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Specific research on transgenic fish considering especially the biology of trout and salmon

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Scientific research on transgenic fish

with special focus on the biology of trout and salmon

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1. Introduction

The development of transgenic farm animals and transgenic fish is lagging markedly behind the development of transgenic crops. Several reasons account for this phenomenon - above all the complex physiology and reproduction biology of more highly developed animals. However, since the 1980s intensive research has been carried out worldwide in the field of transgenic modifications in vertebrates. Especially the development of transgenic fish is of great interest for commercial use.

Since the mid-eighties of the past century the European Union has spent about 7.5 million Euros on eleven different research projects concerning transgenic modifications in fish species like Atlantic salmon, Rainbow trout and Tilapia. The development of transgenic fish has proceeded to the extent that commercial utilisation is possible from a technical point of view. The interest shown especially in transgenic fish may be explained on the one hand by technical reasons. As compared to other vertebrates, genetic manipulations in fish can be carried out quite easily. On the other hand ongoing changes on the world fish market give rise to increased interest in transgenic fish. Since 1984 the production of fish in aquaculture has been growing continuously. Nowadays about 26% of all fish consumed is produced in aquaculture[1]. This is a basic prerequisite for the use of transgenic fish, because hatching and rearing of transgenic fish is only possible in aquaculture.

The European Patent Office granted its first patent on transgenic fish in July 2001: The Canadian company *Seabright* obtained patent EP 0 578 653 B1 on Atlantic salmon and all other fish species carrying an additional gene for faster growth. This patent is effective in 15 European countries including Germany[2]. Applications for the commercial use of these fast growing salmons in the USA, Canada and Chile have already been filed by a private US-Canadian company (Dunham 1999, FAO[3]). In Cuba a GM tilapia is awaiting regulatory approval for food purposes; decisions on approval are still pending. The GM tilapia is a hybrid containing a modified tilapia growth hormone gene to improve growth and conversion efficiency[4].

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[1] FAO – World fisheries and aquaculture atlas CD-ROM (2001) – FAO (ed.).

[2] European Patent Specification for the patent EP 0 578 653 B1 with the title “Gene construct for production of transgenic fish.” European Patent Office – Bulletin 2001/29.

[3] FAO – World fisheries and aquaculture atlas CD-ROM (2001) – FAO (ed.).

[4] FAO – Biotechnology in Food and Agriculture, VI. Background Document to Conference 7: Gene flow from GM to non-GM populations in the crop, forestry, animal and fishery sectors. Conference 2002, from 31st May till 28th June.

In addition to increasing the productivity of fish production by enhanced fish growth, the alteration of meat quality, the enhancement of disease resistances and the improvement of frost tolerance or tolerance various contaminants are economically interesting targets of transgenic fish research (Piker et al. 1998, Levy et al. 2000).

The commercial hatching and breeding of transgenic fish strains in open waters is highly controversial because many uncertainties exist concerning its potential ecological risks. Many scientists, environmental organisations and fisheries management associations claim that the potential hazards of breeding transgenic fish have not been sufficiently investigated yet (Hallerman & Kapuscinski 1992, Shelton 1996, Jönsson et al. 1998, Muir & Howard 1999, Breton & Uzbekova 2000). Biosafety studies have only just started. Therefore there is a great lack of research regarding the evaluation of potential negative effects of the release of transgenic fish strains. Concepts for risk assessment and containment strategies have to be developed and tested. This has already been stated by Piker et al. (1998) in the UBA report 33/98 "Compendium of aquatic organisms relevant for deliberate release“ stating that “research on ecological risks of the release of aquatic organisms is widely underrepresented as compared to the efforts made to refine transgenic methods and techniques”. Especially with respect to non-domesticated animal species such as fish many new questions have been arising with regard to possible environmental effects and the effects on biological and genetic diversity.

The possible escape and the dispersal of transgenic fishes from aquacultural facilities can probably not be prevented completely. Existing containment strategies such as the establishment of sterile populations are quite unsafe and do not guarantee the prevention of gene flow (Shelton 1996). Current experience in fish farming has repeatedly shown that fishes will escape from marine as well as from inland facilities (Penczak et al. 1982, Phillips et al. 1985, Gausen & Moen 1991).

In this respect it is important to collect and incorporate the various biological data available on those fish species that have already been subject to genetic modification and that will probably be put on the market soon. These data will provide the required baseline for the assessment of risks incurred in the release of transgenic fish strains. Compilations of basic biological data have already been worked out for different crop and tree species. They have been published in electronic form as “Consensus Documents“ by the OECD and are available worldwide to all countries and public authorities.

2. Overview of international scientific research on transgenic fish

2.1. Targets and description of genetic modifications in fishes – with special focus on the development of scientific research since 1998[5]

The ongoing overfishing of the worldwide fish resources, concerns about satisfying worldwide food demands and the rapid expansion of fish production in aquaculture with an average annual rate of almost 10% since 1984 (Levy et al. 2000) have in recent years given impetus to intensive research and development of transgenic fish, as this technique has been more successful in improving growth rates of fishes than conventional breeding methods (Penman & MacAndrew 2000). Furthermore genetic manipulations on fish can be carried out quite easily compared to other vertebrates. External fertilisation and development, the transparency of embryos seen in many species and the high fecundity of most species are characters that facilitate genetic manipulation in fishes (Iyengar et al. 1994).

The first report on a successful gene transfer in fish was published in 1985 (Zhu et al. 1985, cited from Barrett et al. 2001). Fifteen years later, Reichhardt (2000) reported 35 different fish species that have already been the target of genetic modifications. An almost complete list of the fish species that have been targets of genetic modifications has been provided by Piker et al. (1998) and Tappeser et al. (2000). Only the arctic charr (*Salvelinus alpinus* L.) has to be added to complete these lists (see Pitkänen et al. 1999).

In the nineties of the past century the development of commercially useful transgenic fish strains was focused on growth enhancement (Sin 1997, Sin et al. 1997). According to Pandian et al. (1999) more than 40 fish growth hormone cDNA and genome sequences have already been isolated, characterised and used for construction of “all-fish” gene cassettes for transformation in other fish species.

Since the first attempts of genome manipulation in fishes, the ever-increasing knowledge on structure and function of eukaryotic genes has clearly shown the need for introns, enhancer regions, boundary regions and locus control regions in addition to a suitable promoter in the construction of appropriate vector systems. In the past 17 years much effort has been devoted to isolating the appropriate fish sequences. Considerable improvements have been made in the characterisation of promoters, local control regions,

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[5] In 1998 the German Federal Environmental Agency published a study that provides an overview of biotechnological research on aquatic organisms up to this date (Piker et al. 1998). The data presented by Piker et al. (1998) have been updated in the present study.

enhancers and introns. So far more than 70 fish cDNA and genomic sequences have been isolated and characterised (Pandian 1999). New gene constructs have been developed that are characterised by better transformation and expression rates than the early ones.

Currently efforts are made in fish biotechnology research to characterise disease-resistance genes. Fish losses from infections are a significant problem in aquaculture worldwide. Therefore the development of disease-resistant fish strains is of utmost commercial interest (Sin et al. 1997, Jia et al. 2000, Hew & Fletcher 2001).

Other targets of genetic engineering research in fishes are improved cold tolerance, improved tolerance to pollutants, sterility and improved meat quality (e.g. colour, taste, fat and protein contents) (Piker et al. 1998, Tappeser et al. 2000, Hew & Fletcher 2001a, Lakra 2001). In order to quickly assess potential environmental hazards a few research teams are also working on the establishment of transgenic fish strains for detecting mutagens and other contaminants in aquatic environments (Amanuma et al. 2000, Carvan et al. 2001). Several biotechnological research projects on fish have been initiated to gain new insight in biological development processes and gene regulation. Research activities in these fields have been growing considerably in recent times (see Appendices, Table 8, and e.g. , Long et al. 1997, Uzbekova et al. 2000, Chen et al. 2001, Huan et al. 2001, Kobayashi et al. 2001).

Research on potential side-effects of transformation events in fish has only started a few years ago. Since 1997 several projects were carried out to investigate into potential changes in behaviour, competitive ability, feed intake, feed digestibility, feed conversion and metabolism of transgenic fish strains (see Appendices, Table 8).

2.2. Methodology: Gene constructs and gene transfer

According to Levy et al. (2000) and Sin (1997) the essential steps in fish genetic engineering are:

- design and construction of the artificial gene constructs to be transferred into fish species,
- transfer of the gene construct into fish germ cells,
- the identification of successfully transformed individuals (screening for transgenic fish),
- determination of transgene expression and phenotype,
- study of inheritance,
- and the establishment of stable transgenic lines by selection and breeding.

An artificial gene construct typically consists of three parts: structural gene(s) ("gene of interest"), reporter or marker gene (structural genes but needed for identification) and regulatory sequences containing the promoter, the transcription terminal sequence and if necessary enhancer(s). The **structural gene** ("gene of interest") encodes for the production of a specific protein. In recent decades, researchers have introduced many structural genes into different fish species, e.g. different growth hormone genes (gh). In the beginning of fish genetic engineering these structural genes were mainly derived from other animals such as cows and birds, from bacteria or even from humans. Currently the majority of transferred structural genes come from other fish species such as rainbow trout, Atlantic salmon, Pacific salmon or ocean pout. An overview of structural genes that have been used in more recently published studies is provided in Tables 1, 2 and 8. A list of structural genes that were used in older publications can be found in Piker et al. (1998) and Iyengar et al. (1996).

For these genes to be successfully expressed in the recipient organism, the artificial gene construct must also contain genetic sequences that serve as regulators for their transcription. **Promoters** are regulating the expression of genes. The first promoters used in fish biotechnology were isolated from the genome of viruses (e.g. the Rous Sarcoma Virus (RSV), the Simian Virus (SV40) or the Cytomegalovirus (CMV)), mammals (e.g. mouse metallothionein-1 (mMT-1)), birds (e.g. the β-actin promoter from chicken) or the frog *Xenopus laevis* (1α-enhanced promoter). Because of their low expression rates (Houdebine & Chourrot 1991), the search for more effective fish promoters has been intensified during the last 15 years. More and more **regulatory sequences** have been identified and isolated in recent years (see appendices Table 8). Widely used fish promoters are the metallothionein-promoter (rtMT) from rainbow trout (*Oncorhynchus mykiss*), the metallothionein-promoter (OnMT) from sockeye salmon (*Oncorhynchus nerka*), the histon-3 promoter (OnH3) from sockeye salmon (*Oncorhynchus nerka*), the antifreeze gene promoter (opAFP) from ocean pout (*Macrozoarces americanus*) and the β-actin promoter from carp (*Cyprinus carpio*). Furthermore enhancer-regions are nowadays added to artificial gene constructs. Such enhancer-regions also influence the expression of structural genes (Devlin 1998, Hsiao et al. 2001).

The third group of gene sequences used for the construction of artificial genes are reporter or marker genes. These genes are used to test the success of gene transfer techniques and to study gene expression in specific tissues and during development and to test promoter activity. Usually these genes are linked to the gene(s) of interest. Reporter genes code for a specific detectable and measurable feature, like e.g. the green fluorescent protein gene (GFP) of the bioluminescent jellyfish, *Aequorea victoria*. Successful gene transfer can be identified by the expression of the reporter gene. Since reporter gene and

gene of interest are linked successful transfer can be derived for the gene of interest, too. In the case of the green fluorescent protein gene, expression as well as expression rate of the transferred gene construct can be detected by measuring the fluorescence rate. The reporter genes commonly used in transformation of fishes are the chloramphenicol acetyltransferase (CAT) gene, the ß-galactosidase (lacZ) gene, the luciferase gene and the mentioned green fluorescent protein gene (GFP). The qualities, functions and uses of these reporter genes have been extensively described by Piker et al. (1998) and Iyengar et al. (1996). Iyengar et al. (ib.) describe three further genes: The neomycin phosphotransferase gene (NEO), the tyrosinase gene and the melanin concentrating hormone gene (MCH).

