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Series on Harmonisation of Regulatory Oversight in Biotechnology No. 39

ABSTRACTS OF THE OECD WORKSHOP ON THE BIOLOGY OF ATLANTIC SALMON (Salmo Salar)

Held in Moscow, Russian Federation, 29 November -1 December 2004

English - Or. English

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Series on Harmonisation of Regulatory Oversight in Biotechnology

No. 39

Abstracts of the Moscow Workshop on the Biology of Atlantic Salmon (Salmo Salar)

Moscow, Russian Federation
29 November -1 December 2005

Environment Directorate

Organisation for Economic Co-operation and Development

Paris 2006

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FOREWORD

Background

The OECD's Working Group for Harmonisation of Regulatory Oversight in Biotechnology organised an OECD Expert Workshop on the Biology of Atlantic salmon (*Salmo salar*). It was held in Moscow, 29 November - 1 December 2004, and was hosted by Germany and the Russian Federation. This was the first occasion for the Working Group to address environmental safety issues related to animals. There were 40 participants from 13 countries, which ensure the participation of experts from key member and non-member countries (Chile, China, India, Russia and Tanzania), who have had leading experience with Atlantic salmon.

Objective of the Workshop

The main purpose of this workshop was to take the first steps in considering whether the general approaches which have been used in the past by the Working Group, primarily to address the safety/ risk assessment of transgenic plants, could be applicable to similar work on the safety/ risk assessment of transgenic fish.

The objective of the workshop was to identify and review the kinds of (and availability of) baseline information of (non-transgenic) from traditional fish farming or breeding, and to determine what information might be relevant to risk/ safety assessment; and what information might be needed for the development of a biology document. The overall approach used by the Workshop was similar to that done to first identify and circumscribe the issues about crop species that would inform a risk/safety assessment of the same species after it had been transformed.

The Working Group decided then to work on the Atlantic salmon species for the following reasons:

- As the concept of familiarity allows risk assessors to draw on previous knowledge and experience available with the non-transformed species and to compare with species whose biology is well understood, Atlantic salmon may be chosen since they constitute a "familiar" species.
- There is an abundance of data available on Atlantic salmon in general.
- There has been plenty of research on the biology, distribution and ecology of wild type Atlantic salmon, because their populations are currently at risk.
- The commercial value of farmed and wild Atlantic salmon products is high.
- They are one of the main fish products originating from OECD countries.
- Atlantic salmon has been the subject of a transgenic application (growth enhancement).

Consequently, the workshop addressed many issues related to the biology and ecology of untransformed Atlantic salmon that could provide information on the risk/ safety assessment of a transgenic fish of the same species. At the same time, the workshop took into account recent developments in modern biotechnology, especially applications involving transgenic fish, to ensure that the information identified on the biology of salmonids, is relevant to the types of applications and traits envisaged. The experts presented a wide range of information on the biology and ecology of Atlantic salmon, as well as details of recent developments in salmon breeding and aquaculture.

Outcome

On the basis of the information identified at the workshop, the Working Group was able to determine the level of familiarity existing with current documents and decided that work in the area of fish biology should proceed. During the 16th meeting of the Working group (February 2005), the Working Group agreed that the Steering Group start to prepare an Operational Plan for drafting a consensus document on the biology of *Salmo salar* (Atlantic salmon).

This text is a summary of the Workshop discussions. It contains the abstract of the various presentations. This document is published under the responsibility of the Secretary-General of the OECD. The opinions expressed in this document are those of the participants to the workshop and do not necessarily reflect the official views of the Organisation or of the governments of its member countries.

ABSTRACTS OF THE PRESENTATIONS

1. Characterization of Wild Atlantic Salmon

Dr. Susanna PAKKASMAA
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Sweden.

The Atlantic salmon *Salmo salar* was named by the Swedish biologist Carl von Linné (Linnaeus), who called salmon "the king of fishes". Taxonomically, it belongs to the phylum Chordates, class bony fishes, order Salmoniformes, family Salmonidae and genus *Salmo*. Salmonids evolved during the Pleistocene about 100 million years ago. The family Salmonidae includes the Atlantic and Pacific salmon, the trout, the charr, the grayling and whitefishes. The main distribution of Atlantic salmon is northern including the east and west coasts of the North Atlantic Ocean and the Baltic Sea. Genetically the largest difference is between European and North American populations, but also Eastern Atlantic and Baltic populations are clearly different. Genetic variation is influenced by historical events and differentiation after the last glaciation, recolonization from nearby rivers, release of hatchery-reared fish, and random genetic drift causing differentiation in small populations. There is considerable variation in ecological and life history traits within and between populations. Local populations, that are often adapted to their native rivers, have important conservation value.

The Atlantic salmon and the brown trout (*Salmo trutta*) are morphologically very similar and may be easily confused, particularly because they often occur in the same areas. Juvenile Atlantic salmon has a small mouth (upper jaw does not reach the rear of the eye), pointed head, long pectoral fins, narrow tail stalk and deeply forked, sharp-ended tail, whereas juvenile brown trout is more robust and has blunter head and tail. The differences get more pronounced with growth and ontogeny, and Atlantic salmon has a more fusiform body and fewer spots than brown trout. Hybridization between the two species may occur, often as a consequence of human activities, such as stocking and translocation.

Atlantic salmon reproduces in freshwater in rivers and spends its juvenile stages there, but the main growth occurs in the sea, or in the case of landlocked populations, in lakes. The freshwater phase lasts 1-6 years, and at the end of it the fish go through smolting, which involves changes in physiology and appearance, and prepare for the seaward migration. The sea phase lasts 1-4 years. The distribution in the sea is influenced by temperature, surface currents, genetic factors and food availability, but there is still relatively little information about the behaviour, feeding and predation in the sea. With the help of homing behaviour, the fish return to the natal river for spawning. Most fish return to their natal rivers, but straying creates gene flow between nearby populations. Some males adapt an alternative life cycle and reach sexual maturity at very small size, without migrating to the sea.

