animals initially (i.e., in the Spring months) grow better than triploids and non-maturing diploids but in the later stages, maturing fish cease feeding altogether. This is described in some detail in my paper, but to summarise, all-female triploids grow similarly in fresh water to diploids. In sea water, triploids grow in a similar manner with regard to weight on length as non-maturing diploid fish. Both these latter categories grow less well for weight on length than maturing fish. Thus, triploids will never be the fastest growers since diploids are likely to be the earliest maturers. Rather, they will grow predictably and continuously and more animals should reach the larger, more valuable sizes. This assumes there are no triploidy associated factors which reduce their survivability. In our trials to date, we have not been made aware of any systematic reduction in the rate of triploid survival.

<u>Comment: Boulanger</u>

Our results vary with the strain in use. We do not get as good results with fall rainbow trout as with spring spawners in producing triploids.

Q: Friars	Could you clarify for me the point you were making regarding escapees that they could ruin a wild population. Would this be by production of aneuploids?
A: Boulanger	Heat shock with brook trout results in a lot of aneuploids. There are fewer with pressure shock.
Q: Friars	Do you then have escaped 3N males that interact with wild 2N females and therefore disturb the reproductive potential of the wild population:
A: Boulanger	No, this has not happened in the wild but rather in our hatchery. We have had bad experiences within our hatchery on two occasions in which two batches of 100,000 eggs each were accidentally fertilized with 3N males. This was due to a misclassification of the males by hatchery workers. Within two days, all eggs in these batches were dead. This has happened by fish jumping from one tank to another, thereby mixing up our treatments. These males have been missed by hatchery workers during the stripping and fertilization process. The people who work for me do not seem to make the distinction between sex-reversal and triploidy. This failure is a catastrophe in the hatchery so that is why I say these triploid males could be bad for wild populations. Now in our hatchery, each time we kill a sex-reversal female to take the milt, we do a karyotype on that fish. It takes about two minutes to do and then we can be sure.

Comment: Friars

This causes me considerable concern. We are talking about triploidy as a means of protecting wild stocks from genetic pollution by introduced stocks. The absence of sperm ducts in these triploids males may be our salvation in this regard. However there are behavioural aspects to be concerned about such as courtship patterns. This still could greatly interfere with wild populations.

Comment: Boulanger

It is important, if you do not want to risk destroying natural populations, that you use all-female lines in your triploid induction phase.

DISCUSSION SESSION, FEBRUARY 21

As chairman for the workshop, Vern Pepper opened the third day of the proceedings with a discussion that was intended to reach a consensus regarding the participants' attitudes to the production and use of non-maturing salmonids for aquaculture. The chairman extracted the following highlights from the workshop presentations as a focus for discussions of the "state of the art" of this technology. These highlights are as follows

SUMMARY STATEMENTS FROM WORKSHOP PRESENTATIONS

R. JOHNSTONE, H.A. McLAY and M.V. WALSINGHAM. PRODUCTION AND PERFORMANCE OF TRIPLOID ATLANTIC SALMON IN SCOTLAND.

... individual female fish differed in their susceptibility to a given heat shock, not only in terms of triploid rate but also in terms of survival rate and, therefore of triploid yield, it was decided that heat shock was unlikely to be the method of choice for commercial application.

The difficulties of moving from small batches of eggs at the experimental level to large volumes under commercial conditions initially led to markedly reduced survival rates relative to controls at some farms. These problems have now been overcome and the appropriate handling protocols for large volumes of eggs identified. Larger vessels (4L) have now been built and tested and the triploidisation process should not be rate limiting to the stripping process of females under commercial conditions except at the largest farms. Using a 4L vessel and the present operating parameters, and assuming a constant supply of eggs, some 16-20L (ca 80-100,000) eggs per hour can be processed.

There is some anecdotal evidence that triploids generated by heat shock perform less well than those produced by pressure shock. This may relate to the more variable results seen, in our experience, as a result of heat shock procedures. It might be argued that the best agent for triploidisation is likely to be that in which all eggs are exactly and similarly exposed.

Triploids perform similarly in fresh water to diploids. In sea water, triploids grow less well for weight on length than maturing salmon but in a similar manner to non-maturing fish.

It is concluded that triploid, all-female stocks will be of benefit both for commercial reasons and because they will minimise the effects of genetic transfer that might otherwise occur via accidental releases of farmed animals.

Experience to date suggests that 90% yields of triploids at essentially 100% triploid rates are possible under commercial conditions at most Scottish farms.

Market yields in terms of weight per unit of rearing volume per unit time in triploid stocks should be greater since more animals would be expected to grow to the larger and more valuable sizes. Their functional sterility should be of additional advantage in areas where the possible impact of accidental releases from farming operations is causing concern.

EDWARD M. DONALDSON, FRANCESC PIFERRER, IGOR I. SOLAR AND ROBERT H. DEVLIN. STUDIES ON HORMONAL STERILIZATION AND MONOSEX FEMALE TECHNOLOGIES FOR SALMONIDS AT THE WEST VANCOUVER LABORATORY.

Production of monosex female chinook salmon has... been a major success.

The economic advantage of growing monosex female chinook is such that a very high proportion of total chinook production in British Columbia is now monosex female.

It was concluded that sterile fish remained in the marine environment until they were either captured or died of natural causes.

There are thus a number of avenues that could be pursued during further research on the production of sterile salmonids. There is no doubt that the further implementation and integration of monosex and reproductive suppression technologies into salmonid production systems will become increasingly important to the salmonid aquaculture industry.

There is interest in extending monosex female culture technology in British Columbia to Atlantic, coho and sockeye salmon and in further investigation of reproductive suppression technologies both for culture purposes and for the prevention of reproductive interaction between farmed and wild stocks.

There is potential to induce a high percentage of sterility in salmonids by immersion during the incubation process.

Recently, there has been significant progress in the development of Y-specific DNA probes for chinook salmon.

P.J. JUNGALWALLA. PRODUCTION OF NON-MATURING ATLANTIC SALMON IN TASMANIA.

The only serious drawback to the Industry, of having such a high grilsing rate, is the restriction on the harvest period. The fish are not large enough to harvest before October and the onset of maturity renders the fish unpresentable to market by the following March.

Where a situation arises, as in Tasmania, that virtually a whole year class attains maturity together, it poses a definite time limit to the harvest period, and incidentally an upper limit to the size of fish which can be produced economically.

The disadvantage of such a grilse based industry is the short season of the harvest period. The suppression of maturation is therefore a desirable goal.

The advent of a rapid, non-destructive test for sex-determination is awaited.

Some 30 litres of eggs were put through a regime of 5 minutes duration, at 9500 psi, and at 30 minutes post fertilisation (300° minutes). Assessment by Flow Cytometry in early 1990 showed 100% triploidy in the surviving fry.

Triploid yield (to eyed egg stage) was 85%, and subsequent assessment by Flow Cytometry showed surviving fry to be 100% triploid

Triploid fry require more bottom surface area than diploids and take longer to reach 1 g. After this stage, their growth rate catches up and perhaps exceeds that of diploids.

No significant differences in disease susceptibility between diploids and triploids have been observed over the three year classes now produced. Samples of smolts from each of the year classes were exposed to seawater challenge and subsequent plasma sodium tests. No significant differences were recorded between diploids and triploids.

From Saltas' experience to date, it is believed that in general the triploids are capable of offering at least a fair performance in terms of growth and survival during their freshwater phase. They also appear to smoltify normally.

It is therefore assumed that the higher incidence of pinheading in triploids is a function of the triploid state.

Sex-reversal of broodstock, and triploidy per-se have been successful. From the three year classes handled to date, some differences between the early growth of diploids and triploids have been observed. In general, freshwater performance of triploids seems similar to that of diploids, and therefore commercially acceptable.

From the two year classes which have been sent to sea-cages, it appears that a significant percentage of triploid smolts fail to adapt to seawater. Unaffected fish continue to grow satisfactorily and show no special susceptibility to diseases or environmental degradation.

A jaw deformity, apparently site specific in its severity and not sibling-related, has also appeared in the 1990 smolts, after some three months in the sea cages. The epidemiology of both these syndromes is still unclear.

TILLMANN J. BENFEY. THE PHYSIOLOGY OF TRIPLOID SALMONIDS IN RELATION TO AQUACULTURE

Studies of other physiological processes also indicate remarkable similarities between triploids and diploids.

... triploid fish are remarkably normal in all regards aside from their sterility.

... triploid salmonids are frequently said to perform poorly in aquaculture, especially when reared under sub-optimal conditions or when kept in mixed groups with diploids. Apparent differences between triploids and diploids may be due to behavioural and sensory problems rather than due to direct physiological effects.

