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Genetically engineered fish and their possible environmental impact

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Abstract

This report provides an overview of the potential ecological and genetic effects of using genetically engineered fish in aquaculture. It reviews the recent developments in gene transfer to fish, and considers how genetic engineering could be used in future aquaculture. When evaluating the environmental effects of transgenic fish, I rely on experience from introductions of non-native species and populations of fish that have occurred for more than a century, and on the effects of the recent escapes of farmed fish in Norway. Throughout, the emphasis of the report is on salmonids and in particular on the culture of Atlantic salmon (*Salmo salar*).

The genetic manipulations which are now being explored by gene technologists worldwide, are first of all mediated through the microinjection of foreign DNA into fish eggs shortly after fertilization. This technique does not interfere much with survival, and has led to high integration of the inserted DNA into the fish genome. A number of different species and genes have been used in these experiments. For the purpose of this report, emphasis is placed on the introduction of growth hormone genes and antifreeze protein genes to Atlantic salmon, because these modifications are likely to be among the first to be used on a commercial scale.

A wide variety of outcomes, ranging from no detectable effect to complete introgression or displacement, has been observed following releases of cultured (non-transgenic) fish into natural settings. Where genetic effects on performance traits have been documented, they appear always to be negative in comparison with the unaffected population. These observations raise concerns over the genetic future of many natural populations in the light of increasing numbers of released fish.

Three scenarios for the future use of genetically engineered Atlantic salmon in aquaculture are presented and discussed. The first scenario assumes that transgenic fish will be used in aquaculture according to their profitability, and that no measures are taken to reduce the number of fish escaping from aquaculture. In this scenario, current negative trends for the genetic and ecological integrity of natural salmonid populations will be reinforced. In particular, the use of transgenic cold-tolerant Atlantic salmon would lead to a northwards expansion of aquaculture, so that a number of populations that are now spared from the effects of escaped fish would be at risk. This could lead to loss of irreplaceable genetic resources in Atlantic salmon, and could likewise affect a number of Arctic charr (*Salvelinus alpinus*) populations which are currently the only fish spawning in the northernmost watercourses. Transgenic, growth-enhanced fish could also affect native populations negatively, but this would depend on how their larger appetite affects their survival in food-limited environments.

The second scenario assumes that concerns about the effects of genetically engineered fish lead to better physical containment than in present-day fish farms. This would lead to a reduction of interactions between escaped and wild fish, but would still have the potential for dramatic effects in cases where technical failure or human error led to large-scale escapes. The third scenario assumes that gene technology be used actively in a search for biological containment of transgenic fish, in addition to better physical containment. This would involve transfer of genes to induce sterility (already an option using other methods) and/or to produce fish that would express suicide-genes upon escape. Only in this third scenario is the use of transgenic fish in aquaculture compatible with internationally established goals for genetic conservation.

Key words: Genetically engineered organisms - Biotechnology - Fish - Aquaculture - Ecological effects - Genetic effects - Salmonids - Norway

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Preface

This report was written on the authority of the Norwegian Directorate for Nature Management (DN contract BTEK–1092). It was prepared while the author was on sabbatical at the Museum of Vertebrate Zoology, University of California, Berkeley, on leave from a position at the Norwegian Institute for Nature Research, Trondheim. The support of these institutions is gratefully acknowledged.

I thank Peter Aleström and Audun Nerland for comments on gene transfer methodology, Arne Jensen for providing information on temperature relationships, and Hilde Meland for help with the final preparation of the manuscript.

The scope and coverage of this report admittedly stretch the competence of the author. I would be happy to receive comments on relevant ideas and data that I have missed and correction of errors in those that I have used.

Berkeley, California, May 1993

Kjetil Hindar

Contents

1 Introduction

Genetic engineering offers the possibility to combine, in a single organism, genetic traits from vastly different taxa, say, a tomato and a flounder. The resulting genetically engineered organism challenges our personal views about what is natural and what isn't, and has created much anxiety about the application of this new technology. One area where the concerns are manifold, is the release of genetically engineered organisms into the environment. On an experimental scale, such releases have been carried out since the mid 1980s in the USA and continental Europe, and since 1992 in Norway.

Releases of genetically engineered organisms (GEOs) raise a number of questions about their environmental impact, many of which are of general biological interest:

* What is the invasion potential of the released GEOs in natural and man-made ecosystems?
* What is the possibility for gene dispersal from the GEOs to cultured and wild relatives?, and ultimately,
* What is the full spectrum of ecological and genetic effects of the releases?

In this current report I present and discuss a scenario where genetically engineered (transgenic) fish are used in aquaculture. In performing this task, I use experience following releases and escapes of salmonids as a basis for making predictions about the possible effects of transgenic fish. Throughout, the perspective will be that of applying genetic engineering to the production of species that are currently used in the Norwegian fish farming industry, in particular Atlantic salmon (*Salmo salar*).

This report is in many ways a synthesis of syntheses that have been written on transgenic fish (e.g. Powers et al. 1992), salmon environmental biology (Gibson 1993), ecological and genetic effects of releasing salmonids (Hindar et al. 1991, Krueger & May 1991), and on the environmental effects of releasing transgenic organisms and fish in particular (Tiedje et al. 1989, Kapuscinski & Hallerman 1991). Its primary objective is to combine information from each of these research fields to speculate about the environmental impacts if Norwegian aquaculture were based on transgenic fish. A subsidiary objective is to stimulate serious contemplation of these impacts by scientists representing a wide variety of biological disciplines.

1.1 Genetic engineering in aquaculture

There is considerable interest worldwide in applying genetic engineering to aquaculture (e.g. Chen & Powers 1990). One reason is that as the world catch of wild fish is rapidly approaching the estimated maximum potential harvest at 100–150 million tons (FAO 1989), an increasing demand for marine proteins is likely to be provided by aquaculture. Another reason is that genetic engineering has a potential to tailor fish species for cost-efficient aquacultural production of fish proteins, either alone or in combination with traditional breeding techniques.

In Norway, fish farming is a likely candidate for the application of genetic engineering. Pilot studies for producing genetically engineered Atlantic salmon and rainbow trout (*Oncorhynchus mykiss*) have already been carried out in Norway (Male et al. 1989, Rokkones et al. 1989).

1.2 Environmental concerns

Any use of GEOs must be viewed against the background of introductions of living organisms being a major cause for loss of global biodiversity (Pimm & Gilpin 1989). For example, introductions have contributed to 68% of the extinctions of North American fish species during the past century (Miller et al. 1989). With the large-scale application of genetic engineering in agriculture and aquaculture, it is likely that the pressure on native organisms caused by introductions will increase.

A number of concerns have been expressed on the ecological and genetic effects following the intentional or accidental release of GEOs to the environment (Royal Commission 1989, Tiedje et al. 1989, Mooney & Bernardi 1990, DN 1991, Ginzburg 1991, Levin 1992). Current knowledge based on experiments with GEOs is

still too limited to provide quantitative assessments of the environmental effects of these releases. Unfortunately, even as the general knowledge increases, it is a problem that one cannot make precise predictions about the environmental effects of a particular release (Simonsen & Levin 1988). This mandates a way of thinking about releases that must incorporate the 'precautionary principle' as a guideline for management.

1.3 Salmonids as a model system

It has been argued that fish serve as excellent models for studies of organismic and molecular evolution, and that they have a large potential as experimental model systems for a number of biological disciplines, including genetic engineering (Powers 1989). Here I argue that fish have a similarly high potential for studying the environmental effects of releases, both with respect to the consequences of increasing gene flow between populations (Hindar et al. 1991, Ryman et al. in press), and in the context of altered community dynamics following species introductions (Moyle et al. 1986, M.E. Power 1990).

Gene flow from genetically engineered organisms to other organisms is a major concern when determining the full spectrum of biological effects of releasing GEOs (Ellstrand 1988, Williamson et al. 1990, NMF 1991). The potential for gene flow is particularly high if the organisms that are subject to genetic manipulation have close relatives (populations or species) in the wild. The development of aquaculture in northern temperate areas may be a highly relevant example. With few exceptions it is based on species which have native populations in the same areas: salmonids such as Atlantic salmon, brown trout (*Salmo trutta*), Pacific salmon (*Oncorhynchus* spp.) and Arctic charr (*Salvelinus alpinus*), marine fishes such as Atlantic cod (*Gadus morhua*), turbot (*Scophthalmus maximus*), Atlantic halibut (*Hippoglossus hippoglossus*), wolffish (*Anarhichas* spp.), and several crustaceans and mollusks.

There is a growing interest internationally for using experience from releases of non-modified organisms to predict the consequences of genetically modified ones (Mooney & Drake 1989, Fox 1992a). Salmonid fishes can serve as an excellent model in this context, because they are a target for genetic engineering, and because information from this group provides one of the best data sets for evaluating the ecological and genetic effects of releases of non-native populations to aquatic environments (Hindar et al. 1991).

First, salmonid fishes are well known genetically and ecologically. They are characterized by the existence of a number of local populations which exhibit a large degree of morphological, ecological and genetic differentiation between them (Ricker 1972, Ryman & Utter 1987). Second, it appears that much of the morphological and ecological divergence among local populations reflects local adaptation (Ricker 1972, Taylor 1991). Third, salmonid fishes have been artificially reared and released for more than a century, and in considerable numbers including a variety of life stages (egg, fry, parr, smolt and adult). Fourth, cultured fish typically represent gene pools which are distinct from the natural populations with which they may come in contact. They commonly represent genetically exogenous populations or crosses between them; in addition, the genetic constitution of cultured populations has frequently been altered through inbreeding, selective breeding or domestication (Allendorf & Ryman 1987, Gjedrem et al. 1988). Finally, many releases have been to environments inhabited by indigenous conspecific or congeneric populations (Krueger & May 1991).

A potential for using salmonids as a model system for making predictions about the effects of releasing genetically engineered organisms in aquatic environments, has been recognized in the Swedish research program on ecological hazards of biotechnology (Kjelleberg & Fagerström 1990) as well as in an OECD report about monitoring of genetically engineered organisms in nature (OECD 1991). The Council of Europe has recently chosen salmonids as one of the case studies for a conference on long-term effects of GEOs to be held in Strasbourg in November 1993.

2 Gene transfer to fish

Genetic engineering applied to aquaculture and fish in particular aims at increasing growth rates and feed utilization, increasing environmental tolerance and resistance to diseases, controlling reproduction, and improving food quality characteristics. This development is entirely in line with the development that has taken place using traditional breeding methods. However, new aspects have been added by genetic engineering. First, transfer of genes is possible between species which do not hybridize naturally. Second, genetic traits from one species can be introduced into another species 'in one shot', rather than by the elaborate breeding schemes of traditional breeding.

2.1 Definition

Genetically engineered (transgenic) fish contain copies of novel genetic constructs introduced into their chromosomal DNA by modern genetic techniques (Kapuscinski & Hallerman 1991). These constructs consist of structural genes (DNA sequences encoding a specific protein) linked to regulatory sequences (DNA sequences necassary for successful expression of the structural gene).

Using this definition, the following techniques are considered to give rise to genetically engineered organisms:

* cloning of genes and transfer with the aid of vectors
* direct injection of genetic material
* fusion of two or more cells using techniques that do not occur naturally.

On the other hand, induction of polyploidy, and cell fusion between species that may hybridize naturally, are **not** considered to give rise to genetically engineered organisms.

2.2 Techniques for gene transfer

The methodology for gene transfer to fish has been reviewed by Chourrout (1987), Chen & Powers (1990), Hew & Fletcher (1992), Powers et al. (1992) and Jiang (1993). What follows is an outline of the techniques reviewed in those papers; the reader is referred to the original publications for a more technical treatment of the subject.

2.2.1 Microinjection

Direct microinjection of cloned DNA into fish eggs is by far the commonest method for introducing genes into the germ-line of fishes. Using this method, transgenic individuals have been generated in several fish species, including rainbow trout (Chourrout et al. 1986, Maclean et al. 1987), Atlantic salmon (McEvoy et al. 1988, Fletcher et al. 1988, 1992, Rokkones et al. 1989, Du et al. 1992), common carp (*Cyprinus carpio*; Zhang et al. 1989, 1990), loach (*Misgurnus anguillicaudatus*; Zhu et al. 1986), channel catfish (*Ictalurus punctatus*; Dunham et al. 1987), tilapia (*Oreochromis niloticus*; Brem et al. 1988), goldfish (*Carassius auratus*; Zhu et al. 1985), zebra fish (*Brachydanio rerio*; Stuart et al. 1988), medaka (*Oryzias latipes*; Ozato et al. 1986), walleye (*Stizostedion vitreum*) and northern pike (*Esox lucius*; Moav et al. 1992).

In mammals, the foreign DNA is microinjected into the male pronuclei of the fertilized eggs. In fishes, the male pronuclei are not visible (except in medaka) and the foreign DNA has to be microinjected into the egg cytoplasm (Powers et al. 1992). Usually, 10^6 copies of the gene construct are injected into each egg. The injection is carried out shortly after fertilization, usually between the one-cell and four-cell stages.

The eggs of many fish species have a tough outer membrane (chorion) which makes microinjection with a glass needle difficult. In order to tackle this problem, an opening in the chorion has been made by microsurgery before the glass needle is inserted (Chourrout et al. 1986, McEvoy et al. 1988), or the glass needle has been inserted through the micropyle (Fletcher et al. 1988, Brem et al. 1988), which is the opening through which

sperm enters the egg during fertilization. Other methods include removing the chorion using a forceps (Ozato et al. 1986) or digestion by trypsin (Zhu et al. 1985, 1986), or performing the microinjection before the fertilization and subsequent water hardening of the chorion take place.