Wild stocks of Atlantic salmon have declined dramatically during the last decades. For instance, salmon inhabit only 20 of the original 80 rivers in which they were previously found that drain into the Baltic Sea, and many wild populations are threatened. Farming of Atlantic salmon is extensive in Europe, North and South America, and it produces fish both for stocking and for food. In Sweden 2.1 million Atlantic salmon smolts are stocked annually. There are both ecological and genetic concerns related to aquaculture, and the erosion of natural gene pools through interbreeding with farmed fish is considered as a major threat for the wild populations.

We have plenty of knowledge about Atlantic salmon because of its importance for fisheries, angling and aquaculture. However, there are still gaps in knowledge in both evolutionary issues and conservation and management related issues.

2. Centres of Origin, Geographic Distribution, and Conservation of Genetic Resources I

Dr. Timothy KING
US Geological Survey
United States

In eastern North America, the Atlantic salmon was historically found in river systems and marine waters along the Atlantic coast from the Hudson River drainage in New York, USA north through the Gulf of St. Lawrence and around the whole coast of Newfoundland and along the Labrador, CA coast to the Fraser River. The species' range has generally contracted and fragmented over the last century and a half; due largely to industrialization and adverse water management, particularly in the more southerly parts of the species' range where human activity is concentrated. Anadromous Atlantic salmon undergo extended ocean migrations but exhibit a high homing fidelity to their natal river or tributary. The substantial reproductive isolation between populations fostered by such precise philopatry has facilitated the evolution and persistence of local adaptation. For instance, the number of winters spent at sea varies latitudinally in North America, with US and southern Canadian stocks comprised primarily of multi-sea-winter salmon while the more northerly Canadian stocks consist of one-sea-winter fish (grilse). Even as most North American Atlantic salmon migrate to feeding grounds in the Labrador Sea off Greenland, those spawning in rivers of the inner Bay of Fundy tend not to leave the bay, and furthermore tend to spawn as grilse rather than staying at sea for multiple winters, as do most fish from that latitude. The persistence of variation at these genetically based life history traits suggests that gene flow between stocks has been limited. For more than a century, the principal management tool directed toward Atlantic salmon in Maine, USA has been supplemental stocking with fish from larger but often distant populations. Prompted by diminishing spawning runs and low juvenile densities, U.S. resource managers have recently designated Atlantic salmon in seven Maine rivers as an endangered species under the U.S. Endangered Species Act. Canada is considering similar protections for salmon inhabiting the Bay of Fundy. The increased use of hatchery-reared Atlantic salmon for supplemental stocking and for commercial aquaculture in this region of the species' range underscores the need to characterize the genetic composition of both wild and captive populations. Moreover, proposals to introduce genetically engineered salmon to areas near these critical habitats raises the concern that genetically modified fish may serve to compound the existing threats to wild stocks. The concern is that certain genetic engineering targets traits, which if released into the wild, pose the risk that negative ecological consequences such as selective advantage (*e.g.*, faster growth, greater threat of predation) could result leading to increased (even if only temporary) fitness. Engineered organisms may pose additional risks where a lack of experience exists with a trait (*e.g.*, increased growth) or DNA construct (*e.g.*, an inhibitor) and its interaction with the target organism. Genetic exchange among populations (*i.e.*, introgression) is also a possibility. Given that Atlantic salmon aquaculture facilities are located at some outlets of salmon producing rivers in Maine and in the Bay of Fundy, CA, and that the addition of genetically engineered salmon to aquaculture operations there could have serious additional effects on wild salmon stocks, more research into the possible consequences of such actions is required.

3. Centres of Origin, Geographic Distribution, and Conservation of Genetic Resources II

Dr. Øyvind WALSØ
Directorate of Nature Management
Norway

Status of stocks of Atlantic salmon Historically, there were 2,615 rivers with Atlantic salmon. In 2000 sufficient data existed to categorize 2,005 of these. The categorization showed that four countries (Iceland, Ireland, Norway and Scotland) host approximately 90% of the remaining healthy rivers with Atlantic salmon in the world In the rest of the countries with salmon bearing rivers the majority of the rivers were threatened (vulnerable, endangered, critical and/or extinct). Wild Atlantic salmon have disappeared in Germany, Switzerland, the Netherlands, Belgium, the Czech Republic and Slovakia.

In the report from The International Council for the exploration of the Sea (ICES), Advisory Committee on Fishery Management and Advisory Committee on Ecosystems, regarding the status of stocks of Atlantic salmon in Europe for 2003 it is considered that (i.)Northern European (Finland, Iceland, Norway, Russia and Sweden) one-sea winter (1SW) stocks are not within safe biological limits (BL), while multi-sea winter (MSW) stocks are within safe BL, and that (ii.) both the Southern European (France, Ireland and UK) 1SW and MSW stocks are not within safe BL. Therefore, with the exception of the Northern European MSW stock, these stocks are considered to be outside safe BL.

North Atlantic Salmon Conservation Organisation North Atlantic Salmon Conservation Organisation (NASCO) is the international organisation established under the Convention for the Conservation of Salmon in the North Atlantic Ocean. Its objective is to contribute through consultation and cooperation to the conservation, restoration, enhancement and rational management of salmon stocks taking into account the best available scientific advice. NASCO is divided in three Regional commission areas, North American (Canada and USA), West Greenland (Denmark, Canada, USA and EU) and North-East Atlantic (Denmark, EU, Iceland, Norway and Russia).