Depending upon the particular study, triploids have been shown to grow faster, slower, or at the same rate as diploids.. The question of what effect triploidy has on growth rates is confused by the interrelationship between growth rates and the onset of sexual maturation, and by possible behavioural differences between triploids and diploids.

At the onset of sexual maturation, energy is diverted from somatic growth to gametogenesis, and thus different growth rates between triploids and diploids should be expected in adult fish.

In rainbow trout, growth rates decline with the onset of sexual maturation in diploids of both sexes and in triploid males, whereas triploid females continue to grow well. In this case, triploid females do not exhibit superior growth rates, but simply continue to grow at a constant rate whereas growth rates decline in the maturing fish.

Some studies have found that triploids do not grow well in competition with diploids but do grow as well as diploids when reared separately. However, others have not found such an effect.

What remains to be determined clearly is whether induced triploidy can be used to meet these objectives without compromising the economic viability of commercial aquaculture.

What has become clear is that triploid female salmonids retain the characteristics of immature fish throughout their lives, and may therefore serve a useful function in aquaculture to eliminate problems associated with sexual maturation, such as decreased flesh quality and increased susceptibility to disease.

G.W. FRIARS AND T.J. BENFEY. TRIPLOIDY AND SEX-REVERSAL IN RELATION TO SELECTION IN THE SALMON GENETICS RESEARCH PROGRAM

Grilsification, defined as sexual maturation after one sea winter, is an undesirable trait in aquaculture because flesh quality deteriorates when nutrients and energy are directed toward reproductive rather than carcass tissues. Also, secondary sex characteristics, such as dark colour, that accompany the sexual maturation process, diminish retail value.

Despite the fact that larger pre-grilse are being grown in Tasmania than in the Bay of Fundy, the industry perceives that the avoidance of grilse in the Northern Hemisphere is a requirement of efficient operations at this time.

Although grilsification is not usually a serious problem for cage culturists using SJR fish, the proportion appears to vary between groups and has been reported to be as high as 40 percent at one Nova Scotia site.

Concerns about cage escapees entering rivers and mating with wild stocks have heightened the cage-culture industry's interest in sterile stocks for the future.

A.M. SUTTERLIN AND C. COLLIER. SOME OBSERVATIONS ON THE COMMERCIAL USE OF TRIPLOID RAINBOW TROUT AND ATLANTIC SALMON IN NEWFOUNDLAND, CANADA.

No differences in the hatchery pre smolt growth rates were noted between female diploids and triploids. After 17 months in sea pens, early maturing diploids were largest, late maturing female diploids intermediate and sterile female triploids smallest. Early maturing, diploid females exhibited slightly lower gutting losses with lower visceral fat compensating for the enlarged ovary.

The weights of the various organs expressed as a percent of body weight reveal that early maturing fish have a larger ovary and larger liver but smaller residue (caeca, fat bodies, stomach and intestine) than late maturing fish and triploids. The reduced size of the residue is largely due to the reduction in size of the fat bodies in early maturing fish.

After 14 months of sea culture, all diploid males, all triploid males, and all diploid females were in the early stages of becoming sexually mature as grilse.

... if mixed groups of female diploid and triploid trout are cultured together, the early growth rate of the diploid trout during the pre-maturation phase is superior to triploids; our results are similar. They suggest that the triploids are perhaps disadvantaged in competing for food.

Protruding lower jaws were found almost exclusively in triploid fish, but were not present in all triploid fish within any particular treated group. No diploid fish originating from previously heat shocked eggs had protruding jaws. Protruding lower jaw syndrome, much to the displeasure of the farmers, persisted in a frequency of between 10-30% in triploids of both sexes; these fish were deemed not marketable.

A mixed strategy wherein female diploids and triploids are held in different cages might be considered.

Although we feel that the techniques for producing female triploid rainbow trout are commercially applicable at this time, the existing literature and unpublished experience in Canada prior to this workshop would indicate that a transfer of comparable technology to produce an"environmentally friendly" salmon proceed at a systematic but cautious rate.

EUGENE B. HENDERSON. SEX-REVERSAL AND INDUCTION OF TRIPLOIDY IN ATLANTIC SALMON: AN INDUSTRY PERSPECTIVE.

From an industry point of view it could be said that there is no pressing interest in reproductively incapable salmon.

A benefit of non-maturing salmon, from an industry point of view, would be to solve the grilsing problem.

JOANNE STEVENSON. MATURITY SUPPRESSION IN RAINBOW TROUT FROM THE PRODUCER'S PERSPECTIVE.

Growth rate of the triploid rainbow trout is similar to that of diploids up to approximately 230 to 280 g. Thereafter, triploids seem to grow slightly faster. Our heat shock process for inducing triploidy also seems to reduce the time to hatching.

... there is a much higher mortality rate among triploid eggs than among diploids, both up to the eyed stage and thereafter up until hatching

... there is an increased incidence of deformities among triploid fry

... triploid fish seem to grow slightly slower than all-female trout.

YVES BOULANGER. PERFORMANCE COMPARISON OF ALL-FEMALE, DIPLOID AND TRIPLOID BROOK TROUT.

Hydrostatic pressure has proven more appropriate than heat shock in terms of: success (over 95%); reliability; speed of execution (500 000 eggs/hour); survival rate of the individuals; and, quality during the growing period (low rate of aneuploids).

Triploidy inhibits only the reproductive development of female gonads whereas the males grow sexually in a normal fashion, even though their sperm is not viable.

When the triploidy method is used, it is essential to use all-female stock in order to avoid growth and behaviour problems related to the sexual maturation of males.

All-female, triploid growth was continuous for the three years of observation. Diploid females lost weight during spawning (1988-1989).

Improved growth of the all-female (rainbow trout) triploids, relative to the diploids, was readily apparent at 500g.

Food conversion always has been better for the triploids regardless of age.

Average gutting loss was 22% for the triploid females compared to 32% for the maturing diploid females. The difference is due mostly to the gonads.

Reduced production costs, varying between $0.22 and $0.40/kg, have been obtained because of improved food conversion and lower gutting losses among triploid trout.

There was consistent and improved flesh quality in triploid relative to diploid trout.

It is our opinion that such chromosome manipulation (triploidisation)... could bring answers to many problems that hamper commercial producers as well as fauna managers.

It is important, if you do not want to risk destroying natural populations, that you use all-female lines in your triploid induction phase.

HIGHLIGHTS OF DISCUSSIONS OF WORKSHOP PAPERS

Comments: Pepper

... an unfortunate negative bias has developed among the sea farmers because of previous maturity suppression efforts using heat shock on Atlantic salmon in Newfoundland. There was a high incidence of jaw deformities within this experimental batch of salmon. We really have no idea if this was a coincident phenomenon (i.e., had nothing to do with the experimental procedure) or was a result of the heat shock treatment. This previous experience has resulted in an attitude among the salmon farmers that they really are not interested in further experimentation with this technology. Their attitude, toward both technology for suppression of maturation and further experimentation with local salmon stocks, is now solidly entrenched in an attitude of "show us, (at your expense, not at ours) and we will determine if there is an advantage to our economic viability".

In my dealings with industry, I simply have not had the answers to these questions and therefore have not been able to provide the positive advocacy you suggest. In truth, that is what I am looking for and is one of the main reasons why I have organized this workshop. I expect that much of the world expertise on this technology is assembled in this room and will provide us with the objectivity necessary to undertake some positive initiatives to integrate our various regional needs into a program that will meet the needs of the salmonid aquaculture industry as well as the resource managers who must concern themselves with wild fishery management problems.

As a DFO biologist, I have responsibility to provide sound scientific advice to wild fishery resource managers but, in doing so, must not overlook both social and economic needs... This topic of production of non-maturing salmon may provide a means to achieve these goals. Suppression of reproductive potential, if applied to a local Newfoundland grilse stock, may provide a means to overcome the grilsing problem. In turn, improved stock performance could result in a desirable and marketable commodity that has not been attainable with local diploid stocks to date. On the other hand, if this technology is applied to imported salmon stocks, the resulting non-reproductive salmon cannot pose any threat of genetic swamping to local wild salmon stocks. Whatever technology is applied, we want to gain stock performance for sea farmers while protecting the wild stocks against genetic swamping. Therefore, I advocate comparative testing of techniques and various stocks to provide objective decision criteria for industry planning and management.

Comment: Anderson
We had similar deformity problems some years ago. They had nothing to do with triploidy. You should not assume the problems you have observed are necessarily related to the triploidy program.