The neomycin phosphotransferase gene is an antibiotic resistance gene[6] that renders the cell or organism resistant to the antibiotic neomycin or its commonly used analogue G418. This marker gene has shown to be unsuitable in the case of living fish embryos, since F_0 transgenic fish are almost invariably mosaic[7], with transgene expression obtained in only a subset of cells. Such mosaic individuals are consequently killed by the drug despite gene expression in some of the cells (Takeuchi et al. 1999, Tappeser et al. 2000, Hsiao et al. 2001). The tyrosinase enzyme encoded by the tyrosinase gene plays an important role in the chain of events during melanophore development and pigmentation. It can only be used in albinos or other suitable colour mutant strains (Iyengar et al. 1996). Fishes that carry the melanin concentrating hormone gene as a reporter gene are visibly brighter than fishes that do not carry this gene (at least there, where the gene is expressed). As a function of the gene the melanosomes became contracted within melanophors (Iyengar et al. 1996).

The gene transfer itself has to be carried out at a very early stage of egg development, preferably at the one-cell-stage of egg development, to insure that the foreign gene construct will be transferred to the majority of cells. The different methods of gene transfer, microinjection, electroporation, sperm-mediated gene transfer, ballistic transformation (also known as "high-speed particle gun gene insertion" or "microprojectile bombardement"), lipofection methodology and use of retroviruses as gene delivery vehicles are described by Houdebine & Chourrot (1991), Linney et al. (1999), Pandian et al. (1999), Piker et al. (1998) and Sin (1997). A comprehensive description of the advantages and disadvantages of the different methods can be found in Piker et al. (1998) and Sin et al. (1997). The most established method for gene transfer in fish is microinjection. The greatest disadvantage of this method is that it is very time-consuming and that mass transfer is not possible. Despite

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[6] Another antibiotic resistance gene used as a reporter gene by Amanuma et al. (2000) in transgenic *Brachydanio rerio* is the kanamycin gene.

[7] Mosaic individuals are individuals that are composed of cells with and without the transferred gene construct.

these technical difficulties this method is the most-used approach in genetic engineering on fish. The survival rate of fish embryos manipulated by this method is between 35 and 80%, the integration rate varies between 10 and 70% (Piker et al. 1998).

According to Piker et al. (1998) the use of the microinjection method results in higher survival rates for manipulated fish embryos than the electroporation method. Data from more recently published studies would appear to confirm this findings. However, a thoroughly worked out comparison of the survival rates of different gene transfer methods is not possible due to missing data in most studies.

As an alternative method to the time-consuming microinjection method the retroviral vector infection is discussed. First successful attempts have been carried out, but there is still missing basic knowledge on species specific retroviruses (Linney et al. 1999).

2.3. Targets and description of genetic modifications in *Salmo salar* L., *Oncorhynchus mykiss* Wal. and *Salmo trutta* L.

With regard to world fish production in aquaculture the two species Atlantic salmon (*Salmo salar* L.) and rainbow trout (*Oncorhynchus mykiss* Wal.) are playing a very important role. Marine finfish aquaculture in Europe is currently dominated by production of Atlantic salmon mainly produced in Norwegian, UK (Scottish), Faroese and Irish coastal waters. Rainbow trout is the second well-established salmonid species for marine aquaculture (Youngson et al. 2001). Therefore it is not surprising that there is a great economic interest in developing strains of these species that have certain advantages for fish production. Since conventional breeding techniques are very time-consuming, the interest in transgenic strains for enhanced aquaculture productivity has increased continuously. Until now the development of commercially valuable transgenic strains of Atlantic salmon and rainbow trout has focused **on two main goals** (see Tables 1 and 2).

The first one is **increasing the productivity** of fish production by enhanced fish growth. The development of such growth-accelerated species would reduce the time required to raise fish to market size[8]. According to Devlin (1997) more than half of the research on transgenic salmonids has been conducted with gene constructs designed to influence growth. This has been mostly achieved by transferring an additional growth hormone gene construct into fertilised fish eggs. Nowadays such growth hormone gene

[8] At the present time, it takes e.g. approximately 16-18 months of sea pen culture to produce marketable Atlantic salmon in Atlantic Canada. If growth rates during this phase could be doubled, it might be possible to market the salmon following a single growing season and obviate the need for overwintering in seapens.

constructs are developed from the genome of other fish species. These so-called “all fish” gene constructs have also been used to produce transgenic Atlantic salmon and rainbow trout. According to Hew & Fletcher (1997) and Du et al. (1992) “all-fish” gene constructs showed an increase in growth enhancement of Atlantic salmon, on average three- to five-fold, with some individual fish being 20- to 30-times larger in the early phase of growth. Cook et al. (2000a) and Saunders et al. (1998) observed an increase in growth enhancement of transgenic Atlantic salmon on a scale of 2- to 3-fold, furthermore growth-accelerated transgenic salmons undergo precocious smoltification (the physiological adaptation which allows survival in sea-water environments) up to two years before the natural transformation (Devlin 1997). This effect may have considerable commercial value since one limiting factor in the production of salmonids is the juvenile rearing phase.

In rainbow trout “all-fish” gene constructs showed an increase in growth enhancement on a scale of 3.2- to 17.3-fold (Devlin 1997 and Devlin et al. 2001). However, Devlin et al. (2001) found that the growth of transgenic wild-strain rainbow trout did not surpass that of a fast growing non-transgenic domesticated strain of trout used in aquaculture. Introducing the growth-hormone construct into this domestic strain did not cause further growth enhancement. These results indicate that similar alteration of growth can be achieved both by selection and transgenesis in rainbow trout, but that the effects are not always additive.

In addition there have been also attempts to improve feed efficiency in rainbow trout by transferring human and rat gene constructs which code for special enzymes (Pitkänen et al. 1999). But these studies were only carried out with the first generation of transgenic fish. Due to the high rate of mosaicism commonly observed in the first generation of transgenic fish, substantial changes in carbohydrate metabolism were not expected, and any definite conclusion on the efficiency of the gene constructs used could not be drawn.

With regard to the development of improved fish strains **the second main goal** of transgenic research in Atlantic salmon is **the improvement of cold tolerance** by transferring a set of antifreeze protein genes. Antifreeze proteins (AFP) are produced by a number of marine teleosts that inhabit waters at sub-zero (zero to –1.8 °C temperatures). These proteins are produced in the liver and secreted into the blood. They serve to reduce the freezing point of the fish to safe levels. Antifreeze protein genes have been identified and analysed from winter flounder (*Pleuronectes americanus*) and have been transferred to Atlantic salmon (Hew et al. 1991). The genes were successfully integrated into the salmon chromosomes, expressed, and found to exhibit Mendelian inheritance (Hew et al. 1999). However the level of antifreeze proteins in the blood of these transgenic salmons were quite low and unlikely to be sufficient to confer any significant increase in freeze resistance on the salmon (Fletcher et al. 2000 and 2001). A commercially interesting advantage of such frost-

tolerant salmon would be that they could be reared in colder climate[9]. But up to now the trial results have not reached any commercial stage.

Another target of utmost commercial interest is the development of disease(s)-resistant fish strains, since high density culture conditions are enhancing the susceptibility of fishes to infections (Hew & Fletcher 1997). According to Hew & Fletcher (1997) several approaches are feasible using transgenic technology. Antisense and ribozyme technologies could be used to neutralise or destroy the viral RNA such as the infectious haematopoetic necrosis virus (IHNV) which causes extensive mortality in salmonids. Another possibility could be to express the viral coat proteins in the host membranes. The expression of this viral protein might titrate out the receptors for the virus, thus minimising viral penetration. The disadvantage of these two methodologies is that they are restricted to one or related pathogens. Alternative methods include boosting the host´s own immune control and overexpressing antimicrobial or antibacterial substances in transgenic fish (Fletcher & Hew 1997, Jia et al. 2000). However, the development of disease-resistant fish strains is still at the beginning and there is still a lot of basic research to do.

Research has been done also in the development of transgenic sterile strains of rainbow trout (Smith et al. 2001). Sexual maturation was hindered by inhibition of gonadotropin-releasing hormone (GnRH) mRNA using antisense technology. First attempts have been successful. F1 and F2 progeny have been produced of transgenic rainbow trout. However, the problem to obtain fidelity of transgene expression still remains unsolved (Smith et al. 2001).

[9] The aquaculture industry along the east coast of Canada e.g. face the problem that most of these coastal waters are characterised by ice and sub-zero temperatures in the winter months. These temperatures are lethal to salmonids. Therefore, sea cage aquaculture of salmon is almost entirely restricted to a relatively small area in the most southerly part of the region (Hew et al. 1995). There are two potential ways in which transgenic technologies could be used to solve the problem of overwintering salmon in sea cages in Atlantic Canada. The first one is to produce freeze-resistant salmon by giving them a set of antifreeze proteins, and the second one is to shorten the time in which the salmon reach market size. This can be achieved by enhanced growth rates due to growth hormone gene transfer.

Table 1: Genetic modifications in *Salmo salar* L. (Atlantic salmon)

Target of genetic modification	gene construct			reference
	reporter gene	structural gene ("gene of interest")	promoter	
Study of growth rate, feed intake, feed digestibility, feed conversion and body composition of transgenic Atlantic salmon in comparison to non-transgenic Atlantic salmon	-	gh (growth hormone gene from *Oncorhynchus tshawytscha* - chinook salmon)	antifreeze gene promoter (AFP) from *Macrozoarces americanus* (ocean pout)	Cook et al. (2000a)
Study of the effect of food deprivation on oxygen consumption, metabolic rate and body composition of transgenic Atlantic salmon in comparison to non-transgenic Atlantic salmon	-	gh (growth hormone gene from *Oncorhynchus tshawytscha* - chinook salmon)	antifreeze gene promoter (AFP) from *Macrozoarces americanus* (ocean pout)	Cook et al. (2000b)
Comparison of oxygen consumption and metabolic rate of transgenic Atlantic salmon in comparison to non-transgenic Atlantic salmon	-	gh (growth hormone gene from *Oncorhynchus tshawytscha* - chinook salmon)	antifreeze gene promoter (AFP) from *Macrozoarces americanus* (ocean pout)	Cook et al. (2000c)
Study of the inheritance and expression of a line of transgenic salmon	-	wflafp-6 (antifreeze protein gene from *Pleuronectes americanus* – winter flounder)	no details given	Shears et al. (1991), Hew et al. (1999)
Study of the smolt development in growth hormone transgenic Atlantic salmon	-	gh (growth hormone gene from *Oncorhynchus tshawytscha* - chinook salmon)	antifreeze gene promoter (AFP) from *Macrozoarces americanus* (ocean pout)	Saunders et al. (1998)
Study of respiratory metabolism and swimming performance in growth hormone transgenic Atlantic salmon under different conditions in comparison to non-transgenic Atlantic salmon	-	gh (growth hormone gene from *Oncorhynchus tshawytscha* - chinook salmon)	antifreeze gene promoter (AFP) from *Macrozoarces americanus* (ocean pout)	Stevens et al. (1999)

Target of genetic modification	gene construct			reference
	reporter gene	structural gene ("gene of interest")	promoter	
Growth enhancement	-	csgh (growth hormone gene from *Oncorhynchus tshawytscha* - chinook salmon)	antifreeze gene promoter	Du et al. (1992)
Enhancement of cold tolerance	-	afp (antifreeze proteins gene) from winter flunder (*Pseudophleuro-nectes americanus*)	antifreeze gene promoter	Hew et al. (1991)
Growth enhancement	-	hgh (humane growth hormone gene)	MT (metallothionein promoter from mouse)	Rokkones et al. (1989)

Table 2: Genetic modifications in *Oncorhynchus mykiss* Wal. (rainbow trout)