From the early 1990s NASCO has been focusing on environmental impacts from Aquaculture. The first resolution according this topic was adopted in 1994. This resolution was replaced by, what is called The Williamsburg Resolution (CNL(04)54) which was adopted in 2003 and amended in 2004. Two of the focus areas in the resolution are to (1.) minimise impacts of aquaculture and introductions and transfers, and to (2.) protect against potential impacts from transgenic salmonids on wild salmon stocks.

1. To minimise impacts of Aquaculture and Introductions and Transfers each Party shall take measures to:

   - Minimise escapes of farmed salmon to a level that is a close as practicable to zero through the development and implementation of action plans as envisaged under the Guidelines on Containment of Farm Salmon;

   - Minimise impacts of ranched salmon by utilizing local stocks and developing and applying appropriate release and harvest strategies;

- Minimise the adverse genetic and other biological interactions from salmon enhancement activities, including introductions and transfers;

- Minimise the risk of transmission to wild salmon stocks of diseases and parasites from all aquaculture activities and from introductions and transfers.

Movements into a Commission area of reproductively viable Atlantic salmon or their gametes that have originated from outside that Commission area should not be permitted.

2. The Parties should apply the Guidelines[1] for Action on Transgenic Salmon to protect against potential impacts from transgenic salmonids on wild salmon stocks. In view of the current lack of scientific knowledge on the impact of transgenic salmonids on wild salmon stocks, the use of transgenic salmonids should be considered a high-risk activity. There should be a strong presumption against any such use.

Gene banks In 1988 it was recommended to establish brood stock stations, so-called “living gene banks”, for Atlantic salmon” in Norway. The main purpose was to establish a living reservoir for genetic material which could be used for the re-establishment or enhancement of threatened stocks. This should be a measure only for the most seriously threatened Salmon stocks. The Directorate for Nature Management today operates two living gene banks. The threats to the stocks that are kept in these stations are hydropower development, acidification, high proportion of escaped farmed salmon, and the freshwater parasite Gyrodactylus salaris. G. salaris is an introduced species to Norway, and soon after an introduction to a salmon river the Salmon stock in that river is threatened by extinction.

Of the 24 salmon stocks that are, or have been, taken care of in the gene banks, eight have been reintroduced to their rivers and six of them are taken out from the gene banks. Two are kept for safety reasons. Ten stocks are under restoration, while five stocks waiting to be restored after eradication of *G. salaris* from the rivers.

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[1] NASCO Guidelines for Action on Transgenic Salmonids CNL(04)41

THE PARTIES to NASCO are aware of the development of transgenic salmonids. While there may be benefits from the introduction of such salmonids if, for example, they could not interbreed with wild stocks the Council recognises that there are also risks which may lead to irreversible genetic changes and ecological interactions.

The Council considers that there is an urgent need to take steps to ensure the protection of the wild stocks and has therefore agreed to cooperate to develop means such that transgenic salmonids cannot impact upon wild salmon stocks. The following specific steps are agreed.

The Parties will:

a) advise the NASCO Council of any proposal to permit the rearing of transgenic salmonids and provide details of the proposed method of containment and other measures to safeguard the wild salmon stocks;

b) take all possible actions to ensure that the use of transgenic salmonids, in any part of the NASCO Convention Area, is confined to secure, self-contained, land-based facilities;

c) inform their salmon producers of the potentially serious risks to wild stocks of this development and consult with the salmon farming industry on this matter through the Liaison Group established between NASCO and the international salmon farming industry;

d) take steps, as appropriate, to improve knowledge on the potential impacts of transgenic salmonids on the wild salmon stocks and their habitat;

e) examine the trade implications associated with transgenic salmonids in accordance with World Trade Organization Agreements and other instruments of international law.

Furthermore, those Parties to NASCO that are also Parties to the Cartagena Protocol on Biosafety to the Convention on Biological Diversity should take into account the provisions of that Protocol.

4. Ecology of Wild Atlantic Salmon I: Fresh Water Stages

Dr. Jörg SCHNEIDER
Bureau for Fish-ecological Studies BFS
Germany

Atlantic salmon is an anadromous species. The freshwater habitats of juvenile salmon are small streams and a few open and land-locked lakes on the western and eastern North Atlantic coasts. Land-locked populations are the remains of the last glaciation. They occur in some northern lakes and in some rivers. Not all land-locked stocks are “locked”- some do have access to the sea. Preferred spawning areas of salmon are upper reaches of fast flowing rivers including smaller tributaries with temperatures not exceeding 25-27°C. The distribution in North America reaches from Labrador to New England and Maine, and in Europe from Arctic Russia to Portugal (incl. Baltic region). In this wide distribution range significant differences in biotic and abiotic factors are affecting freshwater life and have led to river-specific adaptations.

Homing to the natal rivers (or even stream sections) salmon have left as smolts is the key factor to adaptation and ecologic variability. Homing is very accurate, with 1-5% of the spawners being strayers, mostly from neighbouring rivers (= low genetic distance). *Effective* straying normally does not occur frequently enough to break down genetic differences among populations. Homing can therefore be seen as geographical isolation through differences in migratory behaviour, leading to populations with different genetic composition. Genetic integrity is maintained by low strayer rates, and superior reproductive success of the adapted (local) strain. Homing allows adaptation to river specific conditions such as temperature (spawning time), flow, distance to the sea (migration time), predators (such as mammals, birds, fish and reptiles) etc.

Salmon are flexible and variable in their migration patterns in fresh water - temperature and season (spawning time) seem to be the governing factors. Most salmon return as one or two sea-winter-fish. Smoltification is the metamorphosis of the young to a pelagic marine fish. The process is mainly size dependent. The proportion of early smolts therefore correlates with density and productivity of the stream. Smolt age varies between 1 and 7 years within the distribution range. Timing of smolt run depends on river and sea temperature (March - August), with short suitable periods in the northern range.