Comments: Johnstone
It seems to me necessary to say at this point that the appropriate techniques and strategies for maturity control might vary between species. ...heat shocks appear to give better results for rainbow trout and possibly also for Arctic char than for salmon and brook trout where pressure is preferred. The desire to produce steriles at all will also vary between species being clearly of most benefit in the earliest maturing species or where early maturity prevents animals from being grown to the larger sizes. All-female stocks alone are a relatively complete answer to the maturity problem experienced in rainbow trout culture in the UK since most animals are sold at 200-550g and maturity below this size is largely confined to males. This is less true in Atlantic salmon culture in Scotland where the poorer perception of grilse stems from their generally small size (ca 2 kg) as much as from their narrowed market window. Large grilse are not so disadvantageous. All-female stocks will, at best, be only a partial solution to the problem of early maturity in salmon culture.

Triploid fish take 30 to 50 degree days longer to achieve first feeding. I had assumed that later first feeding would result in slower growth throughout development. Surprisingly, this does not always seem to be the case. Triploid fish may be able to 'catch up' in terms of growth.

Irish producers experience generally higher grilse rates than do Scottish farmers although some apparently use lower maturing Scottish and Norwegian strains. The real problem with early maturity is the unpredictability of the grilse fraction and their necessarily reduced marketing window. The use of triploids would avoid this problem and might enable farmers to put fish to sea cages at different times than at present and enable them to move away from the single input systems as practised at present.

...the recognition of the potential of accidental releases to interact with wild stocks has come more to the fore and is perceived by some as an additional benefit of triploidisation. My results confirm the expectation that female triploids will be sterile. Increased triploid usage would therefore minimise any impact of releases.

You must be careful not to confuse correlation with causation. The occurrence of pinheads and jaw deformities may not be due to triploidy. Jaw deformities have occurred in Scotland in diploid stocks. David Bruno from our laboratory has documented one such occurrence in Veterinary Record. There is also a condition in diploid stocks in Scotland called "failed smolt syndrome" that looks, superficially, to be similar to your "pin head" condition. I have attributed the condition we see to a failure to come onto feed in the immediate post transfer period and believe that social/husbandry interactions are most important in its generation.

To everybody, we should say that triploids are not unnatural animals but that they do occur, albeit rarely, in the wild. To the farmers, we should say that the sterility aspect of triploids is likely to be of benefit by making rearing and marketing strategies more predictable. This in turn should make producers more efficient. We must avoid saying that we have all the answers or that there will not be problems that will need to be addressed in future research but.. the prospects are... sufficiently encouraging to justify further evaluation. To stock managers and to those concerned with the potential for alteration of the genetic structure of existing stocks that might follow from accidental releases, we should say that the use of triploids should minimise these concerns. Finally, we might say to consumers that triploids might ensure uniformity of product quality and supply. The seasonal deterioration that takes place in diploid maturers and which conditions their marketing window, will not be a feature of triploid production.

In our hands, heat shocks produce more deformed survivors than appropriate pressure shocks.

Comment: Farmer
Location of farms and type of environment influence grilsing. In Nova Scotia, an evaluation of three different stocks including St. John River, have given higher grilsing rates than in N.B. (20-30%) with high proportions of female grilse from S1 smolts. Also over maturity of post smolts from S2.

Comments: Jungalwalla
The chronological progression we have followed was something in the order of 2,000 eggs in 1988, 50,000 in 1989, and 300,000 in 1990. The number of triploid fish in commercial production is increasing steadily. This progression reflects our ability and confidence to produce sufficient triploids to make it all worth while. The idea is to provide the industry with at least 30% of its requirements as triploids. At this point in time, I have no hesitation in saying that triploidy is the way to go. Any difficulties we have encountered along the way we consider transient. We intend to proceed with our triploidy program and to deal with circumstances as they develop.

Question: Friars
Could you clarify for me the point you were making regarding escapees that they could ruin a wild population. Do you then have escaped 3N males that interact with wild 2N females and therefore disturb the reproductive potential of the wild population.

Answer: Boulanger
No, we have not seen this in the wild. We have had bad experiences within our hatchery on two occasions in which two batches of 100,000 eggs each were accidentally fertilized with 3N sperm. This was due to a misclassification of the males by hatchery workers. Within two days, all eggs in these batches were dead. This has happened by fish jumping from one tank to another, thereby mixing up our treatments. These males have been missed by hatchery workers during the stripping and fertilization process. The people who work for me do not seem to make the distinction between sex-reversal and triploidy. This failure is a catastrophe in the hatchery so that is why I say these triploid males could be bad for wild populations. Now in our hatchery, each time we kill a sex-reversal female to take the milt, we do a karyotype on that fish. It takes about two minutes to do and then we can be sure.

It is important, if you do not want to risk destroying natural populations, that you use all-female lines in your triploid induction phase.

Comment: Friars
This causes me considerable concern. We are talking about triploidy as a means of protecting wild stocks from genetic pollution by introduced stocks. The absence of sperm ducts in these triploid males may be our salvation in this regard. However there are behavioural aspects to be concerned about such as courtship patterns. This still could greatly interfere with wild populations.

Comment: Henderson
It is important that research be done and results evaluated before industry is pressured to produce such sterile fish. Bear in mind, that this is perceived as a non-problem by the industry and producers are content with the performance of the fish now being used.

There is a problem with the notion of hormone treatment. The public has been sensitized to such concepts. Keep in mind that perception is often reality to the public.

Comment: Sutterlin
In promoting an "environmentally friendly" salmon, would not terms such as genetically manipulated, and triploid cause the consumer some concern. While these techniques to overcome seed production are common practices in agriculture, certainly no one advertises peas or bananas as such, except in a seed catalogue.

Comment: Johnstone
Try to emphasize such products as something natural, more like wild salmon and define situation by explaining that triploids actually occur naturally in the wild.

Comment: Jungalwalla
Use terms such as non-reproductive as opposed to triploid or sterile.

Comment: Donaldson
Even with direct suppression of gonad development, steroids levels are lower in aquaculture salmonids than in wild fish; with levels in dairy products being much higher.

PARTICIPANT DISCUSSIONS OF SUMMARY STATEMENTS

On the basis of the highlights from the workshop papers, the chairman opened a discussion session to categorize the extracted highlights relative to their anticipated concerns to potential users of this technology. The goal of this exercise was to determine if the various highlights would be perceived as positive, neutral, or negative (i.e., would the potential recipient, of technology for the production of non-maturing salmonids, consider the statement as advantageous or something to be worried about relative to the economics of operating a salmonid farm).

It became apparent that interpretation of any given statement among workshop participants was not always consistent and depended on the orientation of the individual who voiced the opinion. The following are a few examples:

Statement: Studies of other physiological processes also indicate remarkable similarities between triploids and diploids. ... triploid fish are remarkably normal in all regards aside from their sterility.

Benfey - From a physiological perspective, this is positive. Problems with triploids can be expressed more in poor husbandry conditions.

Ritter - This should be considered as negative since it may mean triploids are inappropriate in marginal culture conditions.

Davidson - If there is no physiological difference between diploids and triploids, this statement should be considered neutral in that it implies no real advantage or disadvantage.

Statement: ... if mixed groups of female diploid and triploid trout are cultured together, the early growth rates of the diploid trout during the pre-maturation phase is superior to triploids; our results are similar. They suggest that the triploids are perhaps disadvantaged in competing for food. A mixed strategy wherein female diploids and triploids are held in different cages might be considered.

Sutterlin - This is positive since it extends the harvesting season for the farmer. Diploids can be harvested first.

Johnstone - Extension of the marketing window could be achieved in salmon my manipulation of input times, sizes and feeding regimes.

Friars - The most efficient system will be one where sorting and grading are eliminated. A cage should be marketed as a unit of production, all fish going to market at the same time.

Henderson - Some producers in New Brunswick already market "a cage at a time". From a production point of view, the important thing is profitability. Anything than can be a means to increase the profit margins, will be useful.

Statement: What has become clear is that triploid female salmonids retain the characteristics of immature fish throughout their lives, and may therefore serve a useful function in aquaculture to eliminate problems associated with sexual maturation, such as decreased flesh quality and increased susceptibility to disease.

Benfey - This is related more to non-maturing fish and not necessarily to triploid fish.

These statements reflect that there was some difference of opinion among the workshop participants. However, the majority of presentation highlights were viewed as positive, both from the point of view of what maturity suppression technology has to offer to the aquaculture industry and protection of wild stocks from consequences of interbreeding with escaped aquaculture salmonids. Since the statement rating exercise was not intended to be quantitative, and ultimately the balance of 'positives' and 'negatives' will vary according to the views of the reader, no attempt is made here to reproduce the balance sheet as developed in this workshop discussion session. Rather, attention is focused on items that most workshop participants perceived as negative. The main concerns with technology for the suppression of reproductive potential, and the discussion of these concerns, was as follows:

Statement ... there is an increased incidence of deformities among triploid fry...