As pointed out by Powers et al. (1992), the success of the microinjection method in transferring foreign genes into fish embryos cannot take away the drawback lying in the tedious and time-consuming procedure of handling eggs individually. There is, therefore, an interest in developing mass gene transfer technologies for use in fish.

2.2.2 Other techniques

One method with a potential for mass gene transfer is the use of retroviruses, which can transfer DNA to hosts by viral infection and mediate transgenesis if foreign genes have been inserted into the viral genome. This method requires characterized species-specific retroviruses, which are available for mammal and bird species, but not yet for fish (Powers et al. 1992).

Electroporation is another method with a potential to mediate mass gene transfer. It involves the use of short electrical pulses to permeabilize the cell membrane, thereby gaining entry of genes into the cell. Inoue et al. (1990) obtained transgenic medaka by electroporation, and detected expression of the inserted rainbow trout growth hormone. But the efficiency of gene transfer was low. Recently, experiments with electroporation have shown a much higher efficiency of gene transfer (Xie et al. 1993).

Liposomes produced from phospholipids have the potential to transfer foreign DNA into mammalian cells and plant protoplasts, but have not yet shown any success in producing transgenic fish (Chen & Powers 1990). Likewise, bombardment of cells by foreign DNA adsorbed onto small tungsten particles has been shown to produce transgenesis in some cultural plants, but has not shown any success in fish.

Embryo stem (ES) cell engineering and subsequent implantation of the engineered cells into early embryos can produce transgenic individuals. Embryo stem cells are cells which can proliferate in an undifferentiated state in cell culture, and still retain their developmental totipotency (i.e. they contribute to all somatic tissues and germ cells). ES cell engineering has been developed for transgenic mouse (Rossant 1993). Using this method, gene targeting is possible via homologous recombination and analysis of the result before regeneration of the embryo and development of the transgenic animal. The same method is under development in zebra fish.

2.3 Evaluation of success

Microinjection of foreign DNA into the cytoplasm of fish eggs appears not to be harmful to the development of the fish embryo. The survival rates of the injected embryos range from 15 to 80% (**Table 1**; see also Chen & Powers 1990). Moreover, it appears that DNA injected into the cytoplasm may be integrated into the host genome to a relatively large extent; integration rates of up to 75% have been reported.

Even though a number of studies have presented evidence of expression of the foreign DNA, only a few studies have so far documented that the foreign genes are genetically transmitted to the next (F_1) generation and expressed in both the parent (P) and F_1 generation (Chen et al. 1990, Stuart et al. 1990, Zhang et al. 1990, Powers et al. 1991, Fletcher et al. 1992).

Based on the limited evidence available, it appears that gene transfer induces much phenotypic variation in the first generation of transgenic fish. Much of this variation presumably reflects variation among genotypes for integrated transgenes, including 'mosaicism' which signifies the integration of the transgenes in some cell types but not others (Kapuscinski & Hallerman 1990). Two or more generations of selection of transgenic fish will be necessary to produce lines showing more homogeneous transgenic genotypes and expression, and desirable performance in other traits (Kapuscinski & Hallerman 1991).

Table 1. Success of microinjection of linear DNA into the cytoplasm of fish eggs (From Chen & Powers 1990).

Fish species	Gene copies	Survival (%)	Integration (%)	Ref.
Rainbow trout	$2x10^8$	77 (hatching)	75 (embryo)	1–2
Atlantic salmon	10^6	50 (fry)	7 (fry)	3
Zebra fish	$5x10^6$	16 (fry)	5 (adult)	5–7
Carp, catfish	10^6	40 (hatching)	10 (adult)	4

1 Chourrout et al. (1986)
2 Chourrout et al. (1988)
3 Fletcher et al. (1988)
4 Stuart et al. (1988)
5 Chen et al. (1989)
6 Zhang et al. (1990)
7 Powers et al. (1991)

2.4 Examples of 'introduced gene – species' combinations

A number of foreign genes (and gene constructs) have been transferred to fishes. These include: human growth hormone (GH) gene, bovine GH gene, fish GH gene, chicken δ–crystalline gene, fish antifreeze protein gene, fish α–globin gene, bacterial β–galactosidase gene, hygromycin resistance gene, chloramphenicol acetyl transferase (CAT) gene and neomycin phosphotransferase gene (Moav et al. 1992, Powers et al. 1992). In order to enhance expression of the foreign genes, various enhancer–promoter complexes have been used. These include the metallothionein–1 and β–actin enhancer–promoter complexes (from cellular genes) which direct expression of chimaeric genes in a variety of tissues, and viral sequences (e.g. the long terminal repeat portion of the avian Rous sarcoma virus) which can direct expression of chimaeric genes in specific tissues (i.e. those normally infected by the virus; Moav et al. 1992).

In the following, I present some combinations of introduced genes and host species that may be interesting from the perspective of large–scale use in the future.

2.4.1 Growth hormone genes

Growth rate is an economically important trait of fishes used in aquaculture, and a number of experiments have been carried out to determine how various hormones affect growth. In some of these experiments, it has been shown that recombinant growth hormone of fish, chicken, bovid and human origin has growth–enhancing properties when administered to juvenile salmonids by feeding, intraperitoneal injection, and dipping in a saline, GH–containing solution (Gill et al. 1985, Agellon et al. 1988, Danzmann et al. 1990).

There is, however, no simple association between exogenous administration of growth hormone and a high growth rate. The studies reporting a growth–enhancing effect of growth hormone have been undertaken at low, suboptimal temperatures for growth. At 17–18°C, near the optimum temperature for growth of rainbow trout, Danzmann et al. (1990) found that a GH–containing diet did not enhance growth, but led to higher levels of sex steroids and a generally higher metabolic activity. They concluded that at high temperatures, GH administration appears to influence gonadal development and other metabolic functions to a larger extent than somatic growth rates. Growth hormone has other functions as well. In particular, GH increases sea water adaptability. This has been demonstrated by administration of

recombinant salmonid GH to Atlantic salmon (Boeuf 1993).

It is as yet unclear whether exogenous administration of growth hormone to fish is cost effective in the long run (and it is moreover prohibited in some countries including Norway). There is therefore an interest in producing transgenic strains of fish that express higher levels of growth hormone, and, once they have been generated, transmit the enhanced growth rate to the next generations (Du et al. 1992, Powers et al. 1992).

Transgenic fish microinjected with growth hormone genes have been shown to grow faster than the controls. In one set of experiments, Zhang et al. (1989, 1990) introduced a recombinant plasmid containing the long terminal repeat sequence of avian Rous sarcoma virus and a rainbow trout GH cDNA into carp embryos. The transgenic fish produced were on average 22% larger than their sibling controls. Moreover, transgenic progeny of these fish grew faster than their non-transgenic siblings, and also much faster than their parents (Powers et al. 1992).

In another experiment, Du et al. (1992) transferred a GH gene construct to Atlantic salmon by linking an antifreeze protein gene promoter from ocean pout (*Macrozoarces americanus*) to a chinook salmon (*Oncorhynchus tshawytscha*) GH cDNA clone. They showed that the average increase in transgenic fish size was five-fold over controls at one year of age (**Table 2**).

Transfer of growth hormone genes to fish created considerable media interest in Norway some years ago when human GH genes were microinjected into Atlantic salmon and rainbow trout eggs. The experiments showed that the inserted gene was expressed at the egg and fry stages (Rokkones et al. 1989), but were terminated before it was clear whether the inserted gene was incorporated into the fish genome or not.

Another Norwegian research group isolated and microinjected into Atlantic salmon eggs growth hormone genes from salmon themselves (Male et al. 1989), producing 10 transgenic salmon which were crossed and slaughtered in autumn 1990. Their progeny have been studied to see whether the inserted genes were inherited or not. Preliminary results using PCR-based screening techniques (e.g. Saiki et al. 1988) suggest that the inserted genes were not inherited. This may be caused by the transgenic individuals being mosaic and that the extra growth hormone gene copies were not present in the germ line. But the possibility remains that because of recombination the screening technique used does not detect transgenic offspring (Audun Nerland, Norbio A/S, Bergen, Norway, pers. comm.).

In a second experiment, Atlantic salmon eggs were injected with a modified growth hormone gene. The genetic modification involved no amino acid changes, but changes in the DNA sequence which could be detected by PCR. One transgenic individual was detected in this experiment (Audun Nerland, pers. comm.), showing the potential for this technique for rapid screening of transgenic individuals. The Atlantic salmon studies reported from Norway have now been terminated.

Table 2. *Comparison of weight and growth rate of transgenic Atlantic salmon juveniles (N=6) and their non-transgenic siblings (N=43) following microinjection of a growth hormone construct in November 1989 (From Du et al. 1992).*

Experimental group	Weight (mean±s.e.; grams)		Growth rate
	Oct 4/90	Jan 12/91	(% per day)
Transgenic	20.9±5.1	47.3±9.5	0.84±0.19
Siblings	7.4±0.3	9.5±0.6	0.21±0.02
Significance of difference	<0.05	<0.01	<0.001

2.4.2 Antifreeze protein genes

Tolerance to cold water is another trait which has economic interest in aquaculture, because the success of the marine phase of salmonid aquaculture is in many areas restricted by cold winter temperatures (e.g. in northeastern USA and Canada, and northeastern Norway). Especially problematic is the winter survival in net pens in the supercooled sea water which occurs in these areas.

During winter time, seawater temperatures in the Arctic may go down to −1.4 to −1.9°C. Atlantic salmon and numerous marine fishes cannot tolerate the cold water and freeze to death at temperatures lower than −0.7°C. However, some species of fish are adapted to extremely low water temperatures by the evolution of antifreeze genes which encode proteins that keep their blood from freezing (DeVries 1971). Polar fish express antifreeze proteins (AFP) throughout the year, whereas northern temperate species, like the winter flounder (*Pseudopleuronectes americanus*), express antifreeze proteins only in winter. The available data suggest that the annual cycle of AFP production is endogenous, with photoperiod acting via the pituitary gland to regulate the time of onset of AFP production (reviewed by Fletcher et al. 1992). The pituitary factor responsible for this regulation has been identified as growth hormone.

Water temperature does not appear to control the onset of AFP production in winter flounder, but it affects the rate of disappearance of AFP from the blood. For example, in the supercooled sea water off Newfoundland during spring, plasma AFP levels remain at winter values despite the fact that AFP synthesis has stopped. Only when the water temperatures increase above 0°C will AFP levels decline (Fletcher et al. 1992).

Atlantic salmon and other salmonids do not have antifreeze proteins. However, when AFP purified from the blood plasma of winter flounder were injected into rainbow trout, their freezing resistance increased in direct proportions to AFP levels (Fletcher et al. 1986). Later, a number of experiments have been carried out to transfer antifreeze genes to Atlantic salmon. These experiments showed integration of the antifreeze genes into the salmon genome, but did not provide evidence for expression of antifreeze proteins (Fletcher et al. 1988). More recently, the same research group has found evidence of antifreeze protein expression, although at levels that are 100–fold less than would be required to provide freezing protection (Fletcher et al. 1992). It is probably only a question of time until adequate antifreeze protein expression is achieved in order for this transgenic system to be of commercial interest.

2.4.3 Other genetic traits of interest

As referred to above, a number of other genes have been introduced to fishes. Most of these experiments are so far of interest in basic research only, and have been carried out with the aims of (1) finding promoters that will express the gene of interest, and (2) introducing genes that, if expressed, will easily distinguish between transgenic and non–transgenic individuals and thereby rapidly determine the success of the method.

In aquaculture (as well as in other areas of bioproduction), there is a continual interest in controlling reproduction and in increasing resistance to diseases (DN 1991). Salmonid aquaculture each year looses large proportions of its potential production to early sexual maturation, and to diseases such as furunculosis, vibriosis, bacterial kidney disease, and a number of viral diseases. There are genetic components to both early sexual maturation and disease resistance (Refstie 1987), but so far the poor knowledge of how individual genes affect these traits limits the use of transgenesis to improve them.

Early sexual maturation, which occurs primarily in males, is costly to the aquaculture industry. Sexually mature individuals grow slowly and have poor flesh quality, and their numbers cannot always be predicted well enough in advance to limit the economic loss due to sexual maturity. Currently, the occurrence of early sexual maturation can be reduced on a large scale in at least six ways: by induction of triploidy, selective breeding for later maturity, androgen treatment, irradiation, interspecific hybridization, and by elimination of the early–maturing sex (Devlin & Donaldson 1992).

I am not aware that genetic engineering has yet succeeded in controlling sexual maturity, although this

may be a future possibility if genes promoting maturity can be identified and silenced. One line of thought which is actively pursued by a Norwegian research group, is the immunization of fish against gonadotropin releasing hormone (GnRH) which – if effective – may lead to regression of the reproductive system (Andersen et al. 1992, Aleström et al. 1992). The objective of this research is to explore the possibility for producing transgenic "anti-GnRH" fish which will be sterile (Peter Aleström, Agricultural University of Norway, pers. comm.).

Disease resistance is another trait of interest to the aquaculture industry (Fjalestad et al. 1993). At present, it appears that the application of gene technology to disease problems primarily lies in providing rapid identification of, and vaccines for, selected pathogens. For example, recombinant DNA vaccines for infectious hematopoietic necrosis virus have been produced (IHNV; Gilmore et al. 1988, Engelking & Leong 1989). It has been suggested that insertion of viral coat protein genes into the germ-line of salmonids may in the future be used to produce transgenic virus-resistant fish (Kapuscinski & Hallerman 1991, Jiang 1993).

It may well be that disease resistant fish will be the most heralded application of genetic engineering in aquaculture. But as long as disease resistance genes have not been identified, transgenic disease resistant fish remain only an intriguing possibility for the future (Maclean & Penman 1990). In Norway, one approach to this problem is by studying the disease resistance properties of transferrin. The transferrin gene in Atlantic salmon has recently been isolated (Peter Aleström, pers. comm.). Another opportunity probably lies in manipulating the major histocompatibility complex (MHC), a group of closely linked loci implicated in resistance to certain diseases in higher vertebrates (Chevassus & Dorson 1990).