Spawning sites are often located at the head of riffles or rapids. The female usually digs a 10 to 30 cm deep redd, deposits the eggs in the gravel (while one or more males fertilize the eggs) and covers them with gravel. Often several non-anadromous precocious males (with only 6-25 cm body-length) participate in the spawning. They often have a very high reproductive success, which has implications for the genetic structure of the populations. The benefits of the variations in mating strategy for salmon populations are yet not fully understood.

Spawning time has a high heritability in salmonid fishes. The timing correlates with water temperature during incubation - spawning time between “cold river“ and “warm river“ populations differs by 5-6 months. In large river systems spawning in upper (colder) reaches may occur several weeks earlier than in lower reaches.

Incubation, hatching and absorption of the yolk sac takes place some 10 - 30 cm deep in the gravel. Under normal conditions mortality at this stage is low (< 20 %). When absorption of the yolk-sac is almost completed, the fry emerges from the gravel bed starting to feed. Mortality rates are very high (68-88 % in the first 17-28 days) due to displacement, starvation and predation. Incubation and first-feeding are periods of intense selection. In this context adaptive temporal variation of spawning is the key factor for optimizing egg and fry survival. A female's spawning time dictates the thermal regime her embryos experience during development and to a large extent, their hatching and emergence time from gravel as fry. The timing of reproduction ensures optimal timing of hatching and initial feeding for the offspring. Optimal timing of hatching depends on flow conditions, temperature (limit ≥ 7°C), prey abundance, predator abundance, inter- and intra-specific competition (prior residence effect). Wrong timing leads to mass mortality in the first weeks after emergence due to starvation, predation, drift etc. - which explains the high heritability of population-specific spawning periods. Interference of salmon escapees on this level is therefore considered to be a major threat to small natural populations by reducing their reproductive success and/or larval survival.

5. Ecology of Wild Atlantic Salmon II: Salt Water Stages

Dr. Lars Petter HANSEN
Norwegian Institute for Nature Research (NINA)
Norway

Atlantic salmon are distributed over large areas in the North Atlantic, and the species is known to undertake long migrations. The distribution of salmon in the sea is probably dependent on several environmental factors like surface temperature and surface currents. Furthermore, the oceanic distribution of salmon may probably also be determined by genetic components, for example that salmon have developed navigation systems that bring the fish to the right place at the right time, and thus maximize fitness. Salmon spend most of its time pelagic in the ocean close to the water surface, and prey on different pelagic animals like crustaceans, fish and squid. Several authors have suggested that Atlantic salmon are opportunistic feeders, but selective feeding has also been described. The abundance of Atlantic salmon in the Atlantic has declined considerably in recent years. It has been shown that marine mortality accounts for a significant proportion of the decline, and is associated with a decline in the temperature to which post-smolts are exposed during the first months at sea.

There are many factors thought to influence marine mortality of salmon. Although it has been suggested that the highest mortality takes place at the post-smolt stage, there are also indications that heavy mortality may occur later in the life-cycle. Important sources of post-smolt mortality are predation, infestations of parasites and diseases, influences from freshwater life as well as synergistic effects between these and a number of other factors. Furthermore, salmon may respond to changes in the ecosystem by altering their life histories, which may be seen by, for example, changes in growth rates, sea age at maturity and seasonal return pattern.

With the development of salmon farming escaped fish from the farms are observed in fisheries and spawning populations. There are high proportions of farmed salmon in Norwegian coastal commercial salmon fisheries. The proportion of farmed salmon in oceanic areas north of the Faroe islands have been estimated to be high. Results from monitoring salmon fisheries and populations in Scotland and Ireland have suggested a much lower proportion of farmed salmon in that area, but different methods used in the assessments as well as different geographical locations of the farms relative to salmon rivers makes it difficult to compare the figures with Norwegian and Faroese estimates. Escaped farmed salmon have also been observed in fisheries and populations in USA and Canada.

Wild salmon leave their home rivers as smolts in the spring and move quickly into oceanic areas. Results from smolt tagging experiments and post-smolt surveys have strongly indicated that ocean currents are the vectors that force the fish northwards. Wild salmon normally stay in the ocean for 1-4 years before they become sexually mature. The homeward migration may be divided in two phases, an oceanic phase with fast movement from the ocean to coastal areas, and a slower migration from coastal areas to the natal river. Hatchery-reared salmon released as smolts in freshwater in the spring have a similar migratory pattern to wild salmon. Smolts imprint, or learn cues important to recognizing their natal streams, sequentially on their way from the river to the sea, and use that information for homing on the return migration. Information on survival and migratory pattern of farmed salmon from tagging experiments in Norway has indicated that fish escaping at smolt stage tend to survive relatively well, return to the same area from where escaped and enter local rivers in that area to spawn. Salmon escaping in the autumn appear to survive poorly to sexual maturation, whereas salmon escaping in winter show somewhat higher survival. Survivors tend to enter rivers far away to spawn. Escaped large salmon tend to move with the current, and do not appear to have a homing instinct. Based on this it can be speculated that farmed salmon escaping from one geographical region may enter freshwater in other geographic regions to spawn.

6. Domestication, Fisheries Practices/Breeding

Dr. Audun NERLAND
Institute of Marine Research
Norway

Farming of Atlantic salmon in Norway started around 1970. In 2003 the production was more than 500 000 tonnes, comprising more than 30% of all exports of fish products from Norway. There are two aspects that have to be considered in order to explain the success of this industry - improvements of the management and improvements of the genetics of the fish.

Management includes selection of sites for farming, technical equipments like sea-cages and feeding systems, feed quality, vaccines and vaccination regimes, transport and slaughter.

Biological research activities on the different life stages of the fish including endocrinology, digestion, maturation and immunology have created valuable knowledge, which again enables optimisation of the management.

Traditional breeding programs focusing upon traits like growth, feed utilization, disease resistance, have resulted in fish more adapted to farming and given more efficient production.