There was a high incidence of jaw deformities within this experimental batch of salmon. Protruding lower jaws were found almost exclusively in triploid fish, but were not present in all triploid fish within any particular treated group. No diploid fish originating from previously heat shocked eggs had protruding jaws. Protruding lower jaw syndrome, much to the displeasure of the farmers, persisted in a frequency of between 10-30% in triploids of both sexes; these fish were deemed not marketable.

Johnstone - Heat shocking gives more inconsistent and variable results and seems to result in irregularities/deformities at points during development.

Pepper - We really have no idea if this was a coincident phenomenon (i.e., had nothing to do with the experimental procedure) or was a result of the heat shock treatment.

Anderson - We had similar deformity problems some years ago. They had nothing to do with triploidy. You should not assume the problems you have observed are necessarily related to the triploidy program.

Statement Although we feel that the techniques for producing female triploid rainbow trout are commercially applicable at this time, the existing literature and unpublished experience in Canada prior to this workshop would indicate that a transfer of comparable technology to produce an"environmentally friendly" salmon proceed at a systematic but cautious rate.

Participants in the workshop were in favour of the potential benefits of maturity suppression as being of potential advantage to the salmonid farming industry as well as to resource managers. However, this enthusiasm was voiced contingent on implementation of a program to provide adequate resolution of some of the "coincident" phenomena as well as attempting to deal with the needs of the industry. The industry attitude, toward both reproductive suppression technology and further experimentation with local salmon stocks, is exemplified by an attitude of "show us, (at your expense, not at ours) and we will determine if there is an advantage to our economic viability". From an industry point of view it could be said that there is no pressing interest in sterile salmon.

SUMMARY DISCUSSIONS

A recurring theme that arose several times throughout the Atlantic Canada Workshop on methods for the production of non-maturing salmonids was captured by Dr. Ray Johnstone as follows:

> It seems to me necessary to say at this point that the appropriate techniques and strategies for maturity control might vary between species. Thus, we have heard at this workshop that heat shocks appear to give better results for rainbow trout and possibly also for Arctic char than for salmon and brook trout where pressure is preferred. The desire to produce steriles at all will also vary between species being clearly of most benefit in the earliest maturing species or where early maturity prevents animals from being grown to the larger sizes. All-female stocks alone are a relatively complete answer to the maturity problem experienced in rainbow trout culture in the UK since most animals are sold at 200-550g and maturity below this size is largely confined to males. This is less true in Atlantic salmon culture in Scotland where the poorer perception of grilse stems from their generally small size (ca 2 kg) as much as from their narrowed market window. Large grilse are not so disadvantageous. All-female stocks will, at best, be only a partial solution to the problem of early maturity in salmon culture. This is because at normal maturity levels, some grilse are females and at high grilse levels, most females mature. Therefore, sterile stocks have their attractions.

With this clear statement of workshop orientation, there followed an animated discussion for most of the duration of the final day of the workshop. The discussion progressed as follows:

Johnstone — In seawater, triploids do less well initially in terms of length and weight when compared to maturing fish which start off growing faster. Growth of triploids is as good as non-maturing fish through all culture phases. By contrast, growth of diploids peaks just before maturation, then erodes markedly. In this sense, triploidy has positive attributes.

Henderson — Currently, salmon farmers market grilse early, over a relatively short time. The suppression of grilsing through triploidy would have a positive impact. A negative aspect would be that triploids would not have the growth spurt that grilse have during the early stages of maturation.

Johnstone — Scottish farmers like the extra cash flow from grilse which have their pre-maturation growth spurt that accelerates them to a marketable size early in the season. At this time the grilse may be a kilo larger than non-maturing fish. This is an advantage where farmers have non-maturing fish that can be marketed once all the grilse have been sold.

Farmers will become more efficient by producing more uniform fish in each month (2-4 kilo fish in every month of the year). The predictable growth rates of triploids offer an advantage in achieving this marketing goal.

Anderson — The advantages of triploidy can be determined only by practical application, that is by actually growing them under commercial farm conditions.

Johnstone — The best, most successful farmers will be using triploids in several years time because the technique offers advantages to them.

Hill — The industry is driven by economics and if regional industries are to compete in the world market, new technologies must be investigated.

Jungalwalla

Triploid fish extend the market window. This is a positive aspect. The fact that triploid fry take longer to reach one gram and require more tank space is a minor negative point with really no significance.

There appear to be some transient problems associated with the transfer of triploid smolts to seawater. However, these may not be related to ploidy. These transient problems may identify areas of future research.

Triploidy is a valuable tool and it is up to industry as to what use is made of this technology.

Donaldson

In conditions of good growth regimes, there may be advantages of going to an all-female, fast growing grilse stock for part of your production. Does anyone have food conversion data related to triploids?

Boulanger

In trout, triploids have better conversion ratios since they are less aggressive.

Johnstone

Data from Scotland suggest that a triploid stock would grow less well than a diploid, high maturity rate stock, up to the point of the harvest of the diploids. The triploids would, of course, remain in culture past the point of the grilse harvest, to grow on to larger sizes.

Since males generally mature earlier than females, it would perhaps be an all-male stock that might be preferred if fast growing grilse were desired. Such stocks would necessitate the production of YY males. Although the potential exists for producing these animals via the crossing of an oestrogenized "female" of male genotype (XY) with a normal male (XY), I know of no published account of its having been done in salmonids.

Pepper

If we are to pursue this technology, we must be certain that we first develop the technology on a fully researched basis and can be certain of the relative performance characteristics of what we end up with as a result of our endeavours to produce non-maturing fish. This will require comparative performance evaluation data. Can we identify a clear course of action from which to identify a research and demonstration program, complete with realistic time frames for the critical elements. If so, should we then proceed to look at mechanisms for funding. Perhaps Ray Johnstone and Ed Donaldson may be able to provide us with some appropriate approaches or, even better, with a suggested model for Canada based on their experience. What should be the strategy of our program?

Johnstone

As I understand them, the particular difficulties you have in Canada relate to the size of your separate regional industries, to their preferred species, to the regulations relating to the movement of species between regions, and to the rather poor perception that the industry has of sterilization technologies. You should not, in my view, work with mixed sex, (i.e., male) triploids. It is important that you move quickly to establish reversed milt lines in order to generate all-female triploids. Mixed sex populations can be used to evaluate preferred triploid induction regimes and you should do this for the species of interest in order to determine how the biological material available to you can best be triploidised. You have this expertise in Canada. Mixed sex triploids should not be used for two reasons. The first is that male triploids mature endocrinologically at the normal time and are of no industrial benefit. The second is that you leave yourself open to the criticism that accidentally released triploid males may interbreed with wild populations and, as Yves Boulanger has said earlier in this workshop, this cross would result in zero survival. For both these reasons, the use of mixed sex stocks might be poorly perceived.

The problem with the generation of inverted milt is the relatively long lead time. Even starting in 1991, assuming an existing all-female stock is available for androgenisation, it would not be until the middle of the decade that inverted milt from a known all-female line would be available. It might be possible to shorten these lead times by the use of a Y specific probe (Donaldson, this workshop) and/or by environmental manipulation.

However, you do have inverted trout milt from known all-female lines in two species. You might, at the same time as developing towards an Atlantic salmon competence, choose to evaluate the benefits of all-female triploid rainbow trout in sea cage culture using this as an exemplar for other species. I am presuming that the scientific desirability to rear comparative diploid controls for any such trial, or rather their potential to escape, would cause no concern.

Because of predominant problems of male triploids, Canada should work exclusively on all-female stock. This is an obvious thrust that will have guaranteed benefit. If you go the route towards triploid lines, you must decide on what techniques provide the advantages you need. You can explore pressure treatment easily as relatively simple technology but you need to be aware that there are differences between investigators as well as differences in the apparatus used for pressure shocking (i.e., Tasmanian features vs Scottish features).

Donaldson — As well, in order to be representative, an evaluation program must compare various stocks under different grow out conditions.

Anderson — How do you involve industry?

Johnstone — Get it to grow the 3N products. Some farmers will do it if the concept is presented to them in the right light with little risk to their profitability. However, we must be careful with the notion of involving industry in evaluating triploidy. Only those hatcheries that have in excess of 80% survival of eggs (i.e., green to eyed) should be considered for inclusion in a program. Significantly lower survival indicates either lack of interest on the part of the facility management or difficulty with the hatchery itself. Either way, the operation must be credible in order to contribute to an effective program. If these considerations are not met, do not consider using that farm.

Boulanger — The technology is largely "tried" but time lags between research and its application/demonstration hinder its widespread application by industry. Researchers must work closely with industry if fish farm performance benefits are to be apparent to industry. This requires that we educate industry personnel and work closely with them on the farms, perhaps for a 3-6 year time frame.