Target of the genetic modification	gene construct			reference
	reporter gene	structural gene ("gene of interest")	promoter	
Growth enhancement to reduce production time	no information	Ongh1, overexpressing growth hormone gene from *Oncorhynchus*	MT (metallothionein promoter)	Devlin et al. (2001)
Study of the developmental expression of the grf/pacap gene, that encodes for the two hormones GRF (growth hormone-releasing hormone) and PCAP (pituitary adenylate cyclase-activating polypeptide). Both hormones are involved in the growth hormone release from the pituitary.	-	1) hypothalamic (hyp)-grf/pacap gene construct from sockeye salmon cloned into pbluescript II KS +/-	645 base pairs of the grf/pacap promoter region	Krueckl & Sherwood (2001)
		2) pituitary (pit)-grf/pacap gene construct from sockeye salmon engineered in a pUC19 vector	gh promoter from *Oncorhynchus tshawytscha* (chinook salmon)	
Study of the inhibitory effect of antisense mRNA	-	antisense-sGnRH-cDNA from *Salmo salar* (Atlantic salmon) cDNA; (GnRH: gonadotropin releasing hormone)	Pab promoter from the gnrh gene of *Salmo salar* (Atlantic salmon)	Uzbekova et al. (2000)
Improvement of the carbohydrate metabolism efficiency of salmonid fish	-	1) hgluT1 (human glucose transporter type 1 c-DNA)	1) CMV promoter (cytomegalus virus)	Pitkänen et al. (1999)
		2) rhkII (rat hexokinase type II cDNA)	2) OnH3-Histon 3 promoter from sockeye salmon	
			3) OnMT-B (metallothio-nein-B promoter from sockeye salmon	

Target of the genetic modification	gene construct			reference
	reporter gene	structural gene ("gene of interest")	promoter	
Study for testing the utility of different GFP gene constructs as cell-labelling tools and reporters of gene expression in transgenic rainbow trout	GFP (two variants: S65T and eGFP)	-	1) CMV promoter (cytomegalus virus) 2) EF1 (1α-enhanced promoter from *Xenopus laevis*)	Takeuchi et al. (1999)
Functional analysis of the histone H3 promoter	ß-galactosi-dase (lacZ)		histone H3 (sH3) promoter from *Atlantic salmon*	Hanley et al. (1998)
Production of L-ascorbic acid	-	rglo (rat gene for L-gulono-γ-lactone oxidase, the key enzyme of L-ascorbic acid biosynthesis)	OnMT (metallothionein promoter from *Oncorhynchus*)	Krasnov et al. (1998)
Studying and improving of gene transfer technical methods	-	minichromosome of brook trout (*Salvelinus fontinalis*) carrying a pigmentation gene		Peek et al. (1997)
In vivo screening of foreign gene expression	luciferase gene	-	CMV/RSV/SV promoter	Gibbs et al. (1991)
Histochemical detection of foreign gene expression	-	β-galactosidase gene	β-actin promoter (from chicken)	Inoue et al. (1991)
Growth enhancement	-	hgh (humane growth hormone gene)	MT (mouse metallothionein promoter)	Guyomard et al. (1989)
Growth enhancement	-	hgh (humane growth hormone gene)	SV 40 promoter	Chourrot et al. (1986)

Target of the genetic modification	gene construct			reference
	reporter gene	structural gene ("gene of interest")	promoter	
Growth enhancement	-	rgh (rat growth hormone gene)	MT (mouse metallothionein promoter)	Maclean et al. 1987, Guyomard et al. (1989), Penman et al. (1991)
Production of sterile strains	-	gonadotropin-releasing hormone antisense genes	Histone 3 from salmon	Smith et al. (2001)

2.4. Institutions and working groups, including current research projects

Regarding current research activities in the field of transgenic fish the most important scientific working groups and their research topics are listed in the following:

USA

- Department of Molecular and Cell Biology and Biotechnology Center, University of Connecticut (Laboratory Thomas T. Chen); Topics: (1) Application of transgenic fish technology in basic and applied research (e.g. cloning and characterisation of structural fish genes, development of disease-resistant strains, development of monitor-fish strain for environmental pollutants), (2) Studying the molecular endocrinology of fish growth hormone and insuline-like growth factors (IGFs), (3) Molecular toxicology
- Air Force Research Laboratory, Human Effectiveness Directorate (Robert S. Cook), Ohio; Topics: Development of transgenic fish lines for detecting potential environmental hazards
- Great Lakes WATER Institute, University of Wisconsin (Michael J. Carvan); Topics: Development of transgenic fish lines for detecting potential environmental hazards
- Department of Fisheries and Wildlife, University of Minnesota (Anne R. Kapuscinski); Topics: Risk assessment and development of biosafety guidelines
- Departments of Animal and Biological Sciences, Purdue University, West Lafayette (William M. Muir; Richard D. Howard); Topics: Risk assessment of transgenic fish

Canada

- Department of Clinical Biochemistry and Biochemistry, Hospital for Sick Children, University of Toronto (Choy L. Hew); Topics: Enhancement of frost tolerance by transferring antifreeze protein genes, growth enhancement of fish by transferring growth hormone genes
- Fisheries and Oceans Canada, West Vancouver Laboratory, West Vancouver (R.H Devlin); Topics: Growth enhancement of fish by transferring growth hormone genes; osmoregulation and hormonal balance of transgenic growth-altered fish; studies on growth development of transgenic growth-altered fish; studies on morphological changes of transgenic growth-altered fish strains, studies on competitive ability of transgenic growth-altered fish; disease resistant strains
- Department of Zoology, University of Guelph; Topics: Physiological characteristics of transgenic growth-altered fish strains
- Ocean Sciences Centre, Memorial University of Newfoundland (Garth Fletcher); Topics: Enhancement of frost tolerance by transferring antifreeze protein genes, growth enhancement of fish by transferring growth hormone genes
- AquaBounty Farms, Prince Edward Island, Canada (Arnold Sutterlin; J.T. Cook); Topics: Growth enhancement of fish by transferring growth hormone genes; feeding behaviour of transgenic growth-enhanced fish; feed conversion efficiency of transgenic growth-altered fish

United Kingdom

- Division of Cell Sciences, Department of Biology, School of Biological Science, University of Southampton (Norman Maclean, M.A. Rahman); Topics: Feed conversion efficiency of transgenic growth-altered fish; growth enhancement of fish by transferring piscine growth hormone genes; improvement of gene expression; development of sterile transgenic strains, studying gene regulation

Ireland

- National Diagnostics Centre, Bioresearch Ireland, National University of Ireland, Galway (Sean Hanley, Terry J. Smith); Topics: application of transgenic fish technology in basic and applied research (e.g. cloning and characterisation of structural fish genes), improvement of gene expression

Finland

- Institute of Applied Biotechnology, University of Kuopio (Aleksei Krasnov, Tiina I. Pitkänen); Topics: Application of transgenic fish technology in basic and applied research (e.g. cloning and characterisation of structural fish genes), alteration of fish metabolism to improve carbohydrates utilisation via gene transfer

Norway

- Norwegian College of Veterinary Medicine, Oslo (P. Aleström); Topics: Improvement of gene expression; development of sterile transgenic strains

Sweden

- Department of Zoology, Göteborg University, Göteborg (J.I. Jönsson); Topics: Studies on competitive ability of transgenic growth-altered fish

France

- Laboratoire de Physiologie des Poissons, INRA Rennes (Svetlana Uzbekova, Bernard Breton, Patrick Prunet); Topics: Improvement of gene expression, development of transgenic sterile fish strains
- Institut National de la Recherche Agronomique, Jouy en Josas Cedex (L.M. Houdebine, D. Chourrout); Topics: Improvement of transgenic technology in fish

Germany

- BioCentre, University of Würzburg (Manfred Schartl); Topics: Improvement of transgenic technology (gene expression) in fish

China

- Laboratory of Freshwater Ecology and Biotechnology, Chinese Academy of Sciences (C. Fu); Topics: Research on feed efficiency of transgenic growth-enhanced fish
- Academia Sinica, Laboratory of Marine Molecular Biology and Biotechnology – Institute of Zoology (Taiwan) (Mark H.-C. Chen, Chi-Yao Chang), Topics: Cloning and characterisation of structural fish genes
- Institute of Fisheries Science, National Taiwan University, Taipei (Yau-Hung Chen, Chi-Yuan Chou, Chung-Der Hsiao); Topics: Cloning and characterisation of structural fish genes; Improvement of gene transferring methods and enhancing of gene expression

India

- Department of Genetics, School of Biological Sciences, Madurai University, Madurai (T.J. Pandian, T. Venugopal); Topics: Improvement of gene transferring methods and enhancing of gene expression

Japan

- Nagoya University, Department of Molecular Biology (Kimiko Amanuma, Hiroyuki Takeda); Topics: Development of transgenic fish lines for detecting mutations caused by compounds in aquatic environments
- Department of Aquatic Biosciences, Tokyo University of Fisheries, Tokyo (Yutaka Takeuchi); Topics: Improvement of gene transfer technology in fish

Cuba

- Divisions of Mammalian Cell Genetics, Centro de Ingenieria Genética y Biotecnologia, Havanna (José de la Fuente, Isabel Guillén, Marta Gómez-Chiarri, Rebeca Martínez); Topics: Growth accelerations of Tilapia

Israel

- Department of Zoology, Tel Aviv University (Philippa Melamed); Topics: Growth accelerations of Tilapia

2.5. Biosafety aspects

Scientific biosafety studies concerning transgenic fish have only just started. Preliminary data on biodiversity impacts of transgenic fish releases and effects on animal health are available, allowing first statements on the potential risks implied. However, any further statements on the effects and possible hazards resulting from the use of transgenic fish still need extensive risk assessment research to be conducted.

With regard to biosafety studies on transgenic fish, three major aspects have to be dealt with

- Genetic modifications can entail unintended effects such as skull and body deformities, tumours, abnormal gill growth or altered feeding behaviour. These side or pleiotropic effects have all been observed with transgenic salmon and trout (see Table 3).

- The stable expression of transferred genes cannot be guaranteed until now. In certain cases, e.g. in the production of transgenic sterile fish populations, this may become a biosafety problem.
- Escaped farm-raised salmon or trout, conventionally bred as well as transgenic individuals, are able to cross-breed with wild stocks of these species. As a consequence of crossbreeding, transgenes might spread into natural populations. This phenomenon can lead to adverse effects on natural communities, and finally disrupt the whole ecosystem.

2.5.1. Pleiotropic effects and their possible consequences

A vast number of studies show, that the various genetic modifications of fish can entail major pleiotropic effects. Most of the unintentional side-effects are mentioned in connection with growth enhancement due to transgenic modifications.

Sharp increases in the growth of transgenic fishes are often accompanied by skull and other body deformities, tumours, altered coloration[10], altered fin or vertebra shapes, abnormal gill growth, missing body segments, atrophies of nape and tail and altered pituary gland structure (Hew & Fletcher 1997, Devlin 1998, Dunham 1999, Mori & Devlin 1999, Pandian et al. 1999, Barrett et al. 2001, Devlin et al. 2001). Cranial abnormalities detected in transgenic trout were not seen in domestic non-transgenic animals, suggesting that, unlike domestication, transgenesis can affect growth pathways outside the range supported by the homeostatic processes that maintain the fish´s normal morphology and viability (Devlin et al. 2001). As a result of genetic engineering the growth hormone balance changes altogether (Dunham 1999). Even less severe alterations of the morphological shape, like larger skill surfaces, can have far-reaching consequences. Stevens & Sutterlin (1999) found, that larger gill surfaces of transgenic salmons lead to an increase in oxygen absorption. An enlargement of the gill surfaces by a factor of 1.24 increased the oxygen absorption of transgenic salmon by a factor of 1.6 as compared to the control groups. This phenomenon has to be taken into consideration in profitability calculations in the management of aquacultures, because increased oxygen demand of the transgenic animals requires increased pumping power to guarantee sufficient oxygen supply.

[10] For example, skin pigmentation appears to be lightened in salmon containing either the opAFPghc or OnMtgh1 constructs, perhaps by influencing hormonal control of melanocyte development and/or condensation (Devlin 1997).