However, biological studies (including molecular biology) have also provided information that has the potential to change the genetics of the salmon by genetic engineering, including transfer of genes from other species. As these techniques enable genetic changes that maybe never would happen in nature, safety aspects require special attention. The safety consideration would have to include the “food safety” for the consumer and ecological impacts for the environment.

As the definition of GMO is organisms “in which the genetic material has been altered by means of gene or cell technology”[2] that may include changes from a single nucleotide to anything else. It will therefore be impossible to say anything in general about the safety of genetically engineered salmon. Testing and evaluation will have to be performed on a case-by-case basis.

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2. The Act relating to the production and use of genetically modified organisms (Gene technology Act) (Act No 38: 1993) Norwegian Ministry of Environment.

7. Uses and Ecological Impact of Net-Pen Aquaculture and Ocean Ranching of Atlantic Salmon I: European Perspective

Dr. Jennifer Teufel
Institute for Applied Ecology e.V.
Germany

While commercial Ocean ranching of Atlantic salmon is practised only at a very small number of sites in Norway and Ireland, the cultivation of Atlantic salmon in marine aquaculture plays a major role in Europe. It takes place in five different nations: Norway, Scotland, Ireland, Iceland, and the Faroe Islands. The largest producer in Europe is Norway, which is also the world´s largest producer of Atlantic salmon. It provides about 45% of the world production of farmed Atlantic salmon. All over the world and especially in Norway the production of Atlantic salmon in aquaculture systems has grown rapidly and continuously since the seventies.

As intensive fish farming has given rise to a couple of ecological problems during the last decades, it is nowadays regulated by different national acts. Nevertheless following factors have still to be named when listing the ecological and environmental impact of salmon farming:

The use of fishmeal and fish oil for feeding: The current practice of using small, marine fish species for fish feed production cannot be considered to be sustainable. First of all these species forage low in the food chain and almost nothing is known about the impact of this fishery on species at higher trophic levels. Secondly, there is still need for research on the impact of this fishery on non-target species. And finally these fish species show highly variable recruitment dynamics. As a result, the prediction of stock trends over time is difficult.

Escapes of individual farmed salmon: Escaped farmed salmon compete with wild salmon for feed sources and spawning grounds. Another important adverse effect is that farmed salmon interbreed with wild salmon. Therefore local adaptation of wild populations can be disrupted and the genetic diversity of wild salmon populations can be reduced.

Release of organic materials and nutrient salts: During the last years, progress in optimising feeding techniques and feed conversion ratio of fish feed used in salmon aquaculture has been made. These improvements have contributed to the reduction in the release of organic waste from salmon net cages in general. Despite these improvements the growing Norwegian production of farmed salmon is still adding significant quantities of organic material and nutrient salts to coastal areas.

Use of pesticides: Instead of decreasing copper emission from nets used in aquaculture facilities in Europe, the use of copper has been increasing over the last 20 years. This may be seen in connection with a lack of alternative solutions. The increased copper use in fish farming must be viewed in the context of the expansion of the aquaculture industry. Furthermore, the use of organophosphates and pyrethroids in salmon farming to combat the salmon lice, acting as neurotoxins, is still another problem.

8. Uses and Ecological Impact of Net-Pen Aquaculture and Ocean Ranching of Atlantic Salmon II: North American Perspective

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The production cycle for Atlantic salmon in North America provides the context for assessing environmental risks. The first 12-18 months of the production cycle occur in freshwater, including spawning of broodstock, hatching of eggs, rearing of alevins and parr, and smoltification. Salmon then are transferred to saltwater for grow-out to market size over 18-24 months. Marine net pens are commercially proven, but pose environmental impacts. Other grow-out systems – ocean ranching, land-based saltwater systems, recirculating aquaculture systems, and sunken cage systems – are not commercially proven, and pose different mixes of environmental impacts. Atlantic salmon production in eastern North America totals 32,550 tons in Canada (2003) and 16,400 tons in the United States (2000), representing 5% of the 1,000,000 tons produced globally (2000). Siting criteria for net pen operations include technical components such as strong tidal action promoting water exchange, seabed of gravel or cobble, and water temperatures between 0.5^{o}C and 22^{o}C, as well as social components such as a suitable regulatory climate, public acceptance, and supporting infrastructure. Feed composition varies through the life cycle, and includes fish meal and oil, carbohydrate binders, vitamin-mineral premix, astaxanthin pigment, and feeding stimulants such as amino acids. Aquaculture uses 35% of world fish meal supply; salmon production alone uses 9%. Growth rate of salmonids is a complex trait, affected by quantity and nutritional content of feed, body weight of fish, water temperature, and other factors. Feed comprises over 50% of production costs; pressure to reduce feed costs also reduces environmental effects. Producers carefully monitor feeding behavior, nutrition, and feeding regimes. Overall, 61,200 tons of feed are administered annually to Atlantic salmon in eastern North America. Minimizing losses to disease and parasites is important to salmonid production. Over 90% of antimicrobial usage in salmon production is for therapeutic intervention, with over 90% administered with feed. Antimicrobial use is declining as the industry adopts a strategy of promoting fish health. Sea lice are managed by chemical treatments and fallowing of culture sites.

9. Ecological Issues for Cultured Atlantic Salmon

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Fish farming contributes to pollution of continental and coastal waters. In addition wild and hatchery fishes can exchange infection agents. However, as a rule, these problems are local. More widespread ecological problems arise from supportive breeding of Atlantic salmon and escapes of farmed fishes. Cultured or farmed salmon interact with populations of wild salmon as well as with the environment. There are four types of such interactions.