Johnstone — For Atlantic Canada, Dr. Tillmann Benfey of UNB has been most involved with this technology. Considering his collaborative research with the Salmon Genetics Research Program (SGRP) in St. Andrews, it may be appropriate to ask Tillmann to consider ways to provide perhaps 200,000 eggs to a farmer.

Benfey — My present capacity to produce significant numbers of triploid eggs is greatly constrained the small pressure vessel I am using for my research. I have a design for a larger pressure shock system and am willing to undertake an expanded research program as well to as collaborate with growers if we are able to increase our production capacity for triploid eggs. We have already initiated a pilot project on triploidy and sex-reversal with the SGRP.

Friars — Yes. We have approximately 5,000 eggs being used in this project and have just recently started feeding fry to achieve sex-reversal. However, we should not loose track of the realization that what we are talking about is relatively advanced technology and has not yet been adequately demonstrated in Atlantic Canada. We are in the process of examining this technology. So far, the success stories with this technology have taken place with Pacific salmon or with Atlantics in Scotland. I must point out that our St. John stock of Atlantic salmon has been refined through breeding. The value of a breeding program should not be overlooked and, in fact, could be a useful component of a program on all-female and triploid research on salmon.

Stevenson — Regarding Ray's suggestion of 200 K eggs with Tillmann. Tillmann should take this concept to the farmer to transfer the expertise and demonstrate the technology. Why not a smaller scale - say 50 K then step up to 200 K?

Benfey — Unsure of situation with industry. At this time, we do know what is possible or what is desirable.

Johnstone — Where is the inverted milt at present? Must it remain in the province of New Brunswick?

Glebe — Disease analysis protocols require that we sacrifice brood stock for disease analysis and then keep eggs in quarantine (FHPR protocol) until 5g fry size. This is expensive since brood fish are valuable and family lots must be kept separated in quarantine. If there was a reliable supply of eggs, growers likely would be interested in understanding the potential 3N benefits. 50-l00 K eggs likely possible with farmers. The inverted milt could be moved after quarantine - say about 30 days. Unfortunately, there are as yet no sites certified for XX milt. Scotland and other European countries export eggs to Canada and we sell eggs to foreign countries. Triploid eggs would be preferable because we don't lose any of our genetic material to competing countries.

Henderson — We are talking a long way into the future before we start doing things like shipping triploid eggs. This is simply due to generation time. These types of things are still a long way down the road. Are there research opportunities elsewhere? We should identify if there are and what they can contribute.

Pepper — There may be opportunities elsewhere but there are numerous limitations. Our resources are limited. There is a great deal of pressure to get answers right now and we want to determine how we can meet these pressures. There is a growing body of information indicating that there have been interactions between wild salmon and aquaculture salmon. What we do not have as yet is confirmation of the consequences of this interaction. If data, that is accumulated over the next several years, confirms that there are undesirable consequences to wild salmon stocks as a result of salmon that have escaped from aquaculture operations, those of us within DFO who have been assigned responsibility for aquaculture development will not have any influence or flexibility on the issue of importation of "foreign" stocks for salmon farming. We would have to demonstrate either that there is virtually no way for these fish to escape to the wild or we would have to show that they could not influence the gene pool of wild stocks, even if they did escape. Hence, our interest in the suppression of reproductive potential and the efforts of this workshop.

Hill — We (Nova Scotia) are currently active in the field of triploidy with three trout growers participating in the program. We would like to be involved in future research. Data generated by studies performed within the province would be more likely to be well received by the local industry than studies done elsewhere.

Pepper — We first need to deal with some basic considerations. To develop a program we must answer: who, what, when, where, why and how.

Glebe — We have sex reversed sperm now in St. Andrews. What would be the regulatory concern on transfer of triploid males to other locations?

Jungalwalla/Johnstone — We would strongly recommend against this!

Stevenson — When we first started with our triploids, we sent some of the eggs out to fish farmers. Some of the progeny (notably the males) performed very poorly which resulted in a great deal of adverse publicity.

Glebe — Government regulation of triploid transfers could help avoid this.

Donaldson — In doing work with triploid coho, we achieved sex-reversal by direct feminization with steroids (a license is required to perform this type of work). Can smolts be transferred between sites?

Anderson — It is very difficult to transfer smolts given current FHP Regulations.

Donaldson — Some time ago, we had a research program whose goal was to produce $1 pink salmon smolts. We found that by accelerating growth, we could get sperm when we required it.

Pepper — How do we come up with an action plan that will deal with the five W's as well as the "How" of it all?

Donaldson — Before addressing that, how do you currently get salmon stocks to Newfoundland?

Pepper — We use a standardized egg transfer/quarantine procedure. We are limited to 100,000 eggs due to worries about genetic contamination of wild stocks. The industry would like to move towards 500,000 eggs (100,000 is not enough for a viable industry). Can we start moving in a direction to help develop an environmentally friendly broodstock?

Donaldson — Given the genetic concerns, your broodstock would need to be contained but their triploid progeny could be released to cage sites.

Pepper — This is the basic assumption behind my efforts to implement this workshop. We need to clarify methodologies, species, and stocks to be used.

Donaldson — Then, simply decide what you want and move towards it.

Pepper — Again, to do so requires a knowledge of the interested "players" as well as some objectivity as to why those players should be involved. I would much prefer to have people choose to be involved due to benefits they perceive for themselves. I do not believe this will work if I have to coerce anyone.

Boulanger — Why try to duplicate what some of us in the industry are already doing?

Pepper — The focus of my present problem is Atlantic salmon. At the moment, there is no one in Newfoundland working with non-reproductive Atlantic salmon.

Boulanger	Adaptation of the pressure technique to creating triploidy in salmon should be easy. Therefore it could be transferred easily to the salmon industry.
Pepper	Easy to do is easy to say. You may be right but, just as you have found it easier to develop techniques for rainbow trout than for brook trout, there may be problems with Atlantic salmon that require a greater research capability. Also, any plan we may wish to implement must take place within the confines of the Canadian Fish Health Protection Regulations and therefore, may require that work be done within regions rather than on an interregional, integrated basis. For these reasons, at least initially, I would prefer to take advantage of the current work being done by Tillmann Benfey and the Salmon Genetics Research Program. However, your point is well taken. SALTAS developed their own pressure shock treatments. There is no reason to believe Canadian industry cannot do this also.
Donaldson	Assuming compliance with fish health concerns, you could produce eggs in one place and grow them out at different sites. The experimental design would be very important. With adequate demonstration, industry will adopt your technologies very quickly, especially in Newfoundland where you may have limited alternatives.
Pepper	The current Bottleneck is "XX" milt. The only "XX" milt available is St. John River stock origin.
Sutterlin	Steps have been taken at Bay d'Espoir towards masculinization. Female milt may be available this fall from St. John River, Age 0^+ parr.
Anderson	I would like to stress Yves Boulanger's point about getting industry involved to accelerate technology transfer. We should not overlook genetic research in triploids also. The SGRP is industry conscious and driven. Rearing of eggs here in New Brunswick is not currently a problem. We need to have some way of pulling things together in terms of research and technology transfer and the SGRP may be the vehicle for this. We have the "tools", technology, and interest, and are willing to take a strong role.
Pepper	How are we going to integrate people like Yves and Joanne?
Boulanger	Requirements are not great, especially for sperm. It is possible to produce large amounts of sperm from a small number of males.
Friars	You can spread milt a long way.
Stevenson	The main problem that arises between government and private interests is communication. The industry should be kept up to date regarding government policy and direction. This helps quell fears of the unknown within the industry.
Henderson	I will approach the New Brunswick Growers to take a few cages of triploids. Industry has not requested this. However, we fear we may be forced to use triploids by DFO because of concern with impacts on wild stocks. Ultimately, if consequences of aquaculture escapees are detrimental, DFO may insist on the use of triploid stocks.
Davidson	Will triploids (monosex?) in aquaculture protect the wild stocks?
Donaldson	As aquaculture salmon are selected, often after several generations, potential impacts on wild stocks are more severe. We do not yet have a comprehensive assessment but evidence to date suggests that non-maturing, anadromous salmonids will stay in the ocean until they die and will never return to fresh water. This is true of hormonally induced sterile chinook and I suspect it will hold true for triploid salmon as well.

PARTICIPANT RECOMMENDATIONS

Pepper — I would like to clarify the recommendations of the workshop and build a case for triploidy and a consensus from the workshop participants.

Jungalwalla — Open with a statement that the suppression of maturation is important.

Benfey — We should really clarify the difference between suppression of maturation and delay of maturation.