Besides morphological problems also physiological effects and alterations of the behavioural biology have been observed (see Table 3). Devlin et al. (2001) compared the growth and development of different transgenic and non-transgenic rainbow trout strains[11]. The transgenic trout strains had reduced viability, and, in the case of the domestic strain, all transgenic animals died before sexual maturation. Farrell et al. (1997) examined critical swimming speeds of growth-accelerated coho-salmon and concluded that this transgenic strain had an inferior swimming ability. Different authors found that the feeding behaviour in transgenic fish strains differed from wild forms of the same species (e.g. Abraham & Sutterlin 1999). In behavioural experiments with simulated attacks of herons, Jönsson et al. (1996) demonstrated that transgenic trouts were quicker to return to the zones close to the water surface, started feeding sooner and ate more food in general. Devlin et al. (1999) found that dramatically faster growing transgenic coho salmon had extraordinary high plasma growth hormone (GH) levels and consumed 2.9 times more feed pellets than the non-transgenic controls in tanks. Finally it was observed in various studies that transgenic fish strains, compared to control groups, show altered body composition. Changes in the amino acid and cholesterol composition, an increased protein content as well as a reduced fat and water content were measured (see Table 3). The nutritional effects of such alteration of fish composition have not been studied so far. Guillén et al. (1999) and Fuente et al. (1998) examined the environmental and nutritional food risks resulting from transgenic Tilapia containing an additional growth hormone gene in test persons. They concluded that the commercial use of transgenic Tilapia present no risks. However, these biosafety studies were limited both in scope number and insufficient for making valuable statements on the overall biosafety of transgenic fish food. The research teams studied a very small test group of eleven persons over a period of only five days. Berkowitz & Krypsin-Sorensen (1994) also consider the consumption of transgenic fish as safe in general. However, they mention a potential allergy risk.

Another potential problem of food derived from transgenic fish is that the insertion of a transgene into the host genome may induce the production of unexpected toxins, e.g. by causing the expression of a quiescent toxin gene in a normally safe species of fish. Berkowitz & Krypsin-Sorensen (1994) believe that the probability of activating a toxin gene is extremely low, because normally safe fish are very unlikely to have toxin genes that can be activated.

[11] The research group compared four different strains: a non-transgenic wild strain, a non-transgenic domestic strain, a transgenic wild strain and a transgenic domestic strain.

In addition it has to be tested whether the observed changes in body composition of transgenic fish might adversely affect their value as a human food resource. Since transgenic modifications with the target of growth enhancement are often accompanied by reduced fat content (see Table 3), the production of fatty acids, including the valuable omega fatty acids, might be decreased simultaneously. Nürnberg & Ender (2001) showed that even a different kind of feed or feed mixture may cause changes in meat composition so that it is less wholesome for human consumption.

To summarise, numerous studies support that genetic engineering in fish (as well as in other organisms) will usually change non-target traits (e.g. body shape, feeding motivation, appetite) in addition to the target traits (e.g. growth rate), thus confirming the need to look for unintended trait changes when assessing the risk / safety of a genetically modified fish strain. The effects of transgenic modifications may differ in different species (Devlin et al. 2001). Therefore the results obtained for individual species cannot be simply applied to other species.

Table 3: Pleiotropic effects, that could be observed in transgenic fish

Organism	genetic modification	pleiotropic effect	reference
Rainbow trout (Oncorhynchus mykiss)	growth enhancement	skull deformities and reduced viability	Devlin et al. (2001)
Coho salmon (Oncorhynchus kisutch)	growth enhancement	allometric changes of the external contour	Ostenfeld et al. (1998)
Common carp (Cyprinus carpio)	growth enhancement	allometric changes of the external contour	Chatakondi et al. (1994)
Coho salmon, (Oncorhynchus kisutch)	growth enhancement	skull deformities	Devlin et al. (1995a)
Atlantic salmon (Salmo salar)	growth enhancement	larger gill surfaces	Stevens & Sutterlin (1999)

Organism	genetic modification	pleiotropic effect	reference
Tilapia (different subgenera of African Cichliden)	growth enhancement	the sexual organs of transgenic female animals were in relation to body size, smaller than those of non-transgenic animals; furthermore transgenic Tilapia had a lower protein and a higher water content as well as higher feed conversion efficiency	Rahman et al. (2001)
Atlantic salmon (Salmo salar)	growth enhancement	enhanced intestinal growth	Stevens et al. (1999)
Tilapia (different subgenera of African Cichliden)	growth enhancement	reduced sperm production	Dunham & Devlin (1998)
Atlantic salmon (Salmo salar)	growth enhancement	altered metabolism, enhanced oxygen need, altered body composition (enhanced water content, lower fat content, lower protein content and lower mineral content)	Cook et al. (2000a), Cook et al. (2000b), Cook et al. (2000c)
Rainbow trout (Oncorhynchus mykiss)	growth enhancement	altered metabolism, altered feeding behaviour	Jönsson et al. (1996)
Atlantic salmon (Salmo salar)	growth enhancement	altered feeding behaviour	Abrahams & Sutterlin (1999)
Coho salmon, (Oncorhynchus kisutch)	growth enhancement	higher water content and lower protein content	Hill et al. (2000)
Tilapia (Oreochromis hornorum)	growth enhancement	altered amino acid and cholesterol composition	Martinez et al. (1999)
Common carp; (Cyprinus carpio)	growth enhancement	altered amino acid composition, higher protein content, reduced fat content, reduced water content	Chatakondi et al. (1995)

Organism	genetic modification	pleiotropic effect	reference
Common carp, (Cyprinus carpio)	growth enhancement	altered feed conversion	Fu et al. (1998)
Coho salmon (Oncorhynchus kisutch)	growth enhancement	reduced swimming abilities	Farrell et al. (1997)
Japanese medaka (*Oryzias latipes*)	growth enhancement	abnormalities of the head and spine, reduced viability	Muir & Howard (2001)

2.5.2. Stability of expressions

According to Levy et al. (2000) transgenic technology has proved to be successful in several aquatic species, in particular in salmon and trout, in contrast to the relative inefficiency of the technology in the generation of "improved" transgenic farm animals. However, rates of gene integration are often low and to obtain fidelity of transgene expression is still a significant problem in fish biotechnology (Schartl et al. 1998, Breton & Uzbekova 2000, Levy et al. 2000, Nam et al. 2000, Kapuscinski & Brister 2001, Smith et al. 2001)[12].

One phenomenon already mentioned in the present study, which occurs in all transgenic fishes is mosaicism. Furthermore the number of gene copies varies from cell to cell, from tissue to tissue and from individual to individual (Takeuchi et al. 1999, Tappeser et al. 2000). Mosaicism in the F_0 generation is seriously hindering the stable transmission of transgenes. In recent times improvements in the construction of transgenes have been made by several research groups enhancing the integration and stability of transgenes. For example, Hsiao et al. (2001) developed a transgene that were flanked by inverted terminal repeats (ITRs) from adeno-associated virus. A more stable expression of the structural gene was reached by transferring such gene constructs to zebrafish. However, the frequency of genomic integration and germ-line transmission could not be improved.

[12] For example, Devlin et al. (1995b, 1994) obtained growth increases between 100 to 600% in the first generation of transgenic Atlantic salmon, that expressed a foreign growth hormone gene. Regarding the progeny of the transgenic F_0 generation only 2.2 to 18.9 of the offspring expressed the foreign gene.

Sheela et al. (1998) reached a more stable integration by using a Zp (Zona pellucida) fish promoter. The Zp regulatory region was derived from winter flounder (*Pseudopleuconectes americanus*). It is the regulatory region of a female sexual gene, that expresses constituitively throughout the year. Inheritance of the transgene was according to Mendelian laws. However, transmission rate was quite low. Altogether, the development of transgenic fish has made some progress, but the problems of low frequency rates of genome integration and the non-stability of transgene expression still remain unresolved (see e.g. Nam et al. 2000).

2.5.3. Ecological biosafety

Transgenic fish strains are developed for commercial fish production in aquaculture. There are several different systems of aquaculture. The spectrum ranges from simple ponds via net-cage farming (marine systems, as well as freshwater systems) to closed warm- or cold-water systems (the most expensive systems)[13].

Regarding fish production in aquaculture one major ecological concern is the escape or movement of domestic transgenic or non-transgenic individuals into natural communities. Such escapes into the wild are quite common (see 2.7, Table 4) and can be caused by natural events, like e.g. storms or floodings, technical defects or human failure.

Since many aquaculture operations are situated in regions where also feral populations of the fish species raised live, there is a great risk that escaped domestic fish populations interbreed with feral fish populations. Whether transgenic or not, escape of domesticated fish into feral populations and interbreeding with feral populations might adversely affect wild-type populations by introducing alleles that are poorly adapted to natural environments (Kapuscinski & Brister 2001, Muir & Howard 2001a).

The potential impacts of transgenic fishes are not well addressed yet and contradicting scenarios have been described (Tappeser et al. 2000). Scientific investigations have not yet identified all the possible mechanisms by which transgenic fishes might influence

[13] A comprehensive overview of different aquaculture systems is provided by Piker et al. (1998).

ecosystems (Muir & Howard 2002b)[14]. However, it is a fact that escapes of fish from conventional aquaculture systems often occur.

Piker et al. (1998) and Muir et al. (1994) regard the environmental risk posed by transgenic organisms as similar to that of the introduction of non-native (allochthonous) species. The observed effects of such species can partly help to estimate the environmental impact of transgenic strains. According to Welcomme (1998 and 1992) aquaculture has been the most important cause of introduction of non-native fish species to other regions. In general such introductions have adverse effects on the native wild fish species including competition via interference or exploitation, predation, inhibition of reproduction, habitat destruction, introduction of new diseases or parasites, and hybridisation (Folke & Kautsky 1989, Krueger & May 1991, Muir & Howard 2001a). In the USA the introduction of non-native fish from aquaculture facilities is believed to be a factor in the decline of seven fish species listed as endangered or threatened under the federal Endangered Species Act (Lassuy 1995).

Not only non-native species can have adverse impacts on native wild fish population. Also domestic strains of native species can cause ecological harm to wild-type populations if large numbers escape and interbreed with wild-type individuals. Especially the frequent and large escapes of farmed Atlantic salmon (*Salmo salar*) that occur worldwide raise concerns about ecological and genetic impacts on wild-type populations of this species (Gross 1998, Youngson et al. 1998 and 2001). Wild Atlantic salmon is characterised by a large number of genetically distinct populations that are adapted to the specific conditions of local river systems to which they return to spawn (Gausen & Moen 1991, Verspoor 1997, Gross 1998, Youngson et al. 2001)[15]. In contrast, cultured Atlantic salmon are bred to be genetically uniform and to exhibit favourable production traits. According to Gross (1998) domestic and

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[14] The Thai government has discouraged several requests to introduce GM tilapia, partly on account of the absence of case-specific risk assessment data and insufficient capability to assess and control genetically modified organisms. Tilapia are not native to Thailand, but some have escaped into natural rivers and wetlands and established feral populations. In November 2001, the Institute for Social, Economic, and Ecological Sustainability (ISEES) at the University of Minnesota received a four-year, U.S.$ 425,000 grant from the U.S. Agency for International Development (AID) for doing research on the effects of introducing tilapia that has been genetically engineered for growth enhancement. The project will measure the likelihood that genetic material will flow from the introduced GM tilapia to the existing feral populations. The impact of the introduction on other feral popultions will also be evaluated (source: press release of the University of Minnesota – 11701/2001; http://www.eurekalert.org/pub_releases/2001-11/uom-iog110101.php).

[15] The most direct evidence for local adaptation relates to the resistance of the Atlantic salmon populations of Baltic rivers to the parasite *Gyrodactylus salaris*. When the parasite was inadvertently introduced to rivers in western Norway large-scale mortalities resulted. Experimental work has demonstrated that, although the parasite is common with Baltic populations of salmon, populations outside the Baltic that have no history of natural exposure to the parasite show little or no resistance to its lethal effects (Youngson et al. 2001).

wild-type Atlantic salmon are so distinct from each other that the domestic strain should be regarded as a new biological entity – called e.g. *Salmo salar* var. *domesticus.*

Interbreeding between domestic and wild-type strains introduces new combinations of genes to genetically distinct populations of wild-type populations, and may break up local genetic adaptations[16]. However, taxonomically distinct wild-type populations are an irreplaceable reservoir of genes (live gene bank) harbouring co-adapted gene and chromosomal complexes that aquaculture breeders can tap to improve economically important traits, such as disease resistance. Introgressive hybridisation would disrupt these gene complexes as well as dilute rare alleles that contribute to the capacity for evolution and that could be also crucially important for aquacultural performance traits (Kapuscinski & Brister 2002)[17].