Influences of the environment on cultured salmon. The survival of hatchery salmon is decreased in comparison with wild salmon (review: Youngson, Verspoor, 1998). High mortality of cultured salmons results from phenotypic and behavioural defects as well as intentional and unintentional selection that improves genetic adaptation of fish to hatchery conditions but reduces their adaptation to natural environment (review: Artamonova *et al.*, in press). Similarly, the survival of non-native fishes is low because of the absence of adaptation to conditions of a foreign river (Altukhov, 1981).

Influences of cultured salmon on environment. Salmon stocking allows the prevention of spawning ground degradation and conservation of the pearl mussel (pearl mussel larvae is a parasite of Atlantic salmon). However, escaping Atlantic salmon interbreed with brown trout more frequently than wild salmon do, and Atlantic salmon populations may be polluted by interspecies hybrids (Youngson *et al.*, 1993; Hindar, Balstad, 1994).

Influence of cultured salmon on wild salmon. Presence of farmed and hybrid salmon may reduce the production of pure native fishes in a river due to increasing competition between juveniles (Einum, Fleming, 1997) and crossing of cultured and wild fishes. Moreover, cultured salmon may be the source of infection for wild fishes. Examples of parasite invasions are the sea lice in Scotland (Butler *et al.*, 2001) and *Gyrodactulus salaris* in Norway (reviews: Johnsen, Jensen, 1994, 2003).

Influence of wild salmon on cultured salmon. Often, competition between cultured and wild young salmon results in selective mortality of the cultured fish because they are poorly adapted to river conditions. On the other hand the reproductive success of farmed females is dramatically reduced in the absence of wild males. The reproductive success of cultured males is not affected by the presence of wild males, but it is decreased relative to wild males (review: Jonsson, 1997).

The main principles of rational salmon breeding were developed thirty years ago (Altukhov, 1975). Non-native Atlantic salmon stocking has been stopped in Russia in accordance with these principles. However, salmon have been found in the Russian rivers of the Baltic and Barents Sea basins that have escaped from foreign fish farms. Escapes of farmed salmons are inevitable and in order to prevent genetic introgression into natural Atlantic salmon populations, all farmed salmons (especially transgenic salmon) should be sterilized.

10. Crosses and Introgression of Cultured and Wild Atlantic Salmon

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Every year, many Atlantic Salmon escape from aquaculture facilities. It is estimated that escaped farm salmon make up 20-40% of the Atlantic Salmon in the fisheries off the Faroes in the North Atlantic Ocean (Hansen *et al.,* 1999), and a similar percentage of spawners in Norwegian rivers (Fiske *et al.,* 2001). This presentation mainly concentrated on the genetic interactions between wild and escaped cultured salmon.

Genetic effects can be indirect due to behavioural, ecological, and disease interaction with the wild population but can also be due to changes in selection regimes in natural populations through differential impacts on particular size, life history, geographical, or temporal components. The genetic effects of released salmonids on natural populations are typically unpredictable; they vary from no detectable effect to complete introgression or displacement.

Genetic effects on performance traits appear always to be negative *e.g.*, reduced total population sizes and reduced performance in a number of traits which can explain such population declines (*e.g.*, lower survival in fresh and sea water).

The direct genetic effects that are expected to result from interbreeding between wild and farmed animals are changes in number of alleles present (genetic variability), changes in frequency and type of alleles and a reduction of between-population genetic variance.

Currently, there is evidence that interbreeding between wild and escaped cultured Atlantic salmon occurs frequently. This is especially threatening to endangered small wild populations though a process called “outbreeding depression”. This process occurs when two genetically different strains, in this case wild and farmed salmon, interbreed resulting in hybrids with lower fitness.

Two “common garden” experiments have been conducted to show the influence of escapees on wild Atlantic salmon populations. The “Burrishoole experiment” (McGinnity *et al.* 1997, 2003, 2004) provided evidence for an outbreeding depression. From the “Imsa” Experiment (Flemming *et al.* 2000) the lifetime reproductive success (adult to adult) of the farm fishes was estimated 16% that of the native salmon, and gene flow from farm to wild was estimated at m=0.19. The effect of this is that the genetic difference between farm and wild would be halved every 3.3 generations. From those experiments and other published studies, it can be summarized that hybridisation between escaped farm salmon takes place and reduces the genetic variability as well as the genetic differences between populations, but also the productivity of natural populations. Part of the wild production is converted to hybrids, which show lower survival, thus reducing overall population fitness. Hybridisation seems to be the rule rather than the exception.

Transferring these results to an accidental release of transgenic salmon raises the demands that transgenic fish need to be kept securely in containments and have to be sterile to avoid introgression into natural populations in case of an accidental release.

11. Overview of State of the Art and Traits Being Introduced to Transgenic Salmon

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Since construction of the first genetically modified fish in China (1984), studies on gene transfer in aquaculture fish have been implemented by many teams from Europe, North America and Asia. Substantial progress has been achieved in terms of gene transfer technology. Generation of genetically modified fish has become a trivial task and development of novel transgene constructs has been enhanced by the large-scale expressed sequence tags (EST) sequencing projects. At present 340,000 salmonid mRNA sequences are available through the public repositories and a substantial fraction of fish genes have been identified at the molecular level. In addition to protein coding sequences, many regulatory elements have been cloned and characterized. Development of vectors including mobile genetic elements (transposons) has enhanced expression and inheritance of transgenes. At the same time attempts to improve the productive traits of farmed fish via gene transfer have been far less successful. Acceleration of fish growth by over-expression of growth hormone (GH) is the key achievement that remains to be accomplished in this area. GH-transgenes have been tried in many aquaculture fish species; however, dramatic increases in growth rates have been accomplished only in salmonid species of 3 genera: Salmo (Atlantic salmon), Oncorhynchus (Pacific salmon) and Salvelinus (charr). GH-enhanced fish are characterized by prolonged acceleration of growth and improved utilization of nutrients. High levels of GH cause developmental abnormalities, such as malformations of the skull and jaws, however this problem can be alleviated by usage of transgene constructs with weak promoters, which provide only slight increase of hormone levels. Despite the attractive features of GH-enhanced fish, its introduction into commercial farming is hampered by negative public opinion. Furthermore, the advantages of gene transfer are compromised by the fact that similar growth enhancement is achieved with conventional selective breeding. This can be illustrated with the fact that a GH-transgene had little effect on the growth of domesticated rainbow trout, a salmonid species, which has been subjected to intense breeding for several decades. Numerous attempts have been undertaken to improve qualitative traits of salmon, such as freezing tolerance, metabolism and utilization of nutrients, and resistance to infectious diseases. No substantial progress has been achieved so far, although research continues.