2.5 Performance tests of transgenic fish

In aquaculture worldwide, various semi-controlled environments are used for the ongrowing phase of the production cycle. These environments may be freshwater ponds for the production of carp and catfish, and marine net pens for the production of Atlantic salmon. As such, they differ in many respects from the controlled laboratory environment where transgenic fish are produced.

At present, only two experiments are underway where the performance of transgenic fish is tested under production conditions. These experiments involved the release into ponds of genetically engineered carp that had been injected with growth hormone genes from rainbow trout, and a parallel release of transgenic catfish (reported by Fox 1990, 1992b).

2.6 Current limitations

The following methodological problems need to be resolved before genetically engineered fish can be produced on a commercial scale (Powers et al. 1992):

* Techniques for transfer of genes into a large number of eggs.
* Mass screening methods to distinguish between transgenic and non-transgenic individuals at an early developmental stage.
* Targeting of genes to specific areas of the fish genome.
* Regulation of foreign gene expression.

Whereas breakthroughs in these areas may offer opportunities for large-scale production of transgenic fish, the possible environmental problems outlined in this report may be even higher obstacles for sustainable, commercial production. One option which will be discussed here is the application of genetic engineering in an active search for producing transgenic fish which are environmentally safer than traditionally bred fish.

2.7 Gene manipulation without the use of transgenic fish

Transgenesis is not the only way by which fish genomes can be manipulated for aquacultural production. The production of chromosomally manipulated, sterile salmonids can without difficulty be carried out on a large scale (Johnstone et al. 1991). Sterility is induced by

heat- or pressure-induced triploidy shortly after fertilization (Chourrout 1987, Thorgaard 1992). Triploid males, but not females, may develop both secondary sexual characteristics and fragments of gonadal tissue. In aquaculture, this potential production problem can be overcome by the use of all-female lines, which can be created by fertilizing eggs with irradiated sperm and thereafter applying heat shock to produce XX-individuals only (Refstie 1983). When some of these XX-individuals are sex-reversed by hormones to become functional males, aquacultural strains can be established where only females (diploid or triploid) are produced.

Recently, a method for determining genetic sex in chinook salmon has been developed (Devlin et al. 1991). This method rests on the isolation of a DNA probe capable of recognizing a DNA fragment which is present in chinook males but not in females. The application of Y-chromosomal probes to the commercial culture of chinook salmon will simplify the synthesis of all-female strains, because the method rapidly screens for the presence of males in these lines (Devlin et al. 1991). The DNA probe developed for chinook salmon has not been successful in determining sex in other salmonids.

3 Potential use of transgenic fish in aquaculture

3.1 International and Norwegian aquaculture

Fish culture dates back 4-5,000 years in China (carp culture) and 1,000 years in Europe. In 1986, it was estimated that the production of fish by aquaculture reached 12 million tons, which was 12% of the tonnage generated by international fishing (FAO 1989).

Artificial reproduction of salmonids (brown trout) was mastered by Stephan Ludwig Jacoby in Germany in the middle of the 18th century. From the 1850s onwards, this technique was used to supplement populations of several salmonid species all over the northern hemisphere (stock enhancement; Egglishaw et al. 1984).

Currently, salmonid culture involves releases of 5 billion juveniles of Pacific salmon and 8 million smolts of Atlantic salmon in ocean ranching operations (Isaksson 1988, McNeil 1991). The biggest enterprise is the release of chum salmon in Japan. Whereas the number of juveniles released differs by three orders of magnitude for Atlantic salmon and chum salmon, the resulting proportions of cultured fish in the wild of these species are not very different (>80% for both Atlantic salmon in the Baltic and chum salmon in Japan).

In closed culture operations, the largest production is that of rainbow trout and Atlantic salmon. The worldwide production of rainbow trout amounts to 250,000 tons annually (Gall & Crandell 1992), of which 60% is within the countries of the European Community. The production of rainbow trout occurs mainly in freshwater ponds and tanks. Norway has a marine net-pen based production of rainbow trout at between 4,000 and 10,000 tons per year. Production of Atlantic salmon in marine net pens amounts to more than 200,000 tons annually, or equivalent to 50 times the harvest of Atlantic salmon worldwide. Seventy-five percent of the captive production of Atlantic salmon takes place in Norway.

A growing aquaculture industry in the western USA and Canada relies on chinook and coho salmon for captive

production. The largest production is that of chinook salmon in British Columbia, which was 15,500 tons in 1991 (Heen et al. 1993). Captive production of brown trout is being developed in France and production of Arctic charr has been initiated in Canada and Scandinavia. Production of these species is still limited in volume compared with other salmonids. The production of Arctic charr in Norway is less than 1,000 tons per year (Mortensen et al. 1992).

In the future, we will probably witness a proliferation of the number of species used in aquaculture. The majority of the new species will probably be marine ones. In Japan, more than 30 marine fish species are now cultured (Davy 1990). Although the culture of many marine species is faced with technical (and/or biological) problems especially at the egg stage and during onset of exogenous feeding, we should expect to see a considerable production of marine fish species in the next one or two decades (Tilseth 1990). The production of farmed cod in Norway was 300 tons in 1987.

The number of accidentally released fish from aquaculture operations is poorly documented, but it must be considerable. For example, 1.2 million fish escaped from net pens along the Norwegian coast during storms in December 1988 and January 1989, and an even higher number escaped during a few days three years later. The biomass of these fish alone (more than 1,200 tons) is equivalent to the total annual yield of wild Atlantic salmon in Norway in the sea and rivers combined (about 1,500 tons; Anonymous 1989). Escapes also occur from freshwater hatcheries, as indicated by findings of hatchery-reared parr in rivers downstream from the hatcheries (Lund & Heggberget 1989).

As a consequence of the escapes, more than 30% of the Atlantic salmon spawners in Norwegian rivers are of farmed origin (Gausen & Moen 1991, Lund et al. 1991). In areas of intense fish culture, up to about 80% of the spawning population consisted of escapees from net pens. The latest estimates of proportions of escaped fish among the spawners suggest that most rivers in Norway receive escaped fish, perhaps except in the farthest northeast where aquacultural production is still small scale.

3.2 Captive rearing of transgenic fish

The recent developments in genetic engineering outlined above suggest that transgenic fish have a potential for cost-efficient aquacultural production of proteins. Limitations exist with respect to finding efficient methods for gene transfer and gene expression, but these are methodological problems likely to be solved in the near future.

If this holds true, there are two other factors which will determine whether or not transgenic fish will be widely used in aquaculture. The first relates to how transgenic fish will be perceived as food by the public. This is a hotly debated topic accompanying the introduction of genetically engineered foods. Whatever its outcome, it is possible that the discussion will settle over other transgenic organisms than fish. For example, transgenic crop plants such as tomato will be introduced to the public on a large scale long before the first marketing of transgenic fish. The US Food and Drug Administration is now reconsidering its year-old policy that genetically engineered foods should not be labeled (San Francisco Chronicle, May 24, 1993, p. C1). In doing so, the agency considers labeling foods based not on health considerations, but on whether consumers have a "right to know" how foods are produced.

The second factor relates to the environmental concerns following the possible escape of transgenic fish to the environment. These concerns are already a major environmental issue following the escapes from aquacultural production of non-transgenic fish. With the wider range of phenotypes which are possible using transgenesis than using traditional breeding methods, this debate will gain momentum with the large-scale production of transgenic fish. Three outcomes which will be discussed in this report are: (1) the environmental concerns are ignored, and transgenic fish will be used in aquacultural production according to their profitability, (2) the environmental concerns will lead to an increasing awareness of problems related to escape, and result in an improved technology to minimize escape rates, and (3) the environmental concerns will lead to transgenic fish being favored over non-transgenic ones, provided that genetic engineering is used to cripple fish that escape

from captivity, as has been suggested by some authors (Maclean & Penman 1990, Powers et al. 1992).

First generation transgenic fish produced in gene transfer experiments are not themselves usable in production (Kapuscinski & Hallerman 1990). Rather, they will serve as progenitors of new genetic lines. These lines will have to be tested with respect to integration, inheritance and expression of the desired trait, as well as in general performance. Only after several generations of inbreeding and testing would transgenic lines have high frequencies of individuals bearing introduced genes in both chromosomal homologues (Christiansen 1990). For Atlantic salmon, which have a generation time of four years in Norwegian aquaculture (Gjedrem et al. 1988), large-scale production of transgenic fish can hardly be expected within the next fifteen years.

If ever realized, production of transgenic Atlantic salmon in Norway will probably be integrated in the national breeding program of this fish. The Atlantic salmon currently raised in fish farming were derived from a number of wild Norwegian populations in the 1970s, and have since then been selected for economically important traits such as growth rate and age at sexual maturity (Gjedrem et al. 1988, 1991). In the 1970s and 1980s, a shortage of Atlantic salmon smolts in the fish farming industry was compensated by imports from the Baltic countries, Iceland and Scotland, but due to increased Norwegian smolt production and the biological disasters following some of the imports, this policy was abandoned and no import has occurred after 1988 (Egidius et al. 1991). If superior transgenic lines of Atlantic salmon were developed in another country, this would create a renewed interest for import. But if we are to learn from history, such imports would probably not be allowed.

3.3 Intentional release of transgenic fish

I do not find it likely that transgenesis will be applied to fish that are intentionally released to the environment for ocean ranching or stock enhancement purposes. This would probably require economic incentives for the development of transgenic fish that could be released with much less potential for unwanted environmental effects than releases of non-transgenic fish. Such an application of transgenic fish is more likely to occur in well-controlled environments for purposes of biological control, than in the largely uncontrolled releases to the marine environment for sustaining fisheries. Kapuscinski & Hallerman (1990) speculate that transgenic, piscivorous fishes, probably sterile, might be used for releases in lakes in North America.

Most intentional releases of Atlantic salmon in Norway have been for local stock enhancement purposes. The largest point releases were compensation measures taken to reduce the effects of watercourse regulation for hydroelectric power production. Smolt releases for ocean ranching have recently been scaled up to surpass the number of smolts released for other purposes. About 1 million Atlantic salmon smolts resulted from intentional releases in Norway during 1992 (Heggberget et al. in press), as compared with an estimated natural production of about 6 million smolts (Ståhl & Hindar 1988). Current Norwegian legislation requires that all intentionally released salmonids be of local stock only, indicating that the use of transgenic lines for release is unlikely.

4 Background: Atlantic salmon in the wild

In order to explore the potential ecological and genetic impact of basing aquaculture on the production of transgenic organisms, we need detailed knowledge not only about the genes transferred, but about the host species itself and the release environment (Tiedje et al. 1989). This chapter serves as an update on salmon biology for those who do not study salmonids in the wild.

4.1 Distribution and life history

Atlantic salmon are distributed in rivers and in the ocean along both sides of the North Atlantic Ocean (MacCrimmon & Gots 1979). On the European side of the Atlantic, salmon are found from the Douro River in Portugal to the Kara River in Russia. On the American side, they are found from the Connecticut River to rivers of the Ungava Bay in Labrador and the Kapitsigdlit River in Greenland. There is no single overview of how many rivers that contain populations of reproducing Atlantic salmon, but it is likely more than 2,000 (Norway alone has about 500 rivers).

The life cycle of Atlantic salmon consists of the following stages (after Mills 1989): adult Atlantic salmon migrate upstream in rivers during spring and summer; eggs are laid in gravel in late autumn; alevins hatch in early spring and 3–4 weeks later start feeding as fry. Parr remain in fresh water for 2–3 years and migrate to the sea as smolt (12–18 cm and 20–60 g) in the spring of their 3rd or 4th year. Salmon travel long distances in the sea and feed there for 1–4 years before returning to fresh water to spawn in their parental stream at sizes ranging from 40 cm and less than 1 kg to 140 cm and 35 kg. Most (70–80%) adult Atlantic salmon survive spawning, but only 3–6% return to spawn a second time. Not all fish migrate; many males become sexually mature and spawn as parr, thereafter they may (or may not) smoltify and migrate to the sea. Some locations not accessible from the sea have Atlantic salmon populations that lead their entire life in fresh water. Not all salmon home accurately; straying rates to other rivers are commonly less than 4% in natural populations (Stabell 1984) but may be higher among small streams.

Altogether, Norwegian rivers contain in the order of 100,000 anadromous Atlantic salmon spawners per year (Ståhl & Hindar 1988). One river, the Tana, probably contains more than 20,000 spawners; in other rivers, the annual number of anadromous spawners appears to be from fewer than 100 to about 1,000.

The life history of two other anadromous salmonids in Norway, brown trout and Arctic charr, differs from that of Atlantic salmon mainly in that they return to fresh water both for overwintering and for spawning. Moreover, their migrations in salt water are coastal rather than oceanic as in Atlantic salmon. The annual sojourn into the sea lasts only for about 30–50 days for Arctic charr and 60–80 days for brown trout (Berg & L'Abée-Lund 1991, Heggberget 1991). Smolt sizes are commonly 15–20 cm for brown trout and 20–25 cm for Arctic charr. Both species mature sexually after 1 to 4 sojourns into the sea and at sizes ranging from 30 to 65 cm (0.3–3 kg).

Brown trout are native to Europe and western Asia, whereas Arctic charr are circumpolar and have the most northerly distribution of any fish spawning in fresh water (Scott & Crossman 1973). Freshwater resident populations are common in both brown trout and Arctic charr throughout the area of anadromy, which for Arctic charr in Norway is restricted to north of 62°N latitude.