Like non-transgenic farmed individuals, transgenic individuals compete as well with wild individuals on food, mating partners and spawning grounds. However, the release of transgenic fishes into natural environments poses additional risks, because, although transgenic individuals retain most of the characteristics of their wild-type counterparts, they may also possess some novel advantages in competition (Muir & Howard 2001a). For example, the altered feeding behaviour that was observed in different studies (see 2.5.1 and Table 3) or traits like enhanced cold tolerance can reveal a fitness advantage[18]. According to Muir & Howard (2001) and Muir et al. (1994) the cumulative action of natural selection over several generations could even modify the expression of a transgene and make the organism more successful. Like other genes also transgenes are introduced in wild fish populations by interbreeding and can alter the genetic structure of these. Such alterations can be accompied by a loss of genetic diversity and a loss of the capacity of evolution (Hallerman & Kapuscinski 1993, Dunham 1999, Breton & Uzbekova 2000, Kapuscinski & Brister 2002).

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[16] There is mounting evidence that a shift in the gene pool of wild Atlantic salmon populations occurs in different European regions due to introgression of farmed salmon (see e.g. Gross 1998 and Youngson et al. 2001).

[17] Some scientists have argued that mal-adaptation of escaped farmed fish ensures that their genes would be quickly purged from wild populations by natural selection. According to Kapuscinski & Brister (2002) no aquacultural broodstock have become so intensively domesticated as to assure a high death rate in the wild and, thus, rapid purging of mal-adaptive genes.

[18] Habitat enlargement could be the consequence of enhanced cold tolerance. This can have adversely effects on other species that are adapted to cold environments.

Considering the risks of unintended (or intended) release of transgenic growth-accelerated fish the consequences of sexual selection have to be assessed too[19]. Quite often larger males have a mating advantage over small males. This has been confirmed for Atlantic salmon in experiments conducted by Jones & Hutching (2001)[20]. Furthermore the fitness of transgenic growth-accelerated individuals can be enhanced also by size-related advantages in foraging or predator avoidance and earlier attainment of sexual maturity. For assessing the risk of transgene spread to wild relatives, Muir & Howard (1999, 2001, 2002a and b) developed a new methodology. Their approach focuses on estimating the overall fitness of a GMO by collecting data at critical “check points” in its life history (Kapuscinski & Brister 2001). First, controlled experiments have to be conducted to test the transgenic organisms for changes in six "fitness components". These six fitness components are also called “net fitness components” by Muir & Howard (2001), because they are regarded as the major means by which natural selection can alter the frequency of a transgene. The six fitness components are juvenile and adult viability, longevity, age at sexual maturation, fecundity (clutch or spawn size), male fertility, and mating success of both females and males (Muir 2002). The second step is to incorporate the data into a mathematical model that integrates them into a single prediction of gene flow from escapees to wild relatives.

Using the developed methodology, Muir & Howard (1999 and 2001) and Muir et al. (1996) showed how important it is to examine interaction between the six fitness components that can be changed by one transgene. They studied transgenic growth-accelerated Japanese medaka (*Oryzias latipes*), as well as its wild-type counterpart and estimated the fitness components chosen. The transgenic medaka grew faster, reached sexual maturity earlier, and had lower viability than non-engineered controls. Computer simulations combining the data on mating advantage and lower viability gave a worrying result, called the "Trojan gene effects" by the authors. The transgene introduced by interbreeding with 60 transgenic individuals into a wild population of 60 000 individuals spread quickly as a result of enhanced mating advantage, however, the reduced viability of offspring drove the mixed population to half its size in less than six generations and to extinction in about 40 generations. Hedrick (2001) came to similar conclusions by using a deterministic model. He investigated both the effects of introducing a transgene that has a male-mating advantage and a general viability advantage. For 66.7% of the possible

[19] Muir & Howard (2001 and 2002a) and Muir (2001) distinguish between the risk and the hazard of transgenic organisms in natural environments. Transgene risk is defined as the probability that a transgene will spread into natural con-specific populations and hazard as the probability of species extinction, displacement, or ecosystem disruption given that the transgene has spread.

[20] In brown trout larger individuals are socially dominant over small trout (Johnsson 1993).

combinations of the possible mating and viability parameters, the transgene increases in frequency, and for 50% of the combinations, it proceeds to fixation. In addition, by this increase in the frequency of the transgene, the viability of the natural population is reduced.

Studies on the impact of transgenic fish on predators and other members of the aquatic biocoenosis are still completely missing.

To summarise, lack of a systematic biosafety assessment for genetically modified fish poses a hazard to aquatic biological communities. Several studies indicate possible risks. This has also been stated by Kapuscinski & Brister (2001) and Piker et al. (1998)[21]. Furthermore, it should be kept in mind that no overall conclusions can be drawn from single risk assessment studies to the general safety or danger of any transgenic organism. Even in the same species, different transgenic lines are likely to vary in fitness even if the same gene construct is used because of differences in copy number and insertion sites of the transgene (Muir & Howard 2001). According to Muir & Howard (ib.) evaluations should be conducted first in closed laboratory systems, then in experimental mesocosms, and finally in more extensively controlled systems that simulate natural systems, with the results of each step being used in the design of the next experiment.

2.6. Containment strategies, experiences gained

In principle, there are two different containment strategies to prevent gene flow between escaped farmed fishes and wild populations.

The first one is physical containment of farmed fish including physical and mechanical barriers. The goal of this strategy is to design aquacultural facilities that are escape-safe[22]. Physical barriers are constructed so that they induce 100% mortality through such physical alterations as imposing lethal water temperatures or pH to water flowing out of fish tanks or ponds before the effluent is discharged to the environment (Kapuscinski & Brister 2001). For cage-farming of salmon such physical barriers are no option. There is no possibility to install such physical barriers.

Mechanical barriers are devices, such as screens, that hold back any life stage of the fishes from leaving the aquaculture facility (Kapuscinski & Brister 2001). Considering fish

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[21] Besides the risks of escapes and introgression of farmed fish, non-conventional aquacultures pose further environmental risks that are described by Folke & Kautsky (1998), Naylor et al. (1998) and Stewart (1997).

[22] In general, three different types of aquaculture facilities can be distinguished: conventional ponds, net cages and closed systems. Advantages and disadvantages of the different types are well described by Piker et al. (1998).

production in ponds it has to be ensured that such mechanical barriers are constructed in a way that even events like flooding, heavy rain falls or draining are not accompanied by escapes of any life stages of fish (including eggs or early development stages)[23].

Net-cage farming facilities are highly vulnerable to breach. According to Kapuscinski & Brister (2001) materials such as extra predator barrier nets and rigid netting can help but are not sufficient to prevent large escapes of farmed fishes due to storm damage, predator damage, or wear and tear. Floating enclosed bags, a new technology, may work well in quiet waters, but not in marine waters. Furthermore these bags need to be tested thoroughly prior to use. The only option that appears to be 100% safe is land-based fish production in closed systems. According to Smith et al. (2001) total physical containment of farmed fish is an unrealistic option for economic reasons given the huge cost of enclosed systems, particularly for sea-based facilities.

The second possible containment strategy is biological containment. Biological containment involves the production of sterile lines of fish. There are several methods to produce sterile fish populations: production of triploids, exposure of gamets to X-ray or gamma irradiation, production of monosex populations by hormonal treatment or production of sterile transgenics (e.g. by inhibition of the gene that codes for the gonadotropin releasing hormone through antisense technology). A precise description of these methods including their advantages and disadvantages can for example be found in papers from Smith et al. (2001), Breton & Uzbekova (2000), Casebolt et al. (1998) and Donaldson et al. (1993).

Induction of triploidy is widely accepted as the most effective method for producing sterile fish for aquaculture (Kapuscinski & Brister 2001). Triploidy induction disrupts gonadal development to some extent. Typically, gonadal development is more fully disrupted in females than in males. Therefore, the production of all-female lines of triploids in fish and shellfish is the best way to maximise disruption of gonadal development as a biological barrier to reproduction of aquacultural escapees. According to Kapuscinski & Brister (2001) under experienced hands, rates of successful triploidy can be expected in the 90th percentile in large-scale production. However, the success will vary with fish strain, egg quality, age of spawners and induction conditions. The critical risk management issue is whether to screen every individual destined for grow-out for the all-female triploid condition or only a subsample of each production lot. In every case, screening for triploidy must occur in every generation. Kapuscinski & Brister (2001) propose to monitor for permanent sterility in triploids. Razak et al. (1999) suggest too that rigorous breeding studies are necessary prior to commercial use

[23] The risk still remains that fishes or spawn are spread by birds.

of transgenic triploids to ensure that no gene flow occurrs. Reversion to the diploid and fertile condition was recently discovered in triploid oysters (Allen & Guo 1996). No reversion in fish has been reported so far.

The production of sterile transgenic fish populations might not to represent a favourable option because of vulnerabilities known to be inherent in gene transfer. Expression of the transgene responsible for sterility induction could be turned off at any time through methylation. The transgene could also undergo rearrangement in the founders or descendants, thus possibly disrupting the expression needed to induce sterility (Kapuscinski & Brister 2001, see also 2.5.2.).

2.7. Escapes of fish contained in aquacultures, experiences gained

In recent years salmon aquaculture has produced a large annual fish biomass. In 1999, more than 620 000 tonnes of aquaculture salmon were produced in the North Atlantic area, with Norway and Scotland accounting for the lion´s share (ICES 2000). In contrast, total figures recorded for salmon from commercial fisheries in the same area were much smaller, i.e. approximately 2200 tonnes (ICES 2000). These figures do not lend themselves to any direct comparison but obviously even small fractional escapes of commercial aquaculture stock have the potential to result in high frequencies of escaped fish among salmon occurring in the wild (Youngson et al. 2001).

In recent years numerous escapes of farmed salmon occurred (see Table 4). These escapes have not been restricted to single individuals. During a single episode in the winter of 1988-89 approximately 700 000 farmed salmon individuals escaped from only one area in the middle coastal region of Norway (Gausen & Moen 1991)[24]. In the summer of 1996 almost 100 000 Atlantic salmon escaped from a relatively small net-pen industry in the State of Washington (USA) (Mottram 1996, cited from Goldburg 2001). According to Gross (1998) increasing numbers of escaped farmed Atlantic salmon are observed outwith the native range of wild Atlantic salmon in the North American Pacific drainage. Even in the State of Alaska, where Atlantic salmon farming is prohibited, isolated specimens of Atlantic salmon have been caught, probably originating from farms in lower British Columbia and upper Washington State.

[24] Assuming an average weight of 30 kg for an adult salmon, this means that during this single event 21 000 t of salmon escaped. In 1985 about 80 000 t of salmon and trout were produced in Norwegian aquacultures. (Folke & Kautsky 1989).

Analysis of research fishing in the Faroese ocean area from 1980/81 onwards, showed that farmed salmon were present in every year. Frequency of occurrence reached a peak during 1989/90 and 1990/91 when escaped fish accounted for more than 40% of the catch. More recently, frequencies have declined to levels of about 20 to 30% (Youngson et al. 2001).

Monitoring of salmon fisheries in outer coastal areas in Norway since 1989 has shown that the frequency of escaped salmon has remained relatively stable, varying between 44 and 49%. These values are higher than the values for the fjord fisheries where the frequency of escaped fish varied between 10 and 21% (Youngson et al. 2001). The frequency of escaped salmon in fjord and river catches is usually lower due to the fact that the escapees tend to enter fjords and rivers later than wild fish, and only after the fisheries have closed (Youngson et al. 2001). However, in 1995 81% of the female spawners caught in the River Vosso, the second largest watershed in western Norway, were of farmed origin (Sægrov et al. 1997). To minimise the adverse impacts of escaped farmed fish to wild populations improved containment is recommended by the Oslo Agreement developed by the North Atlantic Salmon Conservation Organization (Youngson et al. 2001).