Johnstone — I suggest use of all-female triploid salmon in Newfoundland. However, you will need diploid controls in order to do a proper evaluation. You should start sex-reversing Saint John fry right now in Newfoundland. Perhaps there should be sex-reversed fry reared in the province. You will have to consider the minimum information needed to confirm the findings of this workshop. You would be wise to include the expertise of Benfey and the SGRP in designing your Newfoundland program. In considering this design, you will need to: 1) determine the minimum level effort required to produce objective information to demonstrate benefits of triploidy to the industry; 2) determine what industry wants and what government (DFO) wants; 3) distribute a technical report, of the proceedings of this workshop, to industry. This may help convince industry of the advantage of triploidy.

Ritter — What is needed in the end is:
1) A series of statements that identify the pros and cons of all-female triploids;
2) A clear statement of the strategy we want (i.e., a. all-females only, or b. all-female, triploids);
3) The steps required to take the technology from the present through to wide-scale application (e.g., research; technology transfer (testing); and, marketing the technique through demonstration and communication).
In pursuing these needs, you need to consider the pros and cons of:
- a national strategy
- an Atlantic strategy
- a Newfoundland strategy.
You also should consider what further development, through research and/or technology transfer, will be required to address the <u>HOWs</u> of the recommended strategy.

Johnstone — Concerns should be clarified:
- Newfoundland wants a viable industry
- the industry should have a minimum impact on wild stocks

Donaldson — Start with the Newfoundland problem and extrapolate both the problem and possible solutions to other areas.

Johnstone — Significant advantages need to be stated for triploidy as it relates to both of my previous points.

Pepper — We seem to advocate production of female milt and the conversion to triploidy. Given this, we have discussed different locations and their associated problems. If we define the level of interest among the workshop participants in a program for encouraging non-maturation of aquaculture salmonids, we can define its scope and framework.

Donaldson — We first need a plan for pilot scale testing.

Anderson	ASF (Friars) will develop a proposal to evaluate sex-reversal and triploidy. We will explore willingness of New Brunswick Salmon Growers to participate. SGRP, on behalf of ASF, has two interests: l) Aquaculture; and, 2) Aquaculture escapees and their impact on wild stocks.
Sutterlin	Female milt from Saint John stock could be available in Newfoundland from 0+ parr this fall.
Benfey	This being the case, Newfoundland may be ahead of the SGRP by at least a few weeks at this time. Newfoundland already may be in a position to implement its own program.
Donaldson	I advise keeping the program as simple as possible in the development phase.
Johnstone	Too big a program may just create confusion. The bottom lines for resources should be well established to avoid unreal expectations of your output.
Pepper	What I wonder about is if the St. John River stock is the way to go? Will N.S., P.E.I., and N.B. all agree to this and will it be their preferred direction?
Farmer	The N.S. industry is small. In our broodstock development program we are testing the St. John stock as well as two local (N.S.) stocks. In our tests the St. John stock exhibits a 35% grilse rate. Due to high grilse rates, triploids may be necessary if a viable industry is to develop in N.S. We need to demonstrate to the industry that it is advantageous to use triploids.
Pepper	Would a program based on the St. John stock suffice or would we need to include a local stock? It is my impression that N.S. is interested in participating. Therefore it seems likely to assume that the St. John stock would be accepted.
Farmer	I think Nova Scotia salmon farmers may prefer local stocks. It is difficult to perform research in conjunction with industry here due to the instability (financial) of the industry. While they may want a local stock, all of our tested stocks have a high grilsification rate. Therefore, they may be willing to accept St. John fish.
Pepper	What would be P.E.I.'s view?
Gallant	There is a proposal from the AVC in P.E.I. for funding to produce all-female triploid charr. There are no growout sites for salmon in P.E.I., therefore interest for that purpose would be limited. There would be interest in terms of production of triploid smolts for export and there may be interest in terms of triploid char, brook trout, and rainbow trout.
Dubé	On the Quebec side, I would like to point out that the Baies des Chaleurs Aquaculture site is a large land based facility which is well suited for holding broodstock. This facility may be very interested in participating in a proposed triploid program.

SYNTHESIS OF WORKSHOP RECOMMENDATIONS

Within Atlantic Canada, it is apparent that salmonid aquaculture is a valuable industry. In 1990, Atlantic salmon production from New Brunswick sea cages was of the order of 7,500 metric tonnes with a wholesale value in the order of $70 million (Eugene Henderson, New Brunswick Salmon Growers, pers. comm.). This fact alone indicates that salmonid aquaculture is now well established in Atlantic Canada. Of course, such industry, with its social and economic benefits, is of considerable interest to government.

Government has been anxious to encourage development of sustainable industries that build on existing skills (i.e., boat handling, fishing, farming), particularly in areas of traditionally high unemployment. Aquaculture industry development has presented just such an opportunity. While there is both opportunity and need for industrial development, there also is a concurrent requirement for regulation of industries to assure orderly and responsible development that does not undermine environmental stability or foreclose alternative development opportunities. Appropriate regulation of size, location, rate of expansion, pollution and environmental impacts, is a legitimate role of government.

PRIMARY PURPOSE OF WORKSHOP

Aquaculture industry experience, and environmental awareness, has highlighted the desirability of methods of maturity control in salmonids. Interest in this topic of maturity control has developed because of:

a) production loss due to gonadal maturation. During the maturation process, food energy is diverted from growth into gonad development resulting in both loss of market biomass and reduced quality and pigmentation of flesh. Aksnes et al (1986) 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%. They state "... odour and flavour became much less pronounced, and the texture became watery and tough.". Similar results were reported by Boulanger (this workshop).

b) reduced flexibility of marketing in terms of both quality of product and timing of market entry. As pointed out in the presentation by P. Jungalwalla on experience with Atlantic salmon in Tasmania, "The only serious drawback to the industry, of having such a high grilsing rate, is the restriction on the harvest period. The fish are not large enough to harvest before October and the onset of maturity renders the fish unpresentable to market by the following March... Where a situation arises, as in Tasmania, that virtually a whole year class attains maturity together, it poses a definite time limit to the harvest period, and incidentally an upper limit to the size of fish which can be produced economically.

c) implications of accidental release of aquaculture fish to the wild. Among the environmental concerns that must be addressed by government is the fear that aquaculture fish may escape, may interbreed with wild populations, and may thereby cause changes in genetic structure of those wild populations. It is not yet known if there are long-term consequences of such events, what form these consequences may take, or how serious these consequences might be. These concerns assume only that anthropogenically imposed genetic change in wild populations is undesirable. Data are being accumulated from around the world to address this question. As yet, these data are not sufficient to support quantitative analyses of the consequences of genetic interactions among wild and aquaculture fish. In the absence of definitive conclusions regarding genetic interactions among stocks, resource managers have adopted a pro-active yet cautious approach to salmonid aquaculture industry development. However, recognizing the magnitude and importance of aquaculture development in Atlantic Canada, resource managers are anxious to evaluate and demonstrate methods to circumvent genetic impacts between aquaculture and wild stocks.

In the event that present world endeavours to resolve the genetic impact issue result in greater restriction of introductions and transfers of salmonid species, the foresight of experimentation with methods for producing non-maturing salmonids for use in aquaculture could be critical to the growth of the industry. Suppression of maturation in aquaculture stocks is an important goal for the salmonid aquaculture industry as a means to encourage stability and future development of the industry.

REVIEW OF WORKSHOP PROCEEDINGS

As a result of the workshop presentations and the discussions that took place during the three day event, it is apparent that several methods of reproductive control have been investigated. These methods were identified, discussed and evaluated as follows:

1. Breeding - Friars
2. Surgical castration - no good
3. Hormonal sterilization - Donaldson
4. All-female stocks (uses hormones but not on those destined for human consumption) - Donaldson, Johnstone, Jungalwalla, Stevenson, Boulanger, Sutterlin, Benfey
5. Auto-immune sterilization - no good
6. Gamma irradiation - no good
7. All-female triploidisation - Johnstone, Jungalwalla, Stevenson, Boulanger, Sutterlin, Benfey

It was recognized that there are considerable gains to stock performance that can be realized through breeding programs. Such programs can be enhanced further if linked with technology to suppress maturation. As stated by Jungalwalla (this workshop), "One clear solution to the problem is to continue genetic selection for high growth rate, while suppressing maturation in part of the stock from each year class.". The option that was clearly preferred by workshop participants for suppressing maturation of salmon in Atlantic Canada was all-female, triploid stocks. Adoption of this preferred methodology requires: a) means whereby all-female stocks can be generated; and, b) means whereby they can be rendered triploid. The specific techniques appropriate for achieving this preferred option were documented by Jungalwalla (Fig. 2c, this workshop).