Anadromous Atlantic salmon, brown trout and Arctic charr show a variable pattern of dominance of occurrence along a latitudinal transect in northern Norway and Svalbard (Halvorsen et al. 1991). Whereas brown trout dominate numerically in a majority of coastal streams in the county of Nordland (62–69°N), Atlantic salmon dominate in a small majority of streams in both Troms and Finnmark (69–71°N), and Arctic charr is the only species present on the islands of Svalbard (76–80°N). Part of this difference in distribution and dominance have been related to the different temperature requirements of these species (Jensen et al. 1989, and see below).

4.2 Population genetic structure

Salmonid fishes are typically sub-structured into a series of local populations (Allendorf & Utter 1979, Ryman 1983). The amount of genetic differentiation among these populations as estimated from biochemical genetic data is quite large compared with other vertebrates. Atlantic salmon populations worldwide have a third of the total gene diversity resulting from genetic differences between populations (G_{ST}=36%, Ståhl 1987 and **Table 3**); this differentiation among populations arises firstly from a major dichotomy between populations from either side of the North Altlantic Ocean, and secondly from genetic differences between European populations in Baltic and Atlantic drainages. On a more limited geographic range, say, the Norwegian coast or northern Sweden, genetic differences between local Atlantic salmon populations result in a G_{ST} of about 10% (Ståhl 1981, Ståhl & Hindar 1988), which is still as high as that between the three major races of man (Ryman et al. 1983) and more than ten times as high as the differentiation between populations of many marine fishes from a much wider geographic range (e.g. herring, Ryman et al. 1984). In absolute terms, Atlantic salmon are not highly variable genetically as estimated from protein coding loci; the total gene diversity (H_T) in Ståhl's (1987) study was about 0.04. Salmonid species differ quantitatively in the amount and distribution of genetic variation (Ryman 1983). For example, in the brown trout a large part of the genetic diversity occurs between populations even when they are sampled in a restricted geographical area (**Table 3**). Arctic charr appear to have little genetic variation at enzyme-coding loci, at least as estimated for the freshwater resident populations that have been studied. As a contrast, marine species appear to be more genetically variable than salmonids (i.e. a higher H_T; Gyllensten 1985, and **Table 3**), but with less of the genetic diversity caused by differences between populations (i.e. a lower G_{ST}).

The distribution of genetic variation as estimated from biochemically detected loci needs not reflect that of the variation in morphological or ecological traits that are more subjected to natural selection (Crow 1986). A number of morphological and ecological traits are now known to differ between Atlantic salmon populations.

Table 3. Estimated components of gene diversity for natural populations of four salmonid and one other teleost species (data from protein loci).

Species	Study area	Gene diversity		Ref.
		Total (H_T)	Between populations (G_{ST}; %)	
Atlantic salmon	Europe and North America	0.041	36.1	1
" "	Northern Europe	0.034	21.4	2
" "	Northern Sweden	0.025	8.8	3
Brown trout	Sweden	0.040	36.8	2
Arctic charr	Sweden	0.010	24.4	4
Rainbow trout	Western North America	0.069	15.0	2
Herring	Northern Europe	0.087	0.8	5

1 Ståhl (1987)
2 Ryman (1983)
3 Ståhl (1981)
4 Andersson et al. (1983)
5 Ryman et al. (1984)

The following ones have a genetic basis in addition to being environmentally modified: body and fin morphology, egg number and size, survival to various life stages, individual growth rate, precocious male maturity, age at smolting, age and size at sexual maturity, migratory behavior at sea, timing of adult return from the sea, homing precision, and resistance to bacterial diseases and parasites (Saunders 1981, Refstie 1987, Taylor 1991).

We can summarize this overview by stating that there are genetic differences between salmonid populations both in protein-coding loci where single genes and alleles can be identified and in ecological traits that reflect adaptation to different local environments. Precise homing appears to be one mechanism by which such differences can arise and be maintained. Therefore, one consequence of releases of transgenic or otherwise cultured fish into salmon-producing rivers may be a breakdown of the genetic structure and of local adaptations.

4.3 Environmental and interspecific relationships

Freshwater phase. River-dwelling salmonids are adapted to a diversity of depths, water velocities, substrate and cover at different life history stages (Gibson 1993). Freshwater habitats required by the various life history stages can be classified as those suitable (1) for spawning in the late autumn, (2) for feeding during the growing period in spring, summer and autumn, and (3) for overwintering.

Physical characteristics of a typical Atlantic salmon river are a moderately low (0.2%) to moderately steep (1.2%) gradient with a substrate composed of assorted gravel, cobble and boulder. Salmonids require close to fully oxygen-saturated water.

Spawning takes place at the tails of pools on the upstream edge of a gravel bar. Underyearlings stay in shallow water and are commonly found in rapids. Older parr generally stay in deeper, faster water. Slow-flowing and lentic (lake) habitats may be occupied in the absence of severe competitors or predators. Parr prefer water velocities larger than 0.2 m/s, which may be important for the shelter provided by the broken water surface or for prey availability (Gibson 1993).

At temperatures below 7-9°C Atlantic salmon parr shelter in the substrate in riffle areas, but may move to pools. Towards winter all fish are found in areas of low flow, where they remain largely inactive. In addition, parr may inhabit estuaries.

Survival from egg to hatching in Atlantic salmon has been estimated at 74-91%, from emergence to the end of the first growing season at 9-31%, and per year during the rest of the freshwater period at 40-51% (Gibson 1993).

Growth is controlled by the availability of food and by temperature limitations. Feeding is generally opportunistic. The food consists mainly of insects, primarily taken in the water column, but also from the surface and the bottom. In many northern streams food is limiting, and in these regions stream fertility may have relatively greater effect on growth rates than the temperature regime. Adult salmon do not feed upon return to fresh water.

Atlantic salmon prefer cool temperatures with feeding in the range of 7-22.5°C (Elliott 1991). Among eight salmonids tested, Atlantic salmon had the highest temperature requirements for survival, feeding, and growth (Elliott 1981, and **Table 4**). For example, feeding in brown trout is common at temperatures down to 4°C and in Arctic charr as low as 2°C. Elliott (1991) noted the contrast between Atlantic salmon and brown trout, which show a 3°C difference in lower and upper temperature limits as well as in the optimum temperatures for growth. As these species are often sympatric, small changes in temperature could favor one species at the expense of the other.

Power (1969, 1990) noted that in North America, temperature was the most likely factor limiting distribution of Atlantic salmon. He suggested that an annual growing season of 100 days above a water temperature of 6°C was a minimum requirement for existence of salmon. In a cold Norwegian stream, salmon experience at or above 7°C for only 67 days per year, suggesting that populations in cold freshwater

Table 4. Optimum temperature range, upper and lower critical ranges for four salmonid species (From Elliott 1981, 1991). All temperatures are in °C.

Species	Critical temperature		Optimum temperature	
	Lower	Upper	Range	Preferred
Atlantic salmon	0–2*	20–34	6–20	16–17
Brown trout	0–4	19–30	4–19	13–15
Arctic charr	ca.0	22–27	5–16	12–14
Rainbow trout	0–9	19–30	10–22	12–19

*Values vary with acclimation temperature and possibly with source population

environments have adapted to growing at lower temperatures (Jensen & Johnsen 1986).

Temperature affects survival as well as growth. Overwinter survival in salmonids is positively correlated with temperature and fish size. Summer deaths caused by extremely high temperatures have also been recorded.

Young salmon in running water characteristically are territorial, a behavior which is interpreted as a social behavioral response to a limited food resource, in which interference competition for space has been substituted for a direct (exploitation) competition for food (Chapman 1966). Territory size may be related to visual isolation, and therefore negatively associated with substrate heterogeneity and structure (Kalleberg 1958) and water velocity. Territory size, is also negatively dependent on food abundance, intruder pressure, degree of kinship, and positively dependent on fish size.

At high densities salmonids may abandon territorial behavior and instead form dominance hierarchies, or school, related to habitat, temperature, food, social interactions, predators or other factors. Also, territories may not be defended in deeper and slower waters.

Saltwater phase. Atlantic salmon roam in most of the northern Atlantic ocean. Two major feeding areas are around the Faroe Islands (60–66°N) and northwards to 72°N, and along the coasts of Greenland from Ammassalik (66°N) on the east coast to Kangerluk (70°N) on the west (Shearer 1992).

Direct relationships have been demonstrated between ocean climate, sea surface temperature and the abundance and distribution of salmon at sea. Older salmon can tolerate colder sea–surface temperatures than younger fish; temperatures colder than 3–4°C appear to be avoided if possible.

Smolts have a limited tolerance to low sea water temperatures. At lower temperatures than 6–8°C, they do not regulate the plasma salt levels well and can easily die from dehydration (Sigholt & Finstad 1990). In the northernmost parts of the distribution of Atlantic salmon (e.g. Ungava Bay) sea temperatures during summer may be as low as 0–2°C. Populations in these areas appear to have developed special adaptations to the cold marine environment such as exceptionally large smolt sizes and a high degree of residual (non–anadromous) populations (Power 1969). Sea water temperatures therefore appear to be more limiting to the geographical distribution of Atlantic salmon than freshwater temperatures. In accord with this, the eastward expansion of Atlantic salmon into the Kara Sea (and the Kara River) of Russia in the 1920s appeared to be enhanced by a strong inflow of warm Atlantic water (Jensen & Johnsen 1986).

Mortality in the sea is quite high during the smolt and early post–smolt stages, especially from predation by cod, saithe (*Pollachius virens*) and various species of

birds (Shearer 1992). The growth rate at sea is rapid, and the salmon soon attain sizes where natural mortality is low. In the River Esk, Scotland, the survival rate of cohorts of Atlantic salmon from emigration as smolts until return as adults varied between 14–53% during 1964–85 (Shearer 1992).

When at sea, Atlantic salmon eat a variety of marine organisms including crustaceans, cephalopods and fishes such as lantern fishes (Myctophidae), capelin (*Mallotus villotus*), herring (*Clupea harengus*) and sand eel (*Ammodytes lancea*). The food of Atlantic salmon in the ocean overlap with that of brown trout and Arctic charr, indicating a potential for competition between these species at sea (Grønvik & Klemetsen 1987). This competition must, however, be limited in time since Atlantic salmon and the other two salmonid species have largely non-overlapping feeding areas. Virtually nothing is known about competitive (or predatory) relationships between Atlantic salmon and other fishes in the marine environment.

5 Potential ecological and genetic effects of transgenic fish

Releases of genetically engineered organisms may directly or indirectly lead to changes in the natural environment. The environmental effects may be divided into three broad categories (Williamson et al. 1990): (1) effects caused by the genetically engineered organism itself, (2) effects resulting from dispersal of genes from the genetically engineered organism to other organisms in the environment, and (3) altered practice in the use of an organism because of the genetic modification (for example, expansion of salmon aquaculture to the north following the use of transgenic salmon expressing antifreeze protein genes).

The intended effects of transgenic organisms are usually specific with regard to species, environment and period of time. Evaluations of environmental effects must, however, also include other species and environments, because both the transgenic organisms and their genes can spread in time and space after deliberate or accidental release (TeknologiNævnet 1990). It will therefore always be a formidable task to provide documentation on the environmental effects of a release, and almost impossible to predict them. Moreover, it is important to note that genetically engineered organisms are exposed to natural selection and may evolve better adaptations to the environment (Gould 1991).

In this chapter I shall discuss some real or potential dangers following the release of genetically engineered fishes with emphasis on salmonids. Since experience with transgenic salmonids is still nonexistent, I first focus on examples of environmental effects from the deliberate and accidental release of non-modified salmonids. In doing this, I draw on evidence from a review of the environmental effects that have occurred following releases of salmonids throughout the northern hemisphere over the last hundred years (Hindar et al. 1991). In the latter part of the chapter, I combine this information with knowledge about transgenic fish to speculate about the possible environmental effects of transgenic salmonids.

5.1 Principles for risk assessment

The Ecological Society of America has proposed the following principle for risk evaluations when genetically engineered organisms are released to the environment (Tiedje et al. 1989):

> "Genetically engineered organisms should be evaluated and regulated according to their biological properties (phenotypes), rather than according to the genetic techniques used to produce them."

This means that the release of genetically engineered organisms need not differ from such release of other organisms, as regards their threat to the environment. Others have pointed out that the technique used in the genetic engineering must also be an object for assessment. There is, for example, little control on the final location of a gene that is inserted in the genome of animals and plants. This can have consequences for the stability of the genome, first and foremost with respect to regulating gene expression (Kjelleberg & Fagerström 1990).

There will always be an element of biological gambling involved in the release of genetically engineered and other (non-modified) organisms. Our genetic and ecological knowledge is inadequate to allow us to give *a priori*, precise risk assessments for releases (Simonsen & Levin 1988, Drake et al. 1989). We can provide general guidelines on how to assess environmental effects, but we are unlikely to obtain sufficient knowledge of the ecological interplays within and among species to provide precise predictions. This is because we neither know the genes that are important for adaptations to the environment nor the selective forces acting on those genes. Other information which is necessary for making precise genetic and ecological predictions, including population size, migration of individuals and genes between populations, the evolutionary history of the species, its food habits and natural enemies, are also unknown quantities in nature – even among well-studied organisms (Levin 1992, Ryman et al. in press).

We are unlikely to obtain the necessary ecological and genetic knowledge to make precise risk assessments within the foreseeable future. Hence, the best we can do is to base our opinions on experience and give as precise *a posteriori* risk assessments as possible. An important principle is therefore that the environmental effects of genetically engineered organisms must be evaluated on a case-by-case basis through a stepwise procedure that includes fully enclosed pilot studies (OECD 1986). There is broad international agreement about this principle (Royal Commission 1989, Tiedje et al. 1989).