Table 4: Examples of observed Atlantic salmon escapes from aquaculture facilities

Date / period of time	observed escapes	country / region	reference
Winter 1988/1989	Approximately 700 000 farmed salmon escaped at once from one area alone (in the middle costal region of Norway)	Norway	Gausen & Moen (1991)
1989	184 000 farmed salmon escaped in Loch Eriboll, Scotland	Scotland	Webb et al. (1991)
1989	The proportion of reared salmon reported in one river in Iceland was 30.1%	Iceland	Gudjonsson (1991)
1990	Escaped farmed salmon constituted approximately 20-40% of marine catches in Scotland	Scotland	Webb & Youngson (1992)
1991 /1992	Examinations of scale samples collected in commercial fisheries of West Greenland revealed that escaped farmed salmon was present at a frequency rate of 1-2%	West Greenland	Hansen et al. (1997a)

Date / period of time	observed escapes	country / region	reference
during the 1990s	The progeny of escaped farmed salmon have been found in several Norwegian rivers	Norway; Ireland; Scotland	Webb et al. (1993), Lura & Økland (1994), Clifford et al. (1998)
during the 1990s	Results of research fishing: about 20-40% of the salmon found consisted of escaped farmed salmon	Faroe Islands, North-Atlantic	Hansen et al. (1997a), Hansen et al. (1999)
during the 1990s	From 29% on average to more than 80% of the salmon in some Norwegian spawning populations consisted of escaped farmed salmon	Norway	Lund et al. (1991), Fiske & Lund (1999)
1995	81% of the female spawners caught in the River Vosso, the second largest watershed in western Norway, were of farmed origin	Norway	Sægrov et al. (1997)
1996	About 100 000 farmed Atlantic salmon escaped from a relatively small net-pen industry at the Pacific coast	Washington State, U.S.A.	Mottram (1996), cited from Goldburg (2001)
during 1994-1998	An average of 43 000 Atlantic salmon escaped into British Columbia waters	British Columbia, Canada	Noakes et al. (2000)
1992-1999	In one investigated Canadian river the number of farmed fish returning to spawn was 2-8 times that of wild salmon	Canada	Carr et al. (1997), Whoriskey (2000)
2000	An estimated 32 000-86 000 farmed Atlantic salmon escaped from net-pens	Canada	Muir & Howard (2002a)

3. Transgenic fish – activities of governmental and non-governmental organisations

Aquaculture is the fastest growing food-production industry worldwide. This development has attracted the attention of politicians seeking to supply a fast-growing world population – particularly in Asia – with high-quality animal proteins. Many stakeholders in the field of fisheries and aquaculture are convinced that modern biotechnology can promote

further achievement in this field. However, there are many concerns regarding biosafety aspects of transgenic fish. Facing this controversy several international and multinational organisations, a number of national states, an increasing number of scientists and different industrial, consumer and environmental lobby groups are discussing the potentials and biosafety aspects of this technology. The following chapter of the present study will highlight the most important policy concepts, recommendations, plans and decisions.

3.1. International conventions: The Convention on Biological Diversity and the Cartagena Protocol on Biosafety

In 1992 at the Earth Summit in Rio de Janeiro the **"Convention on Biological Diversity" (CBD)** was finally adopted. It is aimed to work against the erosion of genetic and biological diversity on all levels. Conservation and sustainable use of biological resources are the main goals of the CBD. The convention is still the only internationally **binding** agreement obligating all member countries to undertake measures to protect biological diversity (Tappeser & Baier 2000). Currently it has 183 Parties[25]. According to the CBD "sustainable use means the use of components of biological diversity in a way and at a rate that does not lead to the long-term decline of biological diversity, thereby maintaining its potential to meet the needs and aspirations of present and future generations." Its Article 14 calls upon each contracting party to require environmental impact assessments of proposed projects “that are likely to have significant adverse effects.” Regarding genetic engineering (GE) Article 19 paragraph 3 states the following: "The Parties shall consider the need for and modalities of a protocol setting out appropriate procedures, including, in particular, advance informed agreement, in the field of the safe transfer, handling and use of any living modified organism resulting from biotechnology that may have adverse effects on the conservation and sustainable use of biological diversity." In addition Article 8 calls upon "each contracting party shall, as far as possible and as appropriate: (…) (g) Establish or maintain means to regulate, manage or control the risks associated with the use and release of living modified organisms resulting from biotechnology which are likely to have adverse environmental impacts that could affect the conservation and sustainable use of biological diversity, taking also into account the risks to human health" (UNEP/CBD 1992).

Marine aquaculture was addressed at the fourth Conference of the Parties (COP). During this conference it was decided to establish a so called "Ad Hoc Technical Expert Group on Mariculture". The work of the group is intended to help implement programme

[25] source: http://www.biodiv.org/biosafety , 27th May 2002

element 4 (mariculture) of the programme of work on marine and coastal biological biodiversity. The operational objective of the programme is "to assess the consequences of mariculture for marine and coastal biological diversity and promote techniques which minimize adverse impact." The first meeting of the group will be held in July 2002 in Rome (Italy) (see UNEP/CBD document: UNEP/CBD/AHTEG-MAR/1/1/Add.1; 6th June 2002[26]).

More specific regulations are being negotiated, in particular the **"Cartagena Protocol on Biosafety"** to the CBD. The Protocol has been adopted in 2000 by the Conference of the Parties of the CBD, but with only 19 of 50 necessary ratifications[27] it is still far away from coming into force[28]. Once ratified the Cartagena Protocol will be a legally binding agreement under the CBD. The Cartagena Protocol will be the global legal instrument for the regulation of "the safe transfer, handling and use of living modified organisms [which is a similar term as genetically modified organisms (GMO)] resulting from modern biotechnology" (UNEP/CBD 2000). The adoption of the Cartagena Protocol is intended to lay the foundation for a global system of assessing the impact of genetically engineered organisms on biodiversity, and exchanging information through a Biosafety Clearing House Mechanism. It also contains provisions to encourage capacity building in developing the environmental assessment of genetically engineered organisms (OECD 2000).

One bone of contention throughout the final negotiations of the Cartagena Protocol in Montreal in January 2000 was reference to the Precautionary Principle. Proponents of the biotechnology industry were highly critical of the Precautionary Principle because they fear that it will be invoked to block international trade of GMOs and genetically engineered food. Articles 10.6 and 11.8 of the final text confirm the right of a party of import to apply precaution in deciding whether or not to allow the proposed importation of GMO. However the language is quite convoluted and has already generated divergent interpretations of what it will mean in practice. Some view the final text as a weak version of the Precautionary Principle, providing an importing nation the flexibility to weigh the importance of environmental risk against other factors. Most signatory parties to the Biosafety Protocol are also Parties to the WTO. WTO rules, under its Agreement on Sanitary and Phytosanitary Measures, forbid import bans unless the party of import can demonstrate the risk of a product to health or the environment. In contrast, supporters of the Precautionary Principle

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[26] source: http://www.biodiv.org/doc/meeting.asp?wg=TEMCTRE-01

[27] source: http://www.biodiv.org , 27th May 2002

[28] According to Kapuscinski (2002) in September 2000 74 countries and the EU had indicated their interest to ratify by signing the Cartagena Protocol on Biosafety.

see it as a means to require the party of export to demonstrate safety of the product. The final text of the protocol did not fully resolve whether or not rulings of an unfair trade barrier by the WTO could override a party´s decision under the protocol to bar import on the basis of precaution (Kapuscinski 2002).

3.2. International policies and intergovernmental organisations

International institutions and treaties that are affected by the use of genetic engineering in aquaculture are very heterogeneous. Some are dealing with biodiversity at the global level in a more or less general way, e.g. the CBD, or with a special topic like nutrition, e.g. the FAO of the UN. Other organisations are more regionally-focussed on different topics, e.g. “The Conference on the Protection of the North Sea”, the “North Atlantic Salmon Conservation Organization” or the “Network of Aquaculture Centres in Asia-Pacific”.

The following section describes the most important international and intergovernmental organisations dealing with aquaculture and the use of GM fish.

3.2.1. World Trade Organization

Concerning the trade with biotechnology products the World Trade Organisation (WTO) has three trade rules of particular relevance:

- The WTO Agreement on Sanitary and Phytosanitary Measures (SPS),
- The WTO Agreement on Technical Barriers to Trade (TBT), and
- The WTO Agreement on Trade Related Aspects of Intellectual Property (TRIPS).

Currently the USA and the EU Commission are controversial about how to exactly interprete the mentioned WTO trade rules – particularly the Agreement on Sanitary and Phytosanitary Measures (SPS) and the Agreement on Technical Barriers to Trade (TBT) – in the case of the Commission´s proposals for a regulation on traceability and labelling of GMO's “COM(2001)182final” (EU-Commission 2001a) and the proposals for a regulation on GM food and feed “COM(2001)425 final” (EU-Commission 2001b). Regarding the regulation on GM food and feed, the United States of America commented that "reliably and consistently achieving 100 percent non biotech content is not feasible, but experience has shown that a one percent threshold [as mentioned in the proposal] also cannot reliably be tested". Furthermore the USA wondered "that if any biotech foods allowed on the market will have had to be demonstrated to be safe and the EU has not articulated that bio-engineered

foods are unsafe, how will mandatory labelling help the Commission achieve its objective as stated in Article 1?" [29]

3.2.2. Food and Agriculture Organization

Within the United Nations (UN), the Food and Agriculture Organization (FAO) is the most important organisation in the field of the development of aquaculture. The participants of the 24th meeting of the FAOs Committee on Fisheries (COFI) held on 26th February to 2nd March 2001 decided to establish a **Sub-Committee on Aquaculture** under the COFI. This decision reflects the importance attached to aquaculture development by the FAO member governments. The aim of this subcommittee is to provide a neutral forum for consultation and discussion on aquaculture and to advise COFI on technical and policy matters related to aquaculture and on the work to be performed by the FAO on the subject of aquaculture (FAO 2002a). The Sub-Committee on Aquaculture held its first session in Beijing, China on April 18-22, 2002[30]. Discussing the implementation of aquaculture-related provisions of the "Code of Conduct For Responsible Fisheries" (CCRF), the 100 delegates[31] in Beijing "recognized that good environment and consumer health are key factors that need to be addressed to develop a sustainable aquaculture industry" (FAO 2002a). Many participants stressed the importance of food quality and product safety. To address the problems the meeting suggested carrying out series of environmental, social, and economic risk assessment studies for the aquaculture sector to gather reliable information on the risks of aquaculture operations. Regarding genetically modified fish and shellfish it has been stated by the Sub-Committee on Aquaculture that “transferring molecular methodologies should be used with due protection of aquatic diversity and with due consideration given to potential impacts on the autonomy and economy of rural and subsistence populations”. Furthermore the Sub-Committee holds that prior safety assessment, based on risk assessment and precautionary approach will become increasingly common in the pursuit of products from modern biotechnology (Sub-Committee on Fisheries 2002).

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[29] U.S. Comments, 6th December 2001, regarding: "WTO Notification G/TBT/N/EEC/6" proposed Food & Feed Regulation of the EU-Commission (http://www.foeeurope.org/press/US_comments_G_TBT_N_EEC_6.pdf).

[30] The next meeting of the Sub-Committee will be held in Norway in August 2003 (FAO 2002a).

[31] Representatives from governments, inter-governmental organisations, UN agencies, and international non-governmental organisations participated the meeting in Beijing (FAO 2002b).

The "Code of Conduct For Responsible Fisheries" (CCRF)[32] used as the basis for discussion at the meeting in Beijing was adopted by the FAO COFI in 1995 as a voluntary instrument. "However, certain parts of it are based on relevant rules of international law. The code also contains provisions that may be or have already been given binding effect by means of other obligatory legal instruments amongst the Parties" (FAO 1995). Article 9 of the CCRF discusses the development of aquaculture with respect to modern biotechnology and the genetic improvement of fish stocks: "In particular, efforts should be undertaken to minimize the harmful effects of introducing non-native species or genetically altered stocks" (FAO 1995). To support the implementation of the Code, additional "technical guidelines" have been adopted. The second (of so far eight) of these guidelines, "Precautionary Approach to Capture Fisheries and Species Introductions", states: "The use of introduced species, including genetically modified and genetically selected organisms, may allow for continued or increased production from habitats that have been so altered or degraded that native fisheries are no longer viable. Care should be taken not to use this potential productivity from introduced species as justification for further abuse of habitat or for delaying their restoration. (…) 121. Intended introductions should be controlled. As a consequence, those making an introduction (…) would be expected to demonstrate caution by preparing a proposal covering: (…) (3) [the] analysis of potential impacts at the introduction site, including potential ecological, genetic and disease impacts and consequences of its spread, and (4) a qualitative and, where possible, a quantitative risk assessment" (FAO 1996). Furthermore the technical guidelines of the CCRF agree with the "ICES Code of Practice on the Introductions and Transfers of Marine Organisms 1994" of the international science organisation **"International Council for the Exploration of the Sea"** (see also 3.5.1 “International science organisations”), which "forms a basis for a more precautionary introduction" of foreign and genetically engineered species (FAO 1996).