This workshop consensus of the preferred option is primarily technological and does not explicitly recognize the sociological factors that are critical to practical implementation, demonstration, and adoption of these technological tools by industry. A very significant theme of the workshop was voiced by Dr. Tillmann Benfey as follows:

> "What remains to be determined clearly is whether induced triploidy can be used to meet these objectives (i.e., eliminate problems associated with sexual maturation, such as decreased flesh quality and increased susceptibility to disease) without compromising the economic viability of commercial aquaculture."

PROS AND CONS OF NON-MATURING SALMONID TECHNOLOGY

All-female stocks are later maturing since males mature earlier than females. This method of delaying maturation constitutes a desired production strategy for rainbow trout to pan size and chinook to market size but is less desirable for Atlantic salmon and larger rainbow trout. This approach is not appropriate for brook trout and Arctic charr. All-female, triploid salmonids are functionally and endocrinologically sterile. This is not so for the males. Therefore, all-female, triploid stock, in which maturation is suppressed, is the preferred option for both commercial and environmental reasons.

The final day of the workshop started with a discussion of the pros and cons of the affects of induced maturity suppression among salmonids. Some of the highlights of this discussion are as follows:

<u>Pros</u>
Market yields in terms of value per unit of rearing volume per unit time in triploid stocks may be greater since more animals would be expected to grow to the larger and more valuable sizes. Their functional sterility should be of additional advantage in areas where the possible impact of accidental releases from farming operations is causing concern.

A benefit of non-maturing salmon from an industry point of view would be to solve the grilsing problem.

All-female, triploid growth was maintained for the three years of observation. Diploid females lost weight during spawning.

Improved growth of the all-female (rainbow trout) triploids, relative to the diploids, was readily apparent at 500g.

Food conversion has always been better for the triploids regardless of age. Triploids are less aggressive than diploids.

Average gutting loss was 22% for the triploid females compared to 32% for the maturing diploid females. The difference is mostly due to the gonads.

Reduced production costs, varying between $0.22 and $0.40/kg, have been obtained because of improved food conversion and lower gutting losses among triploid trout.

There was consistent and improved flesh quality in triploid relative to maturing diploid trout.

Cons

There is a much higher mortality rate among triploid eggs than among diploids, both up to the eyed stage and thereafter until hatching.

There is an increased incidence of deformities among triploids.

Although we feel that the techniques for producing female triploid rainbow trout are commercially applicable at this time, the existing literature and unpublished experience in Canada prior to this workshop would indicate that a transfer of comparable technology to produce an"environmentally friendly" salmon proceed at a systematic but cautious rate.

It must be emphasized that experience with technology for the production of non-maturing salmonids is not consistent among locations at which it has been applied. The workshop heard primarily positive evaluations from Quebec (Y. Boulanger, Pisciculture des Alleghanys Inc.) and negative interpretations from Newfoundland (A. Sutterlin, Bay d'Espoir Salmon Hatchery Ltd.). It is apparent that these discrepancies have yet to be resolved. As pointed out by Ray Johnstone:

> "Because of predominant problems of male triploids, Canada should work exclusively on all-female stock. This is an obvious thrust that will have guaranteed benefit. If you go the route towards triploid lines, you must decide on what techniques provide the advantages you need. You can explore pressure treatment easily as relatively simple technology but you need to be aware that there are differences between investigators as well as differences in the apparatus used for pressure shocking (i.e., Tasmanian features vs Scottish features)."

Sufficient expertise exists in Canada to address research and technology demonstration needs. As a result of these workshop proceedings, we have the base from which to design a program and to implement the desired option of all-female, triploid stock for extensive testing at aquaculture locations throughout Atlantic Canada. This program should concentrate on achieving a meaningful scale of production in order to enable meaningful industrial evaluation. While research efforts have been proceeding with this technology, the present scale of this research effort is too small to provide an effective demonstration of the advantages or disadvantages as they relate to the fish farmer.

REGIONAL STRATEGIES

Throughout the workshop discussion sessions, it was apparent that participants accepted that there are definite advantages to the aquaculture industry to be gained from all-female, non-maturing stocks of salmonids. While this typically was expressed as a cautious optimism, the emphasis clearly was on demonstration of the technology and education of the potential industry recipients of the technology. As a result, the consensus was that, while centralized facilities might be used initially to produce the fertilized eggs for use by the salmonid farmers, this rearing would have to be done by the farmers themselves so that individual growers could come to their own conclusions about the performance of non-maturing salmonids relative to the normal aquaculture stocks. Advice to the workshop by Dr. Johnstone, based on his experience with the Scottish salmon farming industry, was that performance demonstration should take place only at selected farms where the farm operators have a well documented history of success. Having once established the credibility of the farm, performance evaluations should be done systematically so that all-female, triploid salmonids are evaluated concurrently with both mixed sex and all-female diploid stocks. Diploid and triploid stocks should not be mixed within the same cage but rather, wherever possible, several cages of each ploidy designation should be farmed in the same location under identical farming conditions. Routine, scheduled and systematic data should be collected from random samples of fish in each of the experimental units and should include: quantitative variables such as weight, fork length, mortality, and food conversion; qualitative information such as fish behaviour, appearance, and the frequency of physical abnormalities among triploids vs. control groups; and environmental data such as water temperature and salinity.

Workshop participants recognized that there are likely to be regional differences in the need and interest in such experimental programs as well as different attitudes among growers within regions. While it would be desirable to have an integrated Atlantic Canada program of experimentation with techniques and demonstration of the benefits of this technology to industry, it was concluded that the logistics of such a program would be formidable. Accordingly, the design and implementation of programs, in support of refining technology and involving industry in experiments and performance evaluations, was left to the regions to pursue in light of their other regional priorities. It was concluded that the proceedings of this workshop will be used as substantiation of the need for further work with all-female, triploid stocks of salmonids for aquaculture and that independent initiatives would be pursued within each region to assure resolution of regional issues and brood stock priorities. As stated by Dr. John Anderson of the Atlantic Salmon Federation (ASF):

> We need to have some way of pulling things together in terms of research and technology transfer and the SGRP may be the vehicle for this. We have the "tools", technology, and interest, and are willing to take a strong role... The ASF will develop a proposal to evaluate sex-reversal and triploidy. We will explore the willingness of the New Brunswick Salmon Growers to participate. SGRP, on behalf of ASF, has two interests: l) Aquaculture; and, 2) Aquaculture escapees and their impact on wild stocks.
>
> The SGRP is industry conscious and driven. Rearing of eggs here in New Brunswick is not currently a problem. We need to have some way of pulling things together in terms of research and technology transfer and the SGRP may be the vehicle for this. We have the "tools", technology, and interest, and are willing to take a strong role.

This is consistent with pilot scale work already being actively pursued between the Atlantic Salmon Genetics Research Program (Dr. G. Friars) and the University of New Brunswick (Dr. T. Benfey). The "interests" expressed by Dr. Anderson are consistent with those of the Newfoundland Region as expressed in this workshop and serve as a model for further government/industry dialogue.

A NEWFOUNDLAND PROGRAM

It is apparent that an adequate program of experimentation and performance testing must be done in close consultation and liaison with industry. Not only are there potential environmental benefits to be gained, there may be significant economic gains to industry as well. As reflected in the statement by Boulanger (this workshop), "Reduced production costs, varying between $0.22 and $0.40/kg, have been obtained because of improved food conversion and lower gutting losses among triploid trout.".

Minimum requirements for performance testing will depend on the number of sites at which salmon are being farmed. The minimum number of diploid and triploid female smolts required for comparative performance evaluation in Newfoundland is 12,000 to 16,000 in keeping with present experiments with performance of local vs imported salmon stock. While the following outline may be of interest to other regions of Atlantic Canada, this framework is intended primarily as a focus for discussions among salmonid aquaculturists and regulatory agencies in Newfoundland.

The performance of all-female, triploid salmon is of particular concern in Newfoundland where marine environmental conditions often are marginal for salmonid farming, especially during the winter. The following statements by Dr. Benfey (this workshop) emphasize the need for careful experimental design:

> "... triploid salmonids frequently are said to perform poorly in aquaculture, especially when reared under sub-optimal conditions or when kept in mixed groups with diploids. Apparent differences between triploids and diploids may be due to behavioural and sensory problems rather than due to direct physiological effects."

All-female Methodology

1. The currently preferred methodology for producing homogametic milt is androgenization of feminized stocks. This would require hormone treatment of an all-female group of fry. All-female lines have not yet been developed in Newfoundland, thereby necessitating an alternative approach for 1991.

2. The alternative option of androgenization of mixed sex stocks has started in Newfoundland. The first homogametic milt is expected to be available from precocious parr in 1991. A systematic program of androgenization could result in the following:
    1992 - first homogametic grilse milt; and,
    1993 - first homogametic salmon (i.e., two sea-year) milt.