Even a risk assessment based on the principles of "case-by-case" and "step-by-step" has limitations such that (1) we have to use short-term experience to assess long-term impacts, (2) we can only test the possible problems we are aware of, and (3) an inability to prove any impact can be caused by problems with the method or that the investigation does not cover all the relevant effects (Simonsen & Levin 1988). Moreover, it has been shown in many cases of deliberate releases that the result largely depended upon how many individuals were released (Drake et al. 1989, Griffith et al. 1989). The results of small-scale experiments need not therefore translate directly to large-scale releases.

5.2 Effects of releasing cultured fish

The growing number of escaped Atlantic salmon in feeding areas and spawning populations has led to considerable conjecture about the genetic, epidemiological and ecological effects of the cultured fish on native populations (Hansen et al. 1991). In Norway, the debate has centered around two themes: (1) the reproductive success of escaped fish, and (2) the full spectrum of biological effects caused by escapees on native populations, whether or not the escapees spawn successfully (Hindar 1992).

5.2.1 Behavior and reproductive success

Atlantic salmon released as smolts in fresh water tend to return to the site of release to spawn, but with somewhat higher straying rates than wild smolts (Jonsson et al. 1991). Smolts or post-smolts escaping from a marine site return to the area in the sea from which they escaped, but as they lack a home river, they will enter several

rivers in that area to spawn late in the season (Gunnerød et al. 1988, Hansen et al. 1989). Post-smolts escaping during winter tend to stray more and farther away than fish escaping during the rest of the year (Hansen & Jonsson 1991). Adults escaping during summer seem to enter rivers at random (Hansen et al. 1987). Post-smolts escaping in late summer, fall and winter have poor survival to the adult stage compared to those escaping in spring (Hansen & Jonsson 1989). Survival, however, increases with increasing fish size, and may be very high for escaping adults.

The argument of 'no documented successful spawning' was for long used in favor of taking no action to reduce the number of escapees (cf. Sattaur 1989). This argument has been shown to be false. Spawning of farmed Atlantic salmon escaping to Norwegian and Scottish rivers has been documented on the basis of observations of distinct pigmentation differences between the eggs of wild and pen-reared fish (Lura & Sægrov 1991a,b, Webb et al. 1991). In three rivers having high proportions of escaped fish among the spawners, the proportion of redds containing eggs of pen-reared fish was between 15 and 50%, clearly indicating the potential for successful reproduction. The reproduction of escaped females did not appear to be considerably different from that of wild fish, either in the River Oselven, Norway (Lura & Sægrov 1991b, Vidar Moen, University of Trondheim, pers. comm.) or in the River Polla, Scotland (Webb et al. 1991).

Other studies have used genetic markers in an attempt to quantify the reproductive success of hatchery-produced or farmed fish. The reproductive success of hatchery-produced steelhead trout (anadromous rainbow trout) was compared with that of native, wild steelhead throughout the life cycle (Chilcote et al. 1986, Leider et al. 1990, Campton et al. 1991). The hatchery-produced fish was found to contribute substantially to the reproduction of steelhead in the river. As estimated at the adult stage of the offspring, Leider et al. (1990) found that the relative reproductive success of hatchery trout was 11-13% of their wild counterparts.

Farmed brown trout carrying genetic markers at a biochemically detected locus as well as in a morphological character were introduced into two native brown trout populations by Skaala (1992, and references therein). Among the resulting offspring, both individuals homozygous for the "farmed" allele and heterozygotes were found, suggesting successful reproduction of the farmed fish as well as hybridization between farmed and wild fish. The reproductive success of farmed fish was estimated at 11-24% of that of wild fish when compared at the end of the first growing season of the offspring (Skaala 1992).

5.2.2 Competition, predation and displacement

Intraspecific. In the River Imsa, southwestern Norway, a number of experiments in the river itself as well as in spawning arenas have addressed the behavior and competitive ability of ocean-ranched and farmed Atlantic salmon. River releases have shown that both Atlantic salmon females and males of ocean-ranched origin spawn upon return to the river. However, the proportion of unspawned individuals was higher among ocean-ranched fish than among wild fish, and more so for males than for females (Jonsson et al. 1990, 1991).

Controlled experiments with Atlantic salmon of River Imsa origin show that spawning success is higher for wild than for hatchery-reared fish, even when the hatchery fish is of River Imsa origin (Bror Jonsson, Norwegian Institute for Nature Research, Trondheim, pers. comm.). The differences are smaller when the hatchery-produced fish were released to the wild as smolts than when they were held in captivity until shortly before spawning. Males of hatchery origin are less successful than females. Ocean-ranched females spawn with about as high reproductive success as wild females, whereas farmed females have significantly lower reproductive success (B. Jonsson et al., in preparation). Similarly, in coho salmon (*Oncorhynchus kisutch*), Fleming & Gross (1993) observed reduced reproductive success of ranched hatchery fish in competition with wild conspecifics. In particular, hatchery males were subordinate and were denied access to spawning females. The breeding success of hatchery fish relative to wild fish decreased with increasing density.

Superimposition of redds is common among salmonids, especially when the density of spawners is high. Late-spawning individuals may dig up the eggs of early-spawning fish, and thereby lower the latter's reproductive

success. It has been observed that farmed females may destroy the redds of wild fish in nature (Lura & Sægrov 1991b). Thus, although escaped farmed salmon themselves may have a low reproductive success, they can nevertheless reduce the success of wild fish and thereby the total salmon production of the system.

Little is known about juvenile interference competition between offspring of hatchery-produced and wild fish. A recent review indicates that release of hatchery coho, chinook, and steelhead (anadromous rainbow) trout into streams with resident salmonids disrupts the social order of the natural populations and reduces production of the wild fish (Riddell & Swain 1991).

Interspecific. A number of introductions of salmonid species outside their native range have taken place both between and within continents. For example, self-sustaining populations of North American salmonids, most notably brook trout (*Salvelinus fontinalis*) and rainbow trout, have been established in Europe, South America, Africa, Asia and Australia. Likewise, the European brown trout has been successfully introduced to all continents except Antarctica.

Introductions of brown trout appears to be the major factor causing declines and disappearance of many brook trout populations in eastern North America. Apparently, brown trout exclude brook trout from preferred feeding and resting positions in streams by interference competition. In other eastern American streams, brook trout have been replaced by rainbow trout introduced from Pacific drainages, although the mechanisms by which this replacement took place remain conjectural (Krueger & May 1991). In western North America, cutthroat trout (*Oncorhynchus clarki*) have been replaced by introduced brook trout, and – although mechanisms are unclear – apparently also by introduced lake trout (*Salvelinus namaycush*), rainbow trout and brown trout.

Knowledge about the mechanisms by which one species replaces another may be useful when extrapolating these observations to speculations about transgenic fish. In streams, competition may occur as species attempt to secure territories for adequate space and, therefore, food and cover (Chapman 1966). Such interference competition is primarily mediated through aggressive behavior towards other individuals. Brown trout appears to be the most aggressive of the salmonids, more than its congeneric Atlantic salmon, and more than all of the *Oncorhynchus* and *Salvelinus*. Among the *Oncorhynchus*, it appears that rainbow trout are more aggressive than cutthroat trout (Nilsson & Northcote 1981).

Size-related competitive ability may be another mechanism by which one salmonid species gains a competitive advantage over another species. It has for example been observed that rainbow trout have an advantage over brook trout because of their faster growth rate, and that brook trout have an advantage over cutthroat trout because of an initial size advantage due to earlier emergence (Krueger & May 1991).

In lakes, competition by interference may be restricted to the littoral zone, whereas competition for food resources that are not easily defended, such as zooplankton and profundal zoobenthos, most likely takes place by exploitation. Charrs of the genus *Salvelinus* are more efficient planktivores than salmonids of the genera *Salmo* and *Oncorhynchus* (Hindar et al. 1988). Among the latter, it appears that sockeye salmon (*O. nerka*), which are lake-dwelling as parr and often form lake-resident populations, are the best adapted to a plankton diet.

Predation is a less well documented phenomenon among salmonids than competition, but may be of importance in the replacement of brook trout by introduced brown trout, and in the replacement of cutthroat trout by introduced lake trout (Krueger & May 1991). Large individuals of both brown trout and lake trout are piscivorous both in their native habitats and where introduced.

5.2.3 Interbreeding

Intraspecific. Two broad conclusions regarding genetic effects on native salmonid populations emerged from a review of the empirical observations following releases of non-native salmonids to the environment (Hindar et al. 1991):

1. The genetic effects of (intentionally or accidentally) released salmonids on natural populations are typically unpredictable; they vary from no detectable effect to complete introgression or displacement.

2. Where genetic effects on performance traits have been detected, they appear always to be negative in comparison with the unaffected native populations.

First, the advent of enzyme electrophoresis in the 1960s made it possible to detect genetic changes at the level of single genes. Such techniques applied to salmonid population genetics have demonstrated a wide variety of outcomes following natural reproduction of cultured fish (Hindar et al. 1991, and references therein). At one extreme is displacement of local stocks from massive introduction of non-native fish (e.g. Altukhov 1981), substantial introgression of native and released stocks of the same species (Taggart & Ferguson 1986, Guyomard 1989), and highly elevated hybridization rates among native and non-native subspecies (Allendorf & Leary 1988). At the opposite extreme are reports of no detectable introgression into native populations despite substantial introductions of non-native fish (Wishard et al. 1984, Vuorinen & Berg 1989). Presumably, the non-native fish have been unable to reproduce in the new environment.

A highly variable rate of interbreeding between released hatchery stocks and native populations have been indicated by several studies (Taggart & Ferguson 1986, Guyomard 1989). This occurred despite the fact that the introductions were based on the same hatchery stock and were carried out according to the same procedure for release. This observation should remind us that our ability to predict the genetic consequences of a particular release is limited.

Second, limited direct evidence, but much indirect evidence, suggests a generally negative impact on native populations following the intentional or accidental introduction of cultured fish to the wild (Hindar et al. 1991, and references therein).

Studies of the juvenile stages in fresh water have shown that survival is commonly better for local wild fish than for released, hatchery-produced ones (Leider et al. 1990, Skaala 1992). A direct genetic basis for reduced juvenile survival was indicated in a study of rainbow trout, where survival was higher in wild fish than in either non-native cultured fish or in wild x cultured hybrids (Reisenbichler & McIntyre 1977). Other studies show that genetic changes occur in hatchery-propagated salmonids that would be expected to reduce their performance in the wild, for example regarding swimming stamina, territorial behavior and concealment behavior. Moreover, disease resistance differs between native and introduced juveniles; always in favor of local fish unless the pathogen (or parasite) itself was introduced (Johnsen & Jensen 1991).

Studies of the post-smolt and sub-adult stages in the ocean are of a less detailed nature, but typically show lower return rates of hatchery-produced fish than of wild fish, and lower return rates of transplanted and crossbred than of native populations (e.g. Ricker 1972). This could be related to lower ocean survival or increased straying rate, or both, and could have both genetic and phenotypic causes. A reciprocal transplantation experiment with masu salmon (*Oncorhynchus masou*) suggests a genetic basis for differences in sub-adult and adult survival between local and transplanted populations (Mayama et al. 1989). The local stock outperformed the transplanted one both with respect to the proportion of fish recovered in or near the river of release, and the subsequent survival in the river prior to spawning. Mayama et al. (1989) did not find differences in straying rate between local and transplanted fish, whereas for pink salmon (*O. gorbuscha*), Bams (1976) showed that straying rate of returning adults were higher for transplanted than for native fish.

The typically negative effects of releases of non-native salmonids are not unexpected; if salmonid populations are adapted to their local environments, any introduced populations or crosses involving introduced populations should be expected to perform worse than the native ones (cf. Templeton 1986). The decline in fitness of intraspecific hybrids is known as 'outbreeding depression'. Comprehensive discussions of outbreeding depression have been given by Templeton (1986) for various organisms and by Waples (in press) for fishes. Few cases exist where outbreeding depression have been

experimentally verified in fishes. Recently, however, outbreeding depression has been indicated in artificial crosses between even- and odd-year pink salmon (Gharrett & Smoker 1991), and in detailed studies of the fitness of first- and later-generation hybrids between native northern largemouth bass (*Micropterus salmoides salmoides*) and introduced Florida largemouth bass (*M. s. floridanus*; Philipp 1991).

Interspecific. Releases of salmonids into areas beyond their natural geographical range appear to be accompanied by increasing hybridization rates with closely related native species. Such hybridization has occurred between released brown trout and native Atlantic salmon in Newfoundland (Verspoor 1988) and between released rainbow trout and native cutthroat trout in western USA (Allendorf & Leary 1988). One study indicates that interspecific hybridization rates may increase as a result of releases of exogenous stocks of one species even in areas where the two species coexist naturally. In Spain, Garcia de Leániz & Verspoor (1989) observed hybrids between Atlantic salmon and brown trout that suggested the Atlantic salmon parent was from an introduced Scottish strain. In Norway, high hybridization rates have been observed in rivers having high proportions of escaped or intentionally released Atlantic salmon, but it is not yet known whether the high hybridization rates are a consequence of the releases (Kjetil Hindar & Torveig Balstad, Norwegian Institute for Nature Research, unpublished data).

Intergeneric hybrids between brook trout and brown trout have been observed in North America following brown trout introductions (Krueger & May 1991), as well as in Norway following plantings of brook trout (Dag Matzow, Aust-Agder County Administration, Arendal, pers. comm.).

5.2.4 Disease introduction

Diseases are a serious problem in salmonid culture throughout the world, and represent a threat to many salmonid populations in nature. Transfer of fish between culture operations has led to pathogens and parasites coming in contact with fish populations to which they were not adapted. These new host-pathogen confrontations have led to unnaturally high fish mortality.