12. Risk assessment of genetically modified Atlantic Salmon

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This paper discusses environmental risks posed by genetically modified (GM) Atlantic salmon based on knowledge about interactions between wild salmon and cultured salmon released into natural environments. GM salmon are, at present, exclusively intended for contained aquaculture. The most relevant traits for genetic modification are growth rate and cold tolerance. To carry out a risk assessment, we need to know the environment that GM fish may escape into and the species/populations they are likely to interact with. For Atlantic salmon, containment of cultured fish seems to be least controllable in the marine environment, but escapes also occur during freshwater stages. A major shortcoming for risk assessment of transgenic Atlantic salmon is a lack of knowledge about the factors limiting population sizes and species range in the wild. Moreover, experiments with transgenic salmon in the marine environment are hardly feasible, as in order to achieve ecological realism the experiments will in practice be uncontained. For the freshwater habitat, it is possible to conduct field experiments in closed habitats or in elaborate experimental ecosystems, and small-scale studies can achieve ecological realism even without containment. For example, implants of growth hormone into experimental (non-transformed) fish seems to be a good model for studying behaviour-ecological effects of accelerated growth-rate. Assessment of hybridisation and introgression needs to rely on laboratory studies and data gathered for the non-transformed species. As long as GM salmon are fertile, gene flow to natural populations must be expected. Environmental risks are believed to be highest for GM salmon that have acquired freeze tolerance. This trait may help salmon invade habitats in the northernmost environments that so far have been spared from the effects of aquaculture. A tentative conclusion is that some of the necessary knowledge needed to carry out a formal risk assessment of GM Atlantic salmon will remain unavailable without taking unacceptable risks on behalf of this species and northern ecosystems.

13. Risk Issues Associated with Modifications of Transgenic Salmon

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Atlantic salmon are capable of accelerated 'compensatory' growth following periods of food deprivation but are not known to present rapid growth as a phenotypic life history strategy in nature. In domestication, rapid growth phenotypes have been developed for commercial cultivation over more than three decades of selection. Currently, a new rapid growth phenotype, developed through the introduction of an exogenous growth hormone transgene, is under pre-market review for food and environmental safety by the U.S. Food and Drug Administration. This presentation reviews differences between these rapid growth phenotypes at important life history stages.

Rapid growth phenotypes share key physiological and behavioral attributes regardless of breeding methodology, including the use of a common endrocrine pathway to accelerate growth; elevated metabolism, feeding motivation and efficiency; increased aggression and foraging activity, and reduced antipredator response. Eighth generation domesticated phenotypes outgrow their wild founding populations by roughly a factor of two, measured from first feeding to maturity. Fourth generation transgenics outgrow their founders by a factor of four and, after outcrossing to commercial phenotypes, outperform farmed salmon by a factor of two.

GH transgenic and domesticated phenotypes do not differ in early development timing between incubation and first-feeding, in growth rates after reaching one kg., or in the size at which they mature, when the females produce similar numbers and sizes of eggs. The transgenics can mature a year earlier than nontransgenic controls, reflecting gains achieved in juvenile-to-postsmolt growth rates during the first year of life. During this period of rapid juvenile growth, they achieve a larger size at age and transition more quickly through ontogenetic life history stages, including precocious smoltification. They require a higher level of dissolved oxygen to breathe, more food and oxygen to perform routine activities, and the metabolic cost of those activities rises with increasing effort. They eat more food, convert food to body mass more efficiently, maintain lower energy reserves and use those reserves more quickly, even under food or oxygen deprived conditions.

The phenotypic effects of GH transgenesis hold mixed implications for their likely environmental and genetic fitness in nature. As in farmed salmon, their enhanced appetite and aggressive behavior may improve their ability to acquire food and territorial resources but the metabolic cost of increased foraging, conflict and exposure to predatation may dampen any realized advantage. Evidence that feral farmed phenotypes can displace wild juveniles from preferred feeding territories despite poor rates of lifetime survival and reproductive success, however, suggests that the timing, as well as the direction of counterveiling effects is significant. Unlike GH domesticated phenotypes, however, transgenic salmon are physiologically capable of rapid progression through prey-size-determined feeding niches and of smoltification in their hatch year. They may move quickly out of direct competition with their own birth cohort and experience more aggressive competition from one and two year older resident parr and may emigrate out of the freshwater system as S0 smolt in their first summer.

The concentration of GH phenotypic effects in juvenile life stages and of vulnerability in wild populations during those same life stages helps to clarify the identification of critical control points for environmental risk management. Mechanisms to deny transgenic phenotypes access to freshwater habitat are likely to be necessary, including robust physical containment of juveniles in land-based hatcheries and effective sterilisation of production fish to prevent both gene flow and juvenile production.