In light of potential crises regarding the use of non-local stocks for salmonid farming development in Newfoundland, the emphasis here is that we cannot afford to wait for the most desirable cross (i.e., 1993 homogametic salmon milt x salmon eggs). If funds are available, it is recommended that the Newfoundland industry proceed with fertilization of androgenized precocious parr x salmon eggs in 1991. This will require extreme caution to assure that normal XY males are removed from the sex-reversed pool. The importance of this was stressed by Boulanger (this workshop), "When the triploidy method is used, it is essential to use all-female stock in order to avoid growth and behaviour problems related to the sexual maturation of males.". While the Y-specific, DNA probe technology being developed for Atlantic salmon by Dr. R. Devlin (DFO, CODE for Biotechnology & Genetics, West Vancouver Laboratory) likely would be the preferred means to assure XY males are removed from the broodstock, this technique may not be available in time. In the absence of this technology, the methods of Jungalwalla (this workshop) may have to be used. This procedure follows:

> "Each presumptive donor (i.e., treated fish showing all-male secondary sexual characteristics) is squeezed, and accepted only if no milt is expressed. If upon dissection the testes appear normal, the fish is again rejected. Only if the testes are lobulated is the fish accepted. The presence of only one lobulated testis, and/or the presence of a vestigial ovary, increases confidence that the donor is indeed a masculinized female."

All-female fry would be first fed in 1992 to produce S1 smolts in 1993, grilse in 1994, and salmon in 1995. A grilse hermaphrodite cross would be a good idea and could be done in 1992.

The recommended precaution of testis examination should be satisfactory in the early generations of a sex-reversal program to generate all-female lines. However, once 3N salmonids are being produced for growout, it may become important to assure that all-female lines for sex-reversal, to produce homogametic milt, do not inadvertently harbour any 3N males. This could happen if diploid males escape detection from the initial stages of androgenization of mixed sex stocks as recommended above. If individual sperm donors can not be scanned for ploidy status by flow cytometry, manual, microscopic examination of blood cells is recommended. Yves Boulanger (pers. comm.) recommends the following procedure:

- cut the gill arch of the presumptive donor;
- secure a blood sample with a pasteur pipette;
- smear the blood sample on a microscope slide;
- stain the smear with Wreight's stain (usually available from suppliers of biological reagents);
- examine at 1000X magnification;
- find a field where the cells are spread uniformly with little overlap;

- measure nucleus diameter. While measurements can be quantified with an ocular micrometer, interpretation also can be done subjectively by comparing the sample nucleus size with the normal 2N nucleus. 3N nuclei are markedly larger than 2N nuclei.

Triploidisation

Newfoundland does not have the equipment to undertake a program to induce triploidy. However, there are still options that should be pursued to implement experiments in 1991. This should not be an insurmountable obstacle. Dr. Johnstone (pers. comm.) suggests that, "...using a two litre vessel, 50,000 ova per hour can be rendered triploid and 100% triploid rates at 90% survival rates, relative to controls, can be achieved. Larger vessels (10 litre) could be manufactured and the pressure method, though costly in relation to heat, should not therefore be rate-limiting to stripping and fertilization operations at most farms. The high equipment cost, when seen in relation to the expected value added to treated ova, if yields are consistently good, is likely to be economic. This is especially appropriate in species such as Atlantic salmon where egg costs are high.". While it may be possible to secure a pressure vessel in 1992, it is likely that Newfoundland salmon farmers will wish to import St. John River stock again in 1991. An arrangement should be pursued, with the Salmon Genetics Research Program, wherein Newfoundland would import both diploid and triploid Atlantic salmon eggs, subject to compliance with the Canadian Fish Health Protection Regulations. An alternative might be the transfer of gametes (homogametic milt and/or diploid eggs) to which pressure shock would be applied subsequent to fertilization at the receiving site. It was apparent during the workshop that Drs. Friars and Benfey were receptive to this collaboration although Dr. Benfey indicated that he will need a larger pressure vessel (2 to 4 litres) to participate in an expanded program. Should the logistics of gamete transfer prove insurmountable, the remaining option would be to use local grilse stock for the initial experiments.

SPECIES OF INTEREST

In order of importance, the Newfoundland aquaculture industry should concentrate on development of non-maturing stocks for: 1. Atlantic salmon; 2. rainbow trout; 3. Arctic charr; and, 4. brook trout. In the absence of essential means to implement a program on Atlantic salmon, rainbow trout could be used as an example of the benefits of this technology. Advice from the workshop indicated that "An outline of research requirements should be included in the proceedings of this workshop.".

OUTLINE OF RESEARCH REQUIREMENTS

It is important to realize that industry has voiced its opinion as follows (Eugene Henderson, this workshop):

> "It is important that research be done and results evaluated before industry is pressured to rear such sterile fish. Bear in mind, that this is perceived as a non-problem by the industry (in New Brunswick) and producers are content with the performance of the fish now being used."

In consideration of this attitude, critical research on non-maturing salmonids should be pursued as follows:

1. A reliable method of confirming a homogametic genotype (i.e., XX sperm) is essential. Consequences of inadvertent occurrence of male triploids are too great to be ignored.

2. Evaluations of physical abnormalities, disease susceptibility, and performance of triploid stocks relative to diploid stocks.

3. Demonstration of the performance of triploid stocks under local salmonid farming conditions. This will require concurrent use of diploid and triploid stocks.

4. Perfection of simple, cost effective techniques for cryopreservation of homogametic milt so that the genetic potential of the sex-reversed sperm donors is can be preserved for breeding programs.

WORKSHOP CONCLUSION

While, "Modified organisms should be evaluated on both their genotypic and their phenotypic characteristics..." (Devlin and Donaldson 1990), and it is desirable for the demonstration phase of the work to take place concurrently at selected salmon farms throughout Atlantic Canada, regional programs first must be designed to meet regional needs. Having once ensured that regional needs have been addressed through adequate government/industry consultation, the feasibility of integrating regional programs into Atlantic Canada initiatives and/or National strategies can be discussed.

These Proceedings of the Atlantic Canada Workshop on Non-Maturing Salmonids constitute a first step towards dialogue with industry. The potential consequences, of the accumulating body of information on impacts of aquaculture salmonids on wild stocks, are not to be taken lightly. In the presence of considerable uncertainty about the future use of preferred brood lines for salmonid farming, further work with non-maturing salmonids is strongly advised.

The DFO regions of Atlantic Canada are represented on local salmonid brood stock committees. It is through these committees that industry representatives should express their views and attempt to design a workable program of collaboration, as well as an appropriate experimental design, that will address the needs and concerns of salmonid farmers and resource managers alike. Within Atlantic Canada, there are sources of funds that might be applied towards government/industry initiatives to demonstrate and refine approaches to incorporating non-maturing salmonids into present farm operations. Some funding programs are intended to support industrial development and long-term stability of Atlantic Canada industry. The potential future stability of salmonid aquaculture, as well as economic gains to the industry, may be enhanced greatly by present investment in this technology.

Having once realized significant economic gains from suppression of salmonid maturity, industry is likely to proceed with wide spread and intensive application of this technology. Herein lies a potential threat if adequate precautions are not taken to assure that absolutely no 3N males are produced that might escape to natural habitats. This requires standardization of procedures and may necessitate the regulatory role of government to assure compliance with standards that guard against triploid males. Ultimately, it may be desirable for industry to specialize so that the higher technology required for effective production of all-female, triploid lines is concentrated in a few, certified disease-free facilities.

This workshop has provided an objective base from which to contend that immediate business decisions should be made to incorporate technologies for the production of non-maturing salmonids into present farm operations. There may be significant market and environmental gains to be made.

ACKNOWLEDGMENTS

Participant comments on completion of the workshop indicate that the event was a success and that a lot of very productive discussion served to convey a much greater understanding of this technology among the participants. The apparent success of the workshop was due primarily to the interest of the participants (Appendix 1). I would like to thank those who attended, those who functioned as rapporteurs, those who reviewed the workshop papers, and particularly those who presented papers. Dr. Ray Johnstone provided me with many helpful suggestions on the text for the section of this report on Synthesis of Workshop Recommendations. His comments have been very helpful. I also would like to thank the Department of Fisheries and Oceans for accessing funding for the workshop from the Aquaculture Science component of the Atlantic Fisheries Adjustment Program, and my colleague, Terry Nicholls for his assistance in assembling this manuscript.

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APPENDIX 1

ATTENDANCE LIST: ATLANTIC CANADA WORKSHOP ON METHODS FOR THE PRODUCTION OF NON-MATURING SALMONIDS

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