Two examples from Norway may illustrate this point: First, Atlantic salmon populations in 35 Norwegian rivers have been greatly reduced since 1975 following introductions of fish from hatcheries infected by the monogenean parasite *Gyrodactylus salaris* (Johnsen & Jensen 1986, 1991). This parasite does not appear to be native to Norway, and was probably imported with salmonid eggs or juveniles from infected localities in the Baltic. Its occurrence in the wild may be traced back to a few infected hatcheries from where fish were transported to other hatcheries or released directly into rivers. Laboratory studies suggest that there is a genetic basis for the different effect of the parasite in Norway and in the Baltic; in the latter area populations resistant to the parasite have been found whereas the Norwegian populations tested are highly susceptible to the parasite (Bakke et al. 1990, Jansen et al. 1991).

Second, furunculosis caused by the bacterium *Aeromonas salmonicida* may lead to similar dramatic effects on natural populations. In 1985, this pathogen was introduced to Norwegian fish farms with infected smolts from Scotland (Håstein & Lindstad 1991), and spread from the first few infected farms to reach 32 fish farms in 1988 and more than 500 fish farms (65% of the total) by the end of 1991. Of the 1.2 million smolts that escaped from fish farms during the winter of 1988-89, more than 250,000 fish were from farms infected with furunculosis. Not unexpectedly, the disease was found among spawning salmon in the autumn of 1989, firstly among farmed escapees and later also among wild fish. By 1991, furunculosis had been registered in 63 Norwegian rivers (Heggberget et al. in press). In at least three rivers, the disease had reached epidemic proportions.

The furunculosis example illustrates another point: On the one hand, it was registered in wild Atlantic salmon in one Norwegian river from 1968 onwards, probably caused by the release of rainbow trout in a tributary to that river (Håstein & Lindstad 1991). No detection of the disease occurred after 1979, and no spread to other rivers was detected. On the other hand, after the rapid build-up of fish farms along the Norwegian coast, the new

introduction of furunculosis to the Norwegian fish farming in 1985 rapidly spread through the whole industry and may reach all salmon rivers within a few years. This shows the enormous potential that aquaculture activities have for the spread of epidemics. Simple epidemiological theory predicts epidemics to follow in the wild under these circumstances, even though (the same theory predicts that) an epidemic is usually not the outcome under natural conditions (e.g. Anderson & May 1986).

5.2.5 Effects on population size

A direct, negative effect on stock size appears to have followed a massive egg transfer between populations of chum salmon (*Oncorhynchus keta*) on Sakhalin Island (Altukhov 1981, Altukhov & Salmenkova 1991). There, the total run to the Naiba River fell by 95% as compared with the undisturbed Kalininka River. Another example relates to an attempt to rebuild coho salmon populations in Oregon by stocking hatchery presmolts (Nickelson et al. 1986). Although the juvenile density in 15 stocked streams surpassed that in 15 unstocked streams during the summers following stocking, the density of returning adults did not differ between the two groups, and juveniles were less abundant in the stocked than in the unstocked streams in the subsequent generation.

In Norway, it appears that introduction of parasites and pathogens is the major cause of the decline of salmonid populations at the moment. The threat posed by infectious diseases has by no means reached its full manifestation in wild salmonid populations. Several diseases now being fought in fish farms have yet to be discovered in the wild, or have only recently been registered in one or a very few wild populations (Håstein & Lindstad 1991).

A parasitic salmon louse, *Lepeoptheirus salmonis*, which for long has been a problem to the salmon farming industry, may well turn out to be a major cause for declining populations of Atlantic salmon and brown trout. The salmon louse, which at high densities may kill anadromous salmonids at the postsmolt stage (Finstad 1992), are now so abundant in fish farms that Atlantic salmon and brown trout smolts may become heavily infected when they enter the sea near a fish farm.

A decline of the local wild population has been observed to accompany releases without any detectable introgression or disease introduction (Hindar et al. 1991). The reasons remain obscure, but it is possible that both competition between released and wild fish, and increased predation rates contribute to the decline. Indirect effects on stock size may also occur where small natural populations are being overharvested in a mixed fishery with more abundant cultured fish (cf. Larkin 1981). Documentation that overexploitation of native stocks occurs, either as a result of increased fishing pressure or as a result of elevated predation rates, is so far limited. However, modelling studies suggest that overexploitation of wild fish may be widespread even in the absence of reproduction of the released, cultured fish (Evans & Willox 1991).

In concert with this rather disturbing picture, an increase in the population size of wild rainbow trout has been reported following the cessation of a stocking program of hatchery-reared rainbow trout (Vincent 1987). Four years after a 15-year long stocking program was closed, the number and biomass of two-year old and older rainbow trout had increased by 809% and 1,016%, respectively.

5.3 Possible effects of releasing transgenic fish

It is evident from the above observations that transgenic salmonids must be evaluated both with respect to their effect at the organismal level, at the gene and population level, and with respect to the effects caused by altered aquacultural practice following production based on transgenic individuals (cf. Williamson et al. 1990).

5.3.1 Ecological novelties

All of the effects that were summarized in the previous sections should be considered when predicting environmental effects of transgenic fishes. Moreover, many transgenic fishes will be likely to pose greater ecological risks than escapes from conventional aquaculture because "organisms with novel combinations of traits are more likely to play novel ecological roles, on average, than are organisms produced by recombining

genetic information existing within a single evolutionary lineage" (Tiedje et al. 1989).

In this section, two types of transgenic salmonids – both of them already a reality in the laboratory – will be discussed with respect to the potential effects on wild salmonids. One is transgenic fish with inserted growth hormone genes for enhanced growth rate. The other is transgenic fish with inserted antifreeze genes for enhanced tolerance to cold sea water.

Enhanced growth rate. According to Tiedje et al.'s description of ecological novelties, it is doubtful whether fish with inserted growth hormone genes qualify for that category or not. On the one hand, transgenic fish with extra copies of species-specific growth hormone genes would not be considered dramatically changed by gene technology. On the other hand, if their growth rates were beyond the range expressed by non-modified individuals of the same species, they could definitely play novel ecological roles upon release or escape.

Since large body size is one mechanism by which one fish gains a competitive advantage over another, faster growth rate in transgenic Atlantic salmon would mean that they could be at an advantage both in intraspecific and interspecific contests in rivers. In the former case, this would affect native Atlantic salmon populations negatively; in the latter, it would first of all affect brown trout coexisting with Atlantic salmon. In non-modified fish, growth rate in fresh water is faster in brown trout than in Atlantic salmon. This rank order would most likely be reversed when comparing brown trout with transgenic salmon.

Based on studies of growth hormone administration to fish, transgenic fish could also have higher appetite (Weatherley & Gill 1987). The food requirements of the transgenic fish would therefore easily lead to starvation in periods of food shortage (Powers et al. 1992). If so, transgenic fish would experience poor survival in the many rivers where food limits growth rate, and most probably even in more productive rivers during periods of food shortage (i.e. usually in late summer).

The combined effect of a transgenic fish that on the one hand has periods of faster growth rate than native fish, and on the other hand has periods of higher mortality, would very much depend on when and how they entered the stream ecosystem. If they entered through the reproduction of transgenic fish, they would survive the start-feeding period depending on whether the timing of emergence matched the peak abundance of food organisms. Those surviving the start-feeding period would most probably find difficulties surviving the first autumn in unproductive rivers and would survive longer in productive ones. If, on the other hand, the transgenic fish entered the river through escapes from freshwater hatcheries, there would probably be larger effects on the river ecosystem, because there would always be new fish entering the river, and some of them would be very large-sized compared with wild fish.

Little or no information exists for transgenic fish on changes in other traits that are genetically or phenotypically correlated with growth rate (Kapuscinski & Hallerman 1991). At present, we have to rely on information gained from exogenous administration of growth hormone to fish.

As noted previously, growth hormone plays a role in osmoregulation in anadromous salmonids (Powers 1989, Boeuf 1993). During migration from fresh water to salt water, levels of growth hormone are elevated, leading to an increase in salt (Na^+) exclusion at the gills. Transgenic, emigrating smolts would therefore be likely to avoid predation better than wild smolts upon entering salt water, because they would adjust faster to the saline environment and thereby escape estuarine and coastal predation. Two factors would contribute to the faster osmoregulatory adjustment of transgenic smolt to salt water: (1) a higher level of growth hormone, and (2) a larger body size.

Other physiological effects of elevated levels of growth hormone would probably be earlier sexual maturation and a shorter life-span (cf. Weatherley & Gill 1987). This suggests that even though transgenic growth-enhanced fish would be larger than wild fish of the same age, they would not necessarily be so as spawners – especially in rivers where the local stock is composed of late-maturing fish. In rivers where the spawning population consists of grilse (salmon maturing after one winter in the sea), transgenic escapees would probably be larger than wild fish and would be more likely to dominate on the spawning grounds.

Most fish that escape from fish culture today do so from net pens in the marine environment. Considering the extremely rapid growth of Atlantic salmon in salt water, I expect there would be only very short-term problems with food shortage for transgenic fish, unlikely to lead to increased mortality. Thus, escaped transgenic fish would be at a selective advantage compared with wild salmonids in salt water. This suggests a generally negative effect on other salmonids through intraspecific and interspecific competition. The effects on marine species can only be guessed upon as long as we know so little about their interaction with Atlantic salmon.

Enhanced cold tolerance. Transgenic salmonids expressing antifreeze protein genes from winter flounder or any other species which possess such genes would definitely fall in the category of 'ecological novelties'. Transgenic salmon incorporating genes that have existed in a different, non-salmonid evolutionary setting for at least a hundred million years could play very different ecological roles than wild salmon. The effects of transgenic cold-tolerant fish would most probably be largest on wild populations of Atlantic salmon and Arctic charr, and on marine fishes which express antifreeze proteins naturally.

Interactions between transgenic salmon and marine fishes can only be superficially treated at the present level of knowledge. Several species of fishes express one or other type of antifreeze proteins (Fletcher et al. 1992), but for most of them we know very little about their ecological interactions with Atlantic salmon. Some species are known as prey species for Atlantic salmon, e.g. smelt (*Osmerus* spp.) and herring, and would be subjected to predation by transgenic salmon in a larger geographic area and/or during a longer period of the year than at present. Other species such as northern cods, winter flounder, ocean pout, and wolffish are more likely to be involved in competitive interactions with Atlantic salmon, if they interact at all.

Antifreeze proteins would confer a selective advantage to salmon in any location where cold sea water constrains their life history today. For example, in Ungava Bay, average smolt sizes and ages of over 25 cm and 5 years have been recorded (Power 1969), and there are indications that the life cycle is curtailed in these rivers through maturation in fresh water and no anadromous migration. A pre-smolt expressing antifreeze proteins would most probably be able to emigrate at a smaller size and a younger age, and gain weight (and fecundity) in the sea to spawn at a younger age than individuals of the local populations. Thus, one can easily envisage how transgenic Atlantic salmon could outcompete native fish in these rivers. This effect would be further strengthened if the use of transgenic freeze-resistant fish moved aquacultural production to the north (see section 5.3.3).

If sea temperatures limit the geographical distribution of Atlantic salmon, transgenic freeze-resistant fish would most likely contribute to an increase in the distribution of the species. This would put Arctic charr populations at risk, especially river populations which are considered to be less competitive in that environment than most other salmonids, and which therefore thrive only in physically extreme environments (Svenning 1991).

Do antifreeze proteins only work at sub-zero temperatures or do they have an effect on metabolism at other low (but above zero) temperatures? In the latter case, transgenic salmon could gain a competitive advantage towards brown trout and Arctic charr, which are active at lower freshwater temperatures than Atlantic salmon. But according to Devlin & Donaldson (1992), antifreeze proteins do not play a role above the freezing point and could even be an unnecessary burden.

Many of the potential ecological effects mentioned here, especially those in rivers, could easily be studied with non-transgenic fish. As fish have been shown to respond to injections (and other exogenous administration) of both growth hormone and antifreeze proteins, experiments could be carried out in stream channels to study the effects of such physiologically altered fish on other riverine fishes and on the freshwater biota at large. Even though such experiments could not mimic the whole spectrum of the effects of transgenic fish, they would go a long way of studying major ecological problems.

5.3.2 Interbreeding

The effects caused by interbreeding between cultured fish and native populations have been described in detail above, and shall not be repeated here. Transgenic fish

could result in the same changes in the natural populations, both with respect to changes in genetic structure and with respect to loss of genetic adaptations to local environmental conditions.

Two new aspects that relate specifically to transgenic fish will be briefly commented upon. First, the hybrids produced by crosses between transgenic and wild fish will be heterozygous for the transgenic trait. The strength of natural selection against (or for) the new trait will depend on the expression of this trait in heterozygotes relative to homozygotes (Crow 1986). A well established single-locus theory can be used to predict the fate of the transgenes at various levels of gene flow from transgenic to wild fish, and at various selection regimes and dominance relationships. It should be noted that when immigration rates into natural populations are very high, the recipient populations may be swamped by the inflowing genes irrespective of the strength of selection (Haldane 1932). These effects can be studied by simulation (for an example, see Hindar & Bakke 1991).

Second, transgenic fish in aquacultural production will have gone through one bottleneck more than traditionally bred fish (Christiansen 1990, Kapuscinski & Hallerman 1991). This results from the inbreeding which occurs when homozygous lines are produced from experimentally established transgenic individuals. Escapes of transgenic fish can therefore lead to an even more rapid loss of genetic variation in the recipient native populations than escapes of cultured fish, other factors being equal (Ryman 1991).

When is there too much interbreeding? A multi-gene model to evaluate the consequences of hybridization between two salmonid populations adapted to different environments was recently developed by Emlen (1991). Given sufficiently different selective regimes for the two stocks, which would certainly be the case if one population is in captivity and the other is wild, he concluded that (1) in a scenario involving periodic mixing of populations, non-trivial reductions in fitness may occur even if the mixture proportion is as low as 5-10%, and (2) recovery of fitness following a single hybridization event may require a large number of generations. In accord with this, Mork (1991) showed that a considerable proportional reduction in genetic diversity between natural populations would result from one generation of intrusion of the same aquacultural stock into all of them.