In addition to the mentioned initiatives the FAO organised a large number of meetings and publications on the topic of aquaculture and the use of biotechnological methods therein. Some of the following instruments may not be mainly focused on the application of biotechnology in aquaculture or agriculture but on related questions. The **Codex Alimentarius** for example is a joint initiative of the FAO and the World Health Organisation (WHO) with its focus on food security. In the early sixties both organisations passed resolutions to establish the Codex Alimentarius Commission (CAC), as "an

[32] The CCRF is global in scope and is directed towards members and non-members of the FAO.

intergovernmental body set up to establish international standards on foods."[33] Even though the standards, guidelines and recommendations of the Codex are voluntary they are recognised by the World Trade Organization (WTO) as a reference in international trade disputes. The participants of the 23rd Session of the CAC held in June 1999 adopted a Medium-Term Plan for 1998 to 2002, which states "Consideration should be given to the development of standards in these areas for foods derived from biotechnology or traits introduced into foods by biotechnology, where this is scientifically justified."[33] They also established an **ad hoc Intergovernmental Task Force on Foods derived from Biotechnology** to implement this Medium-Term Plan. The WHO itself has convened a series of expert consultations within the scope of the Codex and many of these were cosponsored by the FAO. Concerning one of these expert consultations the Task Force "welcomed the initiative of FAO and WHO to convene expert consultations to support the scientific aspects of its work in the area of foods derived from genetically modified (…) fish" (CAC 2001), but to date no information is available regarding these expert consultations. Last but not least it should be highlighted that the safety assessment of the Codex – as it is drafted so far – "is characterized by an assessment of a whole food or a component thereof relative to the appropriate conventional counterpart: a) taking into account both intended and unintended effects" (CAC 2002).

In 2000 the FAO has published the third issue of **"The State of the World Fisheries and Aquaculture"**. Quoting the foreword it provides "a comprehensive, objective and global view of capture fisheries and aquaculture, including associated policy issues." (FAO 2000). Regarding genetically modified salmon the text states: "Although there are theoretical causes for concern, there are no real data to support the recent claim that genetically modified salmon are extremely dangerous to the environment."(FAO 2000). Concerning introductions and transfers of marine organisms references to the ICES Code of Practice can be found (see chapter 3.5.1.). One year later the "world fisheries and aquaculture atlas" followed as a digital companion (FAO 2001). The atlas includes more than 300 papers which were prepared for this edition by FAO staff members and professionals from co-operating institutions and others. This information is provided together with about 3000 links to the world wide web.

As already mentioned, the Code of Conduct for Responsible Fisheries is an important tool for the work of the FAO. To support the implementation of the Code the FAO Committee on Fisheries established a special programme of global partnership concerning the

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[33] WHO: Standard Setting Through the Joint FAO/ WHO Codex Alimentarius Commission (http://www.who.int/fsf/GMfood/codex_index.htm).

implementation of the Code of Conduct For Responsible Fisheries and taking the special difficulties of the less developed countries into account; the programme is called **'FishCode'**. "The overall FishCode objective is to raise the economic, social and nutritional benefits obtained from fisheries, especially by coastal fishing communities, through the adoption of responsible fisheries management and resource conservation policies and practices".[34]

3.2.3. Organisation for Economic Co-operation and Development

In general the target of the Organisation for Economic Co-operation and Development (OECD) is to help governments tackle the economic, social and governance challenges of a global economy. The OECD has extensive experience with safety-related activities dating back to the mid-1980s.

In the field of modern biotechnology, genetically modified organisms and food safety the OECD focus, inter alia, on the standardisation and harmonisation of the regulatory framework in the member countries, particularly in its "Working Group on Harmonization of Regulatory Oversight in Biotechnology" which was established in 1995 (OECD 2000). For example, the Working Group and the OECD, respectively, have published a series of consensus documents for the work on (I) harmonisation of regulatory oversight in biotechnology and (II) on the safety of novel foods and feeds. These consensus documents comprise technical information for use during the regulatory assessment of products of biotechnology. Furthermore the OECD has launched other working groups and task forces and provide the well-known database BioTrack Online (developed by the above mentioned Working Group). This database is one of the best sources of information on member country regulations and regulatory developments, as well as field trials and product approvals (OECD 2000). Genetic modifications in animal species are not a Working Group priority as yet. However, in 1993 the OECD in co-operation with the Norwegian Ministry of Environment organised a workshop on “Environmental Impacts of Aquaculture using Aquatic Organisms derived through Modern Biotechnology”[35] and published the contributions of the participants as an initial study on the environmental impacts of aquaculture (OECD 1995).

In 2001 the OECD organised two major meetings on GMO and related topics in general (Bangkok, Thailand, and Raleigh-Durham (N.C.), USA).

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[34] FishCode is a series of projects which are located in different parts of the developing world (http://www.fao.org/fi/projects/fishcode/default.htm).

The Bangkok Conference on **"New Biotechnology Food and Crops: Science, Safety and Society"** took place in July 2001 and was organised by the OECD and the United Kingdom. It brought together 300 participants from over 50 countries and 5 continents, who concluded the conference “with recommendations that all stakeholders commit themselves to greater transparency on genetically modified organisms and that governments increase their support for independent and public-funded scientific research into the risks and benefits of GM foods and crops” (OECD 2001d). Concerning risk assessment methodologies some participants “considered that the present methods for risk assessment were inadequate. In particular, there was a lively debate about *substantial equivalence*. Some considered substantial equivalence neither useful nor scientific. Other stressed that substantial equivalence was not considered a safety or risk assessment but one possible starting point guiding further food safety assessment steps” (OECD 2001a).

Some 250 participants from 45 OECD member- and non-member countries came together at a conference on **"Living Modified Organisms (LMO) and the Environment"** in Raleigh-Durham (United States) in November 2001. The majority of the participants stated that LMO "contribute to a safer, more secure global food supply but their effects on the environment require continuing scientific investigation” (OECD 2001b). “Some argue that transgenic fish may pose negligible ecological risks as they are unlikely to be selected for in the presence of wild populations. However, large numbers of LMO fish interbreeding with natural populations may present an issue. Recovery after release is unlikely. Studies based on one individual environment, for example in contained facilities, are inadequate to predict behaviour and performance in natural environments.” (OECD 2001c).

In addition it should be highlighted that the OECD`s current co-operative research programme **"Biological Resource Management for Sustainable Agriculture Systems"** emphasises – as one of only four themes for 2002–2004 – the "Quality of Animal Products and Safety of Food": "It deals with animal products and includes aquaculture. The overall objective is to assure food safety by identifying and evaluating risk factors. Examples of topic areas might include:

- New safety indicators and their development.
- Bioengineering of new and safer animal products.

[35] This workshop was held on 9th-11th June 1993 in Trondheim, Norway.

- Risk assessment of transgenic animals producing foreign proteins and use of antibiotics and hormones for improving animal production."[36]

In addition to the internationally working organisations and conventions some regionally working intergovernmental organisations exist that are establishing a regulatory framework for the commercial use of GMO like e.g. the European Union, or that have their focus on the development of aquaculture. In addition to the European Union, the most important ones are the Network of Aquaculture Centres in Asia-Pacific (NACA) with a focus on Asia, the "Conference on the Protection of the North Sea", the Nordic Council of Ministers, and the North Atlantic Salmon Conservation Organization (NASCO).

3.2.4. European Union

The Directive 2001/18/EC on the deliberate release of GMO (EU Commission 2001c) has to be established in the EU Member States on 17 October 2002 and will repeal Council Directive 90/220/EEC. The new directive provides detailed regulations regarding the deliberate release and placing on the market of GMO, including the information of the public, risk assessments and monitoring. However, with regard to the last two points for example, definite agreements are still under discussion. Only recently the EU Commission drafted two guidance notes on the principles applicable to environmental risk assessment (EU Commission 2002a) and on the monitoring plan (EU Commission 2002b), which shall be completed by the date the directive will come into force (see also 3.4.: Non-governmental organisations).

Regarding transgenic fish the European Commission stated in an official reply to the enquire P-1557/01 of Ian Hudghton (The Greens / European Free Alliance[37]) that GM fish has potential to cause irreversible damage to fish stocks and to the marine environment in the event of escape[38]. To date, the Commission did not either receive any notification with respect to experimental releases of GM fish nor any application for commercial releases of GM fish. Such authorisations may only be granted subject to the provision that there is no reason to believe that the release could have any adverse effect on human health or the environment.

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[36] OECD-Document: "Co-operative Research Programme: Biological Resource Management for Sustainable Agriculture Systems – Research Themes" (2000-2004) http://www.oecd.org/pdf/M00024000/M00024971.pdf

[37] The Greens / European Free Alliance is a European parliamentary group.

[38] http://europa.eu.int/eur-lex/en/archive/2001/ce35020011211en.html Document 2001/C 350 E/203

3.2.5. Network of Aquaculture Centres in Asia-Pacific

The Network of Aquaculture Centres in Asia-Pacific (NACA)[39] is an intergovernmental organisation of a coordinated and interlinked system of aquaculture and related institutions working in close cooperation on the development of technology, manpower and information required to increase the contribution of aquaculture to national development goals and to expand sustainable aquaculture development in the region. NACA has three regional lead centres: one in India, one in Thailand and one in China[40].

Together with the FAO, NACA organised in 2000 the Asian aquaculture focused conference "Conference on Aquaculture in the Third Millennium". In the frame of this conference it was stated "in the future the aquaculture sector will confront the issue of biotechnology through: (…) addressing the potential implications for aquaculture of biotechnology, including GMOs and other products, in a precautionary, safe and practical way; and encouraging public awareness and providing information to consumers on the potential applications of biotechnology" (NACA/FAO 2000a).

Further outputs of the "Conference on Aquaculture in the Third Millennium" were two publications. "Aquaculture in the Third Millennium – Technical Proceedings of the Conference" (Subasinghe et al. 2000) is a comprehensive and authoritative review of the status of aquaculture development in the world. In addition the recommendations of the thematic sessions were brought together in the "Report of the Conference on Aquaculture in the Third Millennium" (NACA/FAO 2000b).

3.2.6. Conference on the Protection of the North Sea

The "Conference on the Protection of the North Sea" is a meeting of the Ministers of Environment of the riparian states to the North Sea staged at regular intervals. The last meeting, the “Fifth Conference on the Protection of the North Sea”, was held on 20-21 March 2002 in Bergen, Norway. At the end of this meeting the Ministers adopted a Ministerial Declaration, the "Bergen Declaration” (Bergen Declaration 2002). This declaration is covering a wide range of issues of importance for the Protection of the North Sea. Regarding GMO the Ministers agree to take all possible actions, in accordance with the requirements of the Directive 2001/18/EC and comparable national legislation and with reference to the

[39] In 1999, over 90.9% of the total aquaculture production by weight was produced by the Asian region. China contributed 70.2% to global aquaculture production (Sub-Committee on Fisheries 2002).

[40] http://www.agri-aqua.ait.ac.th/naca/Default.htm

precautionary principle, to ensure **that culturing of GMOs is confined to secure, self-contained, safe land-based facilities** in order to prevent their release to the marine environment (Bergen Declaration 2002). The Ministers invite OSPAR (The OSPAR Commission for the Protection of the Marine Environment of the North-East Atlantic) "to control or eradicate genetically modified organisms which after their release adversely affect the marine environment" (Bergen Declaration 2002). The results of the Fifth North Sea Conference shall be taken forward by Norway to the preparations for the World Summit on Sustainable Development in August/September 2002 in Johannesburg (South-Africa).