14. Evaluation of GH transgenic coho salmon for environmental risk assessments

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Genetically engineered aquatic organisms are being developed in several countries for potential use in commercial aquaculture. In addition to assessment of their utility relative to existing aquaculture strains, transgenic strains require evaluation regarding their safety as food products, and, if there is the possibility for their escape into nature, assessment of potential environmental risks. Primary environmental concerns include the potential for altered fitness of transgenic strains which might allow them to outcompete other organisms in the ecosystem for available resources, and to interbreed with conspecifics resulting in altered genetic structure of otherwise naturally-selected populations. Transgenic fish possessing growth hormone transgenes can show significant growth enhancement relative non-transgenic controls in many species, but also can result in other pleiotropic changes, affecting systems in physiology, morphology, and behaviour. Observed changes in GH coho salmon include altered swimming performance, metabolism, nutrient uptake, endocrine function, feeding behaviour, swimming ability, predator avoidance, and disease resistance, each of which has the potential to affect the fitness of the transgenic organism relative to nontransgenic animals. While such laboratory-derived information can be used to hypothesize fitness differences between transgenic and nontransgenic animals, it is difficult to extrapolate such data to definitive risks. Assessments of fitness of species such as salmon which are derived from large complex ecosystems is also complicated by the need to keep transgenic strains in confined culture environments which, for non-transgenic strains, has been found to significantly affect their phenotype (*e.g.*, growth, spawning behaviour) relative to nature, indicating a strong environmental effect on phenotype. Transgenic strains are necessarily kept only in culture environments, and we thus we do not know the phenotype of this genotype from the environment from which risk assessment information is required. In laboratory populations, transgenic animals do not affect the growth and survival of nontransgenic cohorts when food resources are abundant, but under more competitive conditions with low food availability, transgenic salmon have been observed to initially cause growth suppression of nontransgenic cohorts and ultimately population crashes which did not occur in pure nontransgenic populations. While it is unknown whether such effects would also occur in natural populations, these experiments clearly demonstrate the importance of genotype X environment interactions in influencing risk assessment data. For salmonids, it will be difficult to mimic the marine environment in the laboratory, however, early stages have been assessed in mesocosms where transgenic genotypes have been found to suffer higher predation mortality than wild type. Genetic background effects have also been noted to influence the phenotypic consequences of a transgene, suggesting that transgenes in nature might be associated with continuously changing phenotypes and fitness which would make risk assessments a moving target. The uncertainties associated with risk assessments of confined species makes application of biological containment methods desirable. Currently, sterilisation by triploidy is most feasible as a commercial technology, and while not yet completely effective (99.8%) in experiments with transgenic coho salmon, refinement of protocols and/or combining with other containment strategies may yield completely effective reproductive containment. Since risk assessments must currently be conducted on a case-by-case basis, the data derived from studies of GH transgenic coho salmon clearly should not be used directly in risk assessments of other species. However, these experiments have provided information regarding variables and limitations associated with the generation of fitness information from cultured transgenic salmon in laboratory conditions, some of which may be of utility when evaluating information in other risk assessments.

15. Inventory and classification of salmon rivers in East Fennoscandia

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The task of the study was to make an inventory of salmon rivers, analyze their hydrological parameters and find out correlations with the salmon broodstock numbers. The project was implemented in 1999-2004. Of the 142 watercourses surveyed in the region, wild Atlantic salmon was found to spawn in 86 rivers: 18 belonging to the Barents Sea, 44 – White Sea, 10 – Lake Ladoga and 9 – Lake Onego drainage basins, 3 tributaries of Lake Kuito, and 1 tributary of each of Lake Kamennoye and Segozero. In most large rivers, which account for 5% of the 86 rivers, the breeding part of the salmon population is over 5000 individuals, in some large and most medium-size rivers it is 500-1000 ind. (20%), in some medium-size and all small rivers – 100-400 ind. (75%).

Drawing upon multivariate analysis of hydrological parameters, a classification of salmon rivers into 4 groups and 7 types has been suggested. Each group and type of rivers has its distinctive features predetermining salmon reproduction. This fact is essential for further analysis of the reasons for variation in the population characteristics of the species (number of spawning groupings, timing of ascent, etc.).

The results of the inventory were used in developing the national strategy for Atlantic salmon conservation and the Index of Salmon Rivers of Russia, which is now in preparation.

16. Fish genetics studies at the Russian Federal Research Institute of Fisheries and Oceanography (VNIRO)

Vladimir A. BARMINTSEV
Federal Research Institute of Fisheries and Oceanography (VNIRO)
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The Russian Federal Research Institute of Fisheries and Oceanography (VNIRO) was founded in 1933 and since then has kept the leading position in scientific research, conservation, and reproduction of fish resources in Russia. As of 2000, the VNIRO adopted a new tool – molecular genetic analysis – that can bring many benefits for the fisheries sector of the Russian Federation, including:

- • Identification and certification of DNA origin, in particular for sturgeon species of high commercial value;

- • Studying the genetic structure of marine populations and the impact of artificial reproduction;

- • More effective protection of marine fauna and restoration of its diversity, in particular of endangered or rare species of fish;

- • Improving control over transboundary migration of fish and deciding on fishing quotas.

The Centre of Molecular Genetic Identification, a relatively new division of VNIRO that was established in 2002 to serve, among others, as CITES scientific unit, is well positioned and equipped to make a full use of molecular genetic analysis. It is able to perform molecular profiling, including DNA-fingerprinting (RAPD, AFLP), microsatellite markings, sequencing of total mitochondrial DNA and particular nuclear genes, creation and investigation of genetically modified fish.

One of the latest research projects implemented by the Centre showed that a combination of fragment analysis of DNA and its sequencing is required to identify the DNA origin in both wild and cultured population of sturgeon, salmon and carp species of fish. The fragment analysis allows to amplify various loci (specific and anonymous) in order to establish the DNA type even when the exact primary DNA structure is unknown, while sequencing can determine the string of nucleotides in DNA fragments.

ANNEX 1 – PARTICIPANTS LIST FOR WORKSHOP ON THE BIOLOGY OF ATLANTIC SALMON (*SALMO SALAR*)

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