Another quantitative assessment of the genetic effects of interbreeding was provided by Ryman (1991, Ryman et al. in press), who suggested to use the level of naturally occurring gene flow as a quantitative guideline for permissible introgression rates into wild fish populations. Based on the relationship between observable genetic differentiation among populations (G_{ST}) and the number of effective migrants per generation in Wright's (1969) island model, the acceptable introgression rate for an average G_{ST} of 10% (cf. **Table 3**) would be only 2 individuals per generation. This is a long way off the massive numbers of escaped cultured salmon now spawning in Norwegian rivers, and should mandate an entirely new approach to containment of cultured salmonids.

5.3.3 Altered use of cultured fish

Altered use of the organism following production based on transgenic individuals is the third of the categories of environmental effects identified by Williamson et al. (1990). In my opinion, the possible area expansion of aquaculture following production of transgenic cold-tolerant salmon could lead to the most dramatic environmental effects of the production of transgenic fish. This contention is based on (1) the regrettable fact that the current administration of Norwegian aquaculture does not appear to take the threats posed by escapes seriously (Hindar 1992), and on (2) the observation that dramatic biological effects on native populations can occur whether the escaped fish spawn successfully or not (Evans & Willox 1991, Hutchings 1991).

The natural populations in the northernmost parts of the distribution area of Atlantic salmon on both the American and Eurasian continent have been spared from the effects of aquaculture, simply because aquaculture is not there. It is likely, however, that the use of transgenic fish expressing antifreeze proteins would remove the major constraint on expansion of aquaculture to the north. This would mean that an increasingly smaller number of natural populations would remain reasonably unaffected by aquaculture, and could easily lead to loss of unique genetic resources.

One major area for concern would be the genetic and ecological integrity of local populations of Atlantic salmon in northern rivers. For example, northeastern Norway has the singularly most important river for the natural production of Atlantic salmon in Norway, the Tana (Moen 1991). This river probably holds more than 20,000 anadromous spawners of Atlantic salmon and accounts for between 1/5 and 1/3 of the catches of wild Atlantic salmon in Norway. It is moreover characterized by the existence of a number of genetically different, local populations in tributaries and various parts of the main river (Ståhl & Hindar 1988). We can simply not allow aquacultural practice which results in escapes of farmed fish into this river. Similar arguments could most certainly be put forward for other northern rivers, such as the Petchora River in Russia, and rivers of northeastern Canada.

Another area for concern would be the effects on Arctic charr populations, which thrive in northern streams where other salmonids are absent or rare from temperature limitations. For example, the islands of Svalbard have river systems in areas that were deglaciated 100,000 years ago (Svenning 1991), ten times as long ago as mainland Norway. The Arctic charr populations in these streams – and the whole stream ecosystem – represent irreplaceable biological resources which could easily be destroyed by expansion of aquaculture to the north.

5.4 Beyond salmonids

The primary perspective of this report has been that of interactions between transgenic and wild salmonids in coastal streams, where interbreeding, competition and disease introduction are the main factors involved. Kapuscinski & Hallerman (1990), on the other hand, focus on the possible impact of transgenic piscivorous fishes released into lakes. They point out that one complicating factor in predicting the effects of releases on lake communities, is the fact that fish species go through a number of different trophic positions during their life cycle. As an example, Kitchell & Hewett (1987) forecasted in a simulation study that sterile chinook salmon in Lake Michigan would begin to prey on coregonids and smaller salmonids. They proposed that such prey shifts could lead to major changes in the structure of current food webs in the lake. Even more dramatic effects of introductions of predatory fish are now being demonstrated in African lakes, where introduction of Nile perch may be responsible for the extinction of hundreds of endemic cichlid species (Witte et al. 1992).

In rivers, the effects of fish on other trophic levels has been conjectural. One view holds that physical factors play much stronger roles than species interactions in structuring riverine communities, whereas an alternative view holds that both matter. Recent experimental evidence suggests strong effects of fish on four trophic levels in a river food web, where both predatory and herbivorous insects and macro– and epiphytic algae were affected (M.E. Power 1990). Transgenic fish with enhanced growth rate and larger appetite, and also larger size if they escaped in the late parr stage, would most certainly have different effects on the stream ecosystem than native fish of the same species. First of all their larger size would make them more competitive versus other fishes, and they would be able to consume larger–sized prey that were not available to non–transgenic individuals (Kapuscinski & Hallerman 1990).

Sadly, our knowledge about the effects of fish introductions on biological diversity is almost completely limited to effects on other fish species. This needs not be so, considering the enormous number of fish introductions that have occurred (e.g. hundreds of cases where juvenile brown trout and Atlantic salmon have been released above impassable waterfalls). I hope that the renewed international interest in community ecology from the perspective of biological conservation (Pimm 1991) will foster studies that investigate effects of fish introductions not only on other fish but also on invertebrates and plants.

Expansion of aquaculture to new areas would also require an even broader perspective which is not covered in this report. Some key words for the necessary considerations are organic pollution, the use of antibiotics, and geographical and socio-economic aspects of the aquaculture industry (Barinaga 1990, Heen et al. 1993). A common denominator for these aspects is that they must be considered even when the production facilities for transgenic fish are 100% escape proof.

6 Scenarios for genetic engineering in aquaculture

Ten years ago, two of the pioneers of salmon breeding stated that: "The future evolution of salmonid species now lies chiefly in the hands of the people who are beginning to breed them on a large scale. The task embodies challenges. One challenge is to maintain genetic diversity by providing appropriate sanctuaries to ensure the survival of wild stocks. Another is to utilize the enormous adaptability of these remarkable animals by selective breeding ...." (Donaldson & Joyner 1983). This statement is remarkably relevant to the current situation in Norway. The latter of the two challenges is adequately taken care of in breeding programs (Gjedrem et al. 1988) and may see a future extension in the use of transgenic fish. The former challenge, however, appears not to be appreciated as a task which has to be dealt with by the industry (cf. Sattaur 1989).

Up until now, this report has discussed the effects of transgenic fish in aquaculture as if escapes continued on the same order of magnitude as today. This was done deliberately because no action taken by the Norwegian government has convinced me of anything but a bleak future for the genetic and ecological integrity of natural populations. Perhaps the largest problem for the combined management of salmonids in culture and in the wild is that a specific goal for conservation has not been implemented in the management strategy. In the context of genetic conservation, the internationally established goal is to maintain as much genetic variation within **and** between populations as possible (Ryman 1991, and references therein). This goal acknowledges our ignorance of the economical, ecological and evolutionary value of genes and populations. Moreover, it stresses the importance of variation within populations in order to secure their long-term viability, as well as the importance of genetic variation between populations for the maintenance of local adaptations.

In the following, I present three different scenarios for a salmon aquaculture based on transgenic fish, and examine to what extent they are compatible with the established goal for genetic conservation.

Scenario 1: Profitability. The first scenario is an extrapolation of current trends in Norwegian aquaculture. The fish farming industry foresees the following changes for the future of Norwegian aquaculture (Hindar & Jonsson in press): (1) continued use of net pens with some sort of technical quality control, (2) fish farms moved to more exposed localities, (3) selective breeding schemes expanded to incorporate aspects of disease resistance, (4) development of vaccines, and (5) better feed. Its hope is that these changes will increase profitability by improving the quality of both the fish and their rearing environment. Transgenic fish are not part of the picture painted by the fish farming industry, but adding prospects from genetic engineering to this list would not change much except perhaps by finding profitable ways to improve on points (2), (3) and (4).

Extrapolation of today's trends in Norwegian aquaculture will inevitably lead to more stocks being lost because of disease introduction and replacement by escaped fish. Local adaptations will continue to be eroded because of interbreeding between escaped and wild fish. Moreover, no readaptation to local environments will take place as long as the escapes continue. Expansion of aquaculture into new areas following the use of cold-tolerant transgenic fish can only make this situation worse because more natural populations will be at risk. Of course, such a development is not consistent with the goal for genetic conservation.

Scenario 2: Physical containment. An alternative scenario is one based on development of fish farms where physical containment is much better than in net pens, and where in- and outflowing water can be controlled for pathogens and waste products (Hindar & Jonsson in press). Such fish farms have been constructed for land-based operations, and they have some obvious positive effects on fish farming itself such as the economic benefit of not raising fish that escape. Complete physical containment has a high initial cost which results partly from containment itself, and partly from securing a water supply to a contained environment. It is quite expensive to pump salt water to a land-based operation, but recent experiments suggest that the extra costs are outweighed by the increased profitability of production (Arve Berg, Norwegian Hydrotechnic Laboratory, Trondheim, pers. comm.).

Concerns about the environmental effects of transgenic organisms could well serve as an incentive for better physical containment in aquaculture (e.g. Kapuscinski & Hallerman 1990). But is this good enough? Devlin & Donaldson (1992) point out that physical containment measures are subject to disruption, for example by extreme weather conditions or by human error. Given the result from Emlen's (1991) study that it may take many generations for a natural population to recover from a single hybridization event; to rely on physical containment only cannot be consistent with the goal for genetic conservation.

Scenario 3: Biological and physical containment. The third scenario is based on the suggestion that genetic engineering be used to drastically decrease the fitness of cultured fish, should they escape to the environment. It must be emphasized that transgenic salmonids constitute a 'high-risk' group according to international checklists for evaluating the safety of releases of transgenic organisms (e.g. Tiedje et al. 1989, Kjelleberg & Fagerström 1990). If they are to be used in large-scale production, one of the goals for genetic engineering should be to cripple those fish that may eventually escape. As noted previously, salmonids can be sterilized on a large scale by technologically simple genetic manipulation (Johnstone et al. 1991, Devlin & Donaldson 1992). Such sterile fish would not have direct genetic effects on natural populations, but could have indirect genetic effects through the reduction of population sizes (Hindar et al. 1991, Waples 1991).

It has been suggested that genetic engineering should be used to develop suicide gene constructs that would not only prevent the fish from reproducing but would actually become lethal at an appropriate time (Powers et al. 1992). For example, Maclean & Penman (1990) suggested to develop nutritionally crippled strains of fish which would require a particular amino acid that were not present in the natural diet of salmon.

When and how should such a gene be expressed? Consider the following scenario (which was realized in Norway in the autumn of 1991, except no transgenic fish was involved): A major escape occurs just prior to or within the spawning season. If they had not been sterilized, the fish that escaped would not eat and would therefore not be affected by any nutritional crippling.

Instead, they would enter the nearest stream to spawn and would pass the lethal gene to the next generation. Moreover, the vast majority of matings in that stream would involve transgenic fish as one parent, and entire cohorts could be lost if the lethal gene was dominant (cf. Hutchings 1991). If they had been sterilized, they would nevertheless be able to interfere with the spawning of the native fish if they entered the stream, but could have minimal effects if they migrated to the open ocean. Thus, any use of genetic engineering to cripple fish must be carefully thought out to incorporate all the possible biological situations encountered. But such studies should definitely go along with studies that use gene technology to improve production characteristics. Only when environmental safety is one objective of the experimentation with transgenic fish can production goals be combined with internationally established goals for biological conservation.

7 Norsk sammendrag

Denne rapporten, som ble utført på oppdrag fra Direktoratet for naturforvaltning (DN-kontrakt BTEK-1092), gir en oversikt over teknikker for genmodifisering av fisk, samt av mulige økologiske effekter ved bruk av genmodifisert fisk i akvakultur.

Prosjektet ble gjennomført som en tre-delt litteraturstudie: Den første delen tar for seg de forskjellige muligheter for genmodifisering av fisk som er tilgjengelige i dag (kapittel 2), eller som forventes å komme i nær framtid. Den andre delen vurderer i hvilken grad genmodifikasjon kommer til å bli brukt i akvakultur i framtiden (kapittel 3), og for hvilke arter dette er mest aktuelt i Norge. Den tredje delen vurderer miljøvirkningene av at genmodifisert fisk unnslipper fra anlegg, spesielt med henblikk på effekter av at nåværende genteknologiske forsøk med laks oppskaleres til kommersielt bruk (kapittel 5 og 6). Vurderingene av miljøvirkningene av transgen laks er i stor grad basert på de effektene som nå dokumenteres i forbindelse med rømminger av fisk fra akvakulturanlegg, og på de effektene som har skjedd etter utsettinger av laksefisk i over hundre år. For å støtte opp om vurderingene, gir rapporten også noe bakgrunnsinformasjon om biologien til laks og andre laksefisk (kapittel 4).

Den teknikken for genmodifikasjon av fisk som er mest brukt i dag, er mikroinjeksjon av DNA i egg like etter befruktning. Denne teknikken ser ikke ut til å redusere overlevelsen nevneverdig, og den har gitt rimelig høy (men varierende) grad av integrasjon og uttrykk av det introduserte genet. I noen tilfeller er det vist at det introduserte genet nedarves til påfølgende generasjoner og uttrykkes i avkommet. Det påvises ofte økt fenotypisk variasjon i avkommet av transgen fisk. Dette skyldes blant annet at mikroinjeksjon ser ut til å produsere "mosaikk"-individer, dvs. individer som ikke har det introduserte genet i alle celletyper. Mikroinjeksjon krever at hvert egg behandles for seg, og er svært tidkrevende. Dersom genmodifikasjon skal få betydning i storskala akvakultur, er det nødvendig å utvikle nye metoder for genoverføring til store antall egg.

Laks er den mest aktuelle arten for genmodifikasjon i norsk akvakultur. Internasjonalt gjøres det forsøk med en

rekke andre fiskearter, slik som regnbueørret, karpe, tilapia og gjedde. De mest aktuelle genmodifikasjonene ut fra et kommersielt perspektiv er introduksjon av gener for (1) økt vekst, og (2) økt kuldetoleranse. De eksperimentene som har kommet lengst gjelder introduksjon av gener for laksens eget (eller andre arters) veksthormongen i laks. Slike eksperimenter har vist en sterk økning av veksthastigheten i transgene avkom, sammenliknet med ikke–transgene søsken. Det er også eksperimentelt introdusert gener for frysetoleranse fra polare fiskearter til laks. Disse eksperimentene har kommersiell interesse fordi de kan muliggjøre ekspansjon av akvakultur til områder der kaldt sjøvann er en hindring for akvakultur i dag. Ennå er imidlertid disse eksperimentene ikke kommet langt nok med hensyn til uttrykk av "antifrys"–gener i laks.

I framtiden kan det bli aktuelt med transgen fisk der kjønnsmodningen forsinkes eller hindres. I dag er dette mulig ved hjelp av en rekke genetiske og/eller hormonelle teknikker som ikke involverer genmodifikasjon, og det er godt mulig at samme resultat vil oppnås i framtiden ved genmodifikasjon. En norsk forskningsgruppe arbeider med å introdusere gener som hindrer gonadeutviklingen hos fisk. Transgen, sykdomsresistent fisk er enda lengre fra å bli realisert, siden vi ennå ikke kjenner de genene som gir resistens mot fiskesykdommer.

En detaljert forståelse av de økologiske, epidemiologiske og genetiske effektene som kan oppstå hvis det rømmer transgen laks til naturlige miljøer, krever at vi har bakgrunnskunnskap om laksens biologi og om de artene den interagerer med i naturen. Det vil alltid være en formidabel oppgave å dokumentere alle de ulike miljøeffektene som kan oppstå etter en utsetting, og nesten umulig å forutsi dem. Men ved å bruke erfaringer fra tidligere utsettinger som har overføringsverdi til miljøeffekter av transgene organismer, kan vi lære en hel del.

Det er satt ut laksefisk i over hundre år, og det har etterhvert blitt oppsamlet en del erfaringer som kan brukes til å vurdere effektene av rømt laks (og storutsettinger av fisk av fremmede stammer) på de lokale villfiskstammene. Dette er de to viktigste konklusjonene av en undersøkelse som syntetiserer erfaringer om de genetiske effektene av (ikke–genmodifisert) rømt og utsatt laksefisk på naturlige populasjoner:

* De genetiske effektene er ofte utforutsigbare. I noen tilfeller har den utsatte fisken ført til gjennomgripende genetiske forandringer hos villfisken (fullstendig samavl eller også fortrenging av den lokale bestanden), mens det i andre tilfeller ikke er påvist noen genetiske forandringer.

* I de tilfellene der det er funnet effekter på viktige økologiske egenskaper, ser disse effektene ut til å være negative sammenliknet med den upåvirkede lokale bestanden. Det er for eksempel vist redusert bestandsstørrelse i generasjonen etter utsetting hos to arter av stillehavslaks. Det er videre vist negative forandringer i en rekke egenskaper som kan forklare denne observasjonen, slik som: redusert yngeloverlevelse, dårligere territorialitet og skjulatferd hos yngelen, lavere overlevelse i sjøen og i elvene før gyting, økt feilvandring, og dårligere motstandskraft mot sykdommer.
  Langtidsvirkningene av utsettinger og rømminger av oppdrettsfisk vil derfor være tap av lokale tilpasninger, og homogenisering av de naturlige populasjonene til en eneste stor bastardbestand.

Rømt oppdrettslaks utgjør nå i gjennomsnitt en tredjedel av gytefisken i norske laksevassdrag, og i noen elver er innslaget av rømt fisk mer enn 80%. Det er dokumentert at de kan gyte i norske elver, og noen studier tyder på at hunnfisken ikke trenger å ha noe særlig dårligere gytesuksess enn ville hunnlaks. Rømt hannfisk, derimot, ser i stor grad ut til å bli utkonkurrert av ville hannfisk på gyteplassen. Det må understrekes at selv der oppdrettsfisken selv har svært dårlig gytesuksess, kan den ha betydelige effekter på den lokale villfisken. For eksempel er det vist at rømt hunnlaks har gravet opp gytegroper der det tidligere i sesongen gytte villfisk. Enda mer dramatiske effekter kan skyldes sykdomsintroduksjon til villfiskbestandene.

Så langt vi kjenner situasjonen i Norge, så er sykdomsintroduksjon kanskje den mest dramatiske effekten av at det rømmer eller blir satt ut fisk. Den dødelige lakseparasitten *Gyrodactylus salaris* har nå blitt spredt til mer enn 30 elver, hovedsakelig gjennom utsetting og spredning av fisk fra infiserte anlegg. Den

parasittiske copepoden lakselus er i dag et problem i fiskeoppdrett, og store mengder lakselus har også blitt observert på vill laks i nærheten av oppdrettsanlegg. Hvor store skader vill laks får på grunn av slike angrep er ennå lite kjent. Mange steder ser virkningene ut til å være større på sjøørret enn på laks. Furunkulose er en smittsom fiskesykdom som kom til oppdrettsanlegg i Norge med oppdrettssmolt fra Skottland i 1985. Siden den gangen har den spredt seg til mer enn 500 oppdrettsanlegg og 63 lakseelver. Store mengder død laks som følge av sykdommen har blitt observert i enkelte lakseelver.

Der fisk er introdusert til områder hvor arten ikke fantes fra før, har det ofte skjedd gjennomgripende forandringer i den lokale fiskefaunaen. I USA regner man med at fiskeutsettinger har vært delaktige i 68% av de utdøelsene av fiskearter som er observert der i løpet av det siste århundret.

Vurderinger av de økologiske og genetiske effektene av genmodifisert fisk blir i stor grad de samme som vurderingene av effektene av ikke-genmodifisert fisk. Men nye aspekter kommer i tillegg, spesielt der en genmodifisert fisk har fenotypiske egenskaper som ligger helt utenfor rammen av det som observeres blant ikke-genmodifiserte artsfrender. Da kan den genmodifiserte fisken spille en helt ny rolle i økosystemet. Dette kan skje både hos laks med innsatte gener for økt veksthormonproduksjon, og hos laks med økt kuldetoleranse etter innsetting av antifrys-gener. Rapporten bruker nettopp laks som er modifisert i disse to egenskapene som 'case studies' for å evaluere miljøeffektene av transgen fisk.

Effektene av transgen laks på ville fiskebestander diskuteres i forhold til (1) effektene av laks med endrete egenskaper i fiskesamfunnet, (2) effektene av at genmodifisert laks krysser seg med vill laks, og (3) effektene av at den geografiske utbredelsen av akvakultur kan endre seg som følge av at oppdrettsfisken tilføres nye egenskaper.

Transgen laks med innsatte veksthormongener kan få økt konkurransestyrke i elver ved at de er større enn ikke-transgene artsfrender, og også større enn ørreten, som vanligvis vokser raskere enn laks. Fisk med veksthormongener vil imidlertid også ha større appetitt enn ikke-transgen fisk. Det kan medføre at de lettere sulter i perioder med næringsmangel, som ofte oppstår på ettersommeren selv i produktive vassdrag. Effekten kan være større dødelighet blant transgen fisk enn blant ikke-transgen fisk. Den totale effekten på fiskesamfunnet i elver blir derfor avhengig av når og hvordan den transgene fisken kommer dit. Dersom de først og fremst kommer via reproduksjon av voksne, transgene individer, så kan dødeligheten være svært høy allerede tidlig i livet (under startfôring eller på slutten av første vekstsesong). Men dersom de kommer via gjentatte utslipp fra settefiskanlegg nær elven, kan det komme nye, transgene individer til alle årstider og i alle størrelsesklasser, og effektene kan bli mye større.

Veksthormon spiller også en rolle i den osmoreguleringen som er nødvendig for et liv i sjøvann. Transgen smolt med veksthormongener vil kunne ha en selektiv fordel ved utvandring i sjøen, fordi de lettere kan greie overgangen til saltvann, og dermed unnslippe høy predasjon i estuarier og andre kystnære områder. Både økt evne til osmoregulering og økt kroppsstørrelse i forhold til vill smolt vil bidra til denne fordelen. En annen bieffekt av økt veksthormonproduksjon vil sannsynligvis være tidligere kjønnsmodning. Det vil medføre at selv om den transgene fisken vokser fortere, så vil den ikke nødvendigvis være større enn villfisken på gyteplassen, annet enn i typiske smålakselver.

Mesteparten av oppdrettsfisken som rømmer i dag gjør det fra sjøanlegg. Det betyr at man også må vite noe om effektene av transgen laks i det marine miljøet. Siden laks har så høy vekstrate i sjøen, så er det lite sannsynlig at den transgene fisken ville oppleve økt dødelighet på grunn av for høy appetitt i forhold til næringsgrunnlaget. Jeg forventer derfor at den transgene fisken ville kunne få økt konkurransestyrke i forhold til vill laks i det marine miljøet. Det må imidlertid understrekes at vi vet svært lite om laksens plass i marine næringsnett, og om hva det betyr å introdusere laks med økt veksthastighet.

Transgen laks med økt kuldetoleranse ville først og fremst få økt konkurransestyrke i miljøer med kaldt sjøvann i perioder av året (under 0 grader). Effektene ville bli størst på de marine fiskeartene som uttrykker antifrysgener naturlig, og på populasjoner av laks og røye i nordlige områder. Noen av de fiskeartene som uttrykker antifrysgener er kjent som byttedyr for laks i havet (sild

og krøkle), og de ville bli tilgjengelige som bytte for laks i større geografiske områder og/eller i større deler av året. For andre fiskearter med antifrysgener (nordlige flyndre- og torskefisk og steinbitt) gjelder den begrensningen som ble nevnt over: vi vet svært lite om deres interaksjoner med laks.

Antifrysgener ville gi en selektiv fordel for transgen laks i den nordligste delen av dagens utbredelsesområde for arten. I nordøstre Canada, der sjøtemperaturen ved smoltutvandring kan være rundt 0 grader, har laksen en ekstremt høy smoltalder og -størrelse og en svært stor grad av ferskvannsstasjonære individer av begge kjønn. Det er lett å tenke seg at en transgen kuldetolerant laks i disse områdene kunne utkonkurrere villfisken, fordi den ville kunne utvandre ved en lavere smoltlengde og -alder, og dermed få mye høyere reproduktiv kapasitet i løpet av en gitt tidsperiode. Likeledes kunne antifrysgener være med på å øke utbredelsesområdet til laks mot nord, og derved påvirke de røyepopulasjonene som lever der som eneste fiskeart i ferskvann. Røye er mindre konkurransesterk enn laks i elver, og ville kunne bli utkonkurrert av transgen kuldetolerant laks.

Den største effekten av transgen laks med antifrysgener ville etter min oppfatning være at dette ville føre til en omfattende ekspansjon av fiskeoppdrett mot nord. Mange av de nordlige fiskepopulasjonene er i dag forskånet fra effekter av akvakultur, rett og slett fordi det ikke fins sjøanlegg i nærheten. Disse populasjonene (spesielt av laks og røye) ville da kunne bli utsatt for de samme genetiske, epidemiologiske og økologiske effektene som vi opplever langs den norske vestkysten i dag. I Norge ville dette bety at laksestammene i en elv som Tana, som kanskje huser mer enn 20% av all norsk villaks, kunne bli mye mer utsatt for påvirkning av oppdrettslaks enn de er i dag. Liknende argumenter kunne ganske sikkert føres i marken for elver i det nordlige Canada og Russland.

Tre scenarier for en framtid med transgen laks i akvakultur er diskutert i rapporten. Det første scenariet beskriver en utvikling der transgen fisk blir brukt i forhold til sin lønnsomhet, uten at det medfører andre endringer av næringen. Dette ville gjøre at de effektene vi i dag ser av norsk akvakultur på ville fiskestammer bare ville forsterkes, med tap av uerstattelige genressurser som resultat.

Det andre scenariet beskriver en situasjon der bekymringen for miljøeffekter av transgen fisk medfører at anleggene gjøres rømningssikre. Dette ville kunne redusere effektene på villfisk til et minimum av hva de er i dag, og ville også kunne ha en positiv effekt på oppdrettsnæringen selv (f.eks. ved at den ikke produserer fisk som rømmer). Det kan være vanskelig å garantere den tekniske innesluttingen mot uhell som skyldes ekstreme værsituasjoner eller menneskelig svikt. På bakgrunn av at det er vist at én storstilt hybridisering mellom oppdrettsfisk og villfisk kan ha dramatiske effekter på villfisken, og på bakgrunn av at transgen laks ville bli plassert i en "høyrisiko-gruppe" blant transgene organismer, så burde den tekniske innesluttingen være komplementert med former for "biologisk inneslutting".

Det tredje scenariet beskriver nettopp en slik framtid, der genteknologi brukes aktivt for å sikre en biologisk inneslutting som brukes sammen med forbedret rømningssikring. Det fins metoder i dag som kan sikre sterilisering av fisk i stor skala. Dette ville hindre direkte genetiske effekter på villfisken dersom de rømte, men ville ikke kunne hindre andre effekter (som f.eks. sykdomsspredning). Noen genteknologer har derfor foreslått at den transgene fisken burde ha en slags selvmordsgener, som for eksempel ble uttrykt som følge av at rømt laks ville mangle et næringsemne som ble gitt i fôret til oppdrettsfisk. Dersom dette kan kombineres med sterilisering av fisken, kunne genteknologien være med på å bidra til en situasjon for villfisken som er bedre enn den vi ser i dag. Dette krever at det fins økonomiske insentiver for å bruke genteknologi til å minimalisere miljøeffekter, og ikke bare til å forbedre produksjonsegenskapene til oppdrettsfisk.

8 Literature

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