3.2.7. Nordic Council of Ministers

The Nordic Council of Ministers, formed in 1971, is the forum for intergovernmental co-operation of the Nordic European countries (Sweden, Norway, Denmark, Finland, Iceland), the autonomic territories of Greenland, the Faeroes and Åland Islands and the North-western region of Russia[41]. The Nordic Council of Ministers together with NordRiskGen network[42] organised two conferences in the past years dealing with the potential commercial use of genetic manipulated fish: “Research and Regulation with Regard to GM Fish” held in Iceland from 21-22 September 1996 and “Genetically modified organisms in Nordic habitats – sustainable use or loss of diversity?" held in Helsinki, Finland, 1998. Both conferences focused on environmental effects of GM fish as well as relevant regulation and risk assessment.

3.2.8. North Atlantic Salmon Conservation Organization

The North Atlantic Salmon Conservation Organization (NASCO) is an international organization established under the Convention for the Conservation of Salmon in the North Atlantic Ocean which entered into force on 1 October 1983. The objective of the Organization is to contribute through consultation and cooperation to the conservation, restoration, enhancement and rational management of salmon stocks. Contracting Parties are Canada, Denmark (in respect of the Faroe Islands and Greenland), the European Union,

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[41] http://www.norden.org

[42] The NordRiskGen Network does not exist any more. It was shut down in the mid 90'. No other network has been established instead (personal communication from Birgitte Skjoldager Wøhlk, Senior Advisor Environmental Affairs, Denmark).

Iceland, Norway, the Russian Federation and the United States of America[43]. In 1994, the NASCO Council adopted a resolution – the Oslo Resolution – designed "to minimize impacts from salmon aquaculture on the wild salmon stocks" of the North Atlantic area (NASCO Council Document CNL(94)53). It provides recommendations for a number of categories of possible interaction. Article 2 of the resolution specifically refers to minimizing genetic interactions. The measures proposed are dealt with in more detail in an Annex to the resolution, including measures for minimising the incidence of escaped salmon in the wild. In 1997 at its 14th Annual Meeting, NASCO adopted the "NASCO Guidelines for Action on Transgenic Salmon" (NASCO document CNL(97)48)[44]. In relation to escapes from aquaculture, specifically, this document requires that NASCO Parties should advise the Council of any proposal to rear transgenic salmon, including proposed measures for containment or other measures to safeguard wild fish in the presence of escaped fish. Furthermore the Parties should take all possible actions to ensure that the use of transgenic salmon is confined to secure, contained land-based facilities. And in addtion to other actions, the Parties should take steps to improve knowledge on the potential impacts of transgenic fish on the wild stocks and their habitat.

3.3. National policies

International agreements on transgenic fish have to be harmonised within the scope of national legislation. Therefore the current state of regulation in the most important countries relating to the potential commercial rearing of transgenic fish has been summarized in the following.

3.3.1. Canada

Canada´s framework for regulating products derived from biotechnology was adopted in 1993. Aquatic organisms including transgenic strains are regulated by the Fisheries Act by the Department of Fisheries and Oceans. The Department has prepared a policy paper entitled "Policy on Research with and Rearing of Transgenic Aquatic Organisms" and considers regulations to provide for containment procedures and environmental evaluations. Methods and rules to ensure the safety of food derived from transgenic animals should be based on the "Guidelines for the Safety Assessment of Novel Foods" (Canadian Food

[43] http://www.nasco.org.uk/html/about_nasco.html

[44] http://www.nasco.org.uk/html/guidelines_for_action_on_trans.html

Inspection Agency 1999). "Volume III. Genetically Modified Livestock Animals and Fish", announced for 2000, however, is not yet available.

3.3.2. United States of America

In the United States of America most transgenic animals are regulated under the animal drug provisions of the Federal Food, Drug and Cosmetics Act. The Food and Drug Administration (FDA) is responsible for milk, dairy products, fish, shellfish and animal drug products. The Federal Food, Drug and Cosmetics Act, 21 U.S.C. §§ 371-379d, defines a "drug" to include “articles … intended to affect the structure or any function of the body of man or other animals", 21 U.S.C. § 321g, as an introduced genetic construct will of necessity "affect the structure or … function" of transgenic animals, the genetic construct is a "drug". The genetic construct may also produce a protein that is a drug. To receive an FDA approval for commercialising GE fish, producers must complete a New Animal Drug Application (NADA) and demonstrate the safety and effectiveness of these fish.[45]

Recently several states of the United States of America have initiated or implemented corresponding regulation on their own:

In **California** there are, to date, two different bills on the agenda: "SB 1525" "would make it unlawful to import, transport, possess, or release alive into this state any live transgenic fish, or roe thereof, except under a permit", as well as a list of exotic animals, and "AB 2962" would require the labelling of all transgenic (genetically modified) fish and shellfish "that is to be offered for retail sale, other than by a restaurateur"[46].

Recent federal action in **Maine** has been targeted to ban genetically modified fish, but a corresponding bill has not come into force yet[47].

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[45] On May 3, 2000, President Clinton directed the Council on Environmental Quality (CEQ) and the Office of Science and Technology Policy (OSTP) to “conduct a six month interagency assessment of Federal environmental regulations pertaining to agricultural biotechnology and, if appropriate, make recommendations to improve them.” The assessment was undertaken as part of a larger set of policy measures intended to build consumer confidence and ensure that U.S. regulations keep pace with the latest scientific and product developments. Six case studies are available on the OSTP-web-site (http://www.ostp.gov/html/012201.html . For the "GM-salmon-case study" see: http://www.ostp.gov/html/ceq_ostp_study2.pdf .

[46] www.sen.ca.gov
[: sb1525: http://info.sen.ca.gov/cgi-bin/postquery?bill_=sb_1525&sess=CUR&house=B&site=sen ; ab2962: http://info.sen.ca.gov/cgi-bin/postquery?bill_=ab_2962&sess=CUR&house=B&site=sen]

[47] source: http://www.waterinthewest.org/newsdesk/rivercurrents/011802.htm and http://www.centerforfoodsafety.org/gefish/Articles/Greenwire1-15-02.htm

Maryland adopted a law in 2001 banning transgenic fish from the state's network of waterways. Regarding any future approval for transgenic fish, rearing would only be allowed in contained ponds and lakes[48].

Under a new Administrative Rule the Department of Fish and Wildlife in **Oregon** does not authorize the release of transgenic fish into locations where such fish may gain access to wild fish populations. "Fish that have been modified through genetic engineering and are released into wild populations have the potential of causing adverse ecological and genetic impacts. The Department shall consider releases of transgenic fish to pose a serious risk to wild populations. The Department shall not authorize the release of transgenic fish into locations where such fish may gain access to wild fish populations[49]."

3.3.3. Norway

In Norway genetically modified organisms (GMO) are regulated through the "Gene Technology Act" (GTA)[50]. The Act relates to the production and use of GMO. Management responsibilities for provisions and regulations under the Act have been allocated to two different ministries: The Ministry of Health and Social Affairs is responsible for regulation and management in connection with the contained use of GMO, while the Ministry of Environment is responsible for deliberate releases of GMO. Regarding the impact assessment the Act states that "Applications for approval of deliberate release pursuant to section 10 shall contain an impact assessment setting out the risk of detrimental effects on health and the environment and other consequences of the release" (GTA, 3.11. "Impact Assessment"). Since Norway is part of the EU regulation through the so called European Environmental Agency (EEA) Agreement, it receives all notifications for research releases and applications for commercial releases within the EU/EEA region. "Approval is not required for the placing on the market of a product that is approved for placing on the market in another EEA country pursuant to the rules laid down in Annex XX, Entry 25, of the EEA Agreement (Council Directive 90/220/EEC)" (GTA, see 3.10 "Approval").

In addition to these regulations a "Laymen's Consensus Conference on Genetically Modified Food Products" took place in 2000 as a follow-up conference of a precedent

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[48] source: press release 04/12/2001 Organic Consumer Association (Little Marais, Maryland) http://www.organicconsumers.org/patent/mdfish.cfm

[49] Oregon (USA): Administrative Rules 635-007-0595 "Transgenic Fish" http://arcweb.sos.state.or.us/rules/OARS_600/OAR_635/635_007.html

[50] Act No. 38 of 2nd April 1993 (http://binas.unido.org/binas/country.php3?id=14)

consensus conference held in 1996. The National Committees for Research Ethics, NEM, NENT, and NESH[51], independent bodies with mandates from the Ministry of Education, Research and Church Affairs – organised the Conference in co-operation with "the Norwegian Biotechnology Advisory Board" and "the Norwegian Board of Technology". The panel comprised of 15 persons. The final report of the Consensus Conference 2000[52] called for a moratorium concerning "all cultivation of gene food and gene fodder, with the exception of release into the environment of genetically modified organisms in experimental field-studies. Prohibition of import and sale of genetically modified food and genetically modified fodder. (…) obviously there is some general consensus in the expert community that we know very little of the environmental effects of using GM plants. (…) Commercial research gives little priority to environmental research." Therefore the panel demanded that more official support should be given to biosafety research relating to GM products, and in particular to independent GM research being done outside the internal research environments of the GM industry.

3.4. Non-governmental organisations

The industry, civil society and also environmental lobby groups participate in the discussion on the applications of modern biotechnology. The potential risks and benefits of biotechnology in general and of food production and processing based on GE fish in particular have given rise to specific lobby groups[53].

Environmental and consumer lobby groups

In the United States of America a coalition of around 60 groups started a joint petition process in May 2001[54], "demanding a moratorium on the domestic marketing and importation of genetically engineered fish until Food and Drug Administration of the United States Department of Health and Human Services (FDA) adequately addresses the impacts to the environment and human food safety." The petitions have been submitted to the FDA, the

[51] NEM: The National Committee for Medical Research Ethics; NENT: The National Committee for Research Ethics in Science and Technology; NESH: The National Committee for Research Ethics in the Social Sciences and Humanities.

[52] Referred to here as the Final Report (http://www.etikkom.no/E/gmf2.htm).

[53] Since lobby groups are in general working using other medias than scientists, most of the sources cited in this chapter did not go through a peer-review system. This aspect should be kept in mind reading this chapter.

[54] For a list of the supporting groups, initiatives and people see: http://www.centerforfoodsafety.org/gefish/legal/petitioners/ShortListofPetitioners.htm

Department of Commerce, the United States Department of Agriculture (USDA) and others[55]. Two representatives in the Congress of the United States – Robert DeFazio (Democrat, Oregon) and Dennis Kucinich (Democrat, Ohio) – joined this coalition and Dennis Kucinich tried to amend a bill in order to support the intention of the petition initiative, a one-year moratorium on FDA's approval of GE-fish[56].

Another campaign, launched in co-operation by Friends of the Earth, The Center for Food Safety and Clean Water Action (all United States), appealed to the owners of restaurants and retailers of fish and fish food in the United States, asking them "not to sell engineered fish and oppose its commercial introduction to avoid contamination of their supplies"[57].

In a more general way – not only with a view to the aquaculture sector – and regarding Directive 2001/18/EC of the European Parliament, "Friends of the Earth Europe" (FOEE)[58] are calling for a proper framework for monitoring and risk assessment. The drafts of two guidance notes as mentioned in the annexes of the relevant directive (EU-Commission 2002a und 2002b) have been discussed – for example – at a meeting in March 2002, that has been organised by the EU Directorate-General Environment[59]. This meeting showed that "there is a high level of disagreement among stakeholders, especially concerning monitoring." One of the issues still under discussion is whether gene-flow itself does pose an environmental risk. They "point out the fact that many of the environmental risks of gene flow are still unknown. Therefore the Precautionary Principle should be applied. Consumer organisations are worried that gene flow could greatly reduce the freedom of choice, since conventional crops could get contaminated with GMOs." Further it has not been clarified as yet what effects on the environment will have to be risk assessed and monitored – the EU Commission has not presented a corresponding list of potential effects. So for example, "the industry does not want to monitor the amount of pesticides used on GM crops compared to the amount used on conventional crops, environmental non-governmental organisations like

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[55] Source: http://www.gefoodalert.org/library/admin/uploadedfiles/Consumer_and_Environmental_Groups_File_Formal_.htm

[56] e.g.: Friends of the Earth: http://www.foe.org/foenw/ge/alert.html

[57] Press release Friends of the Earth 18th October 2001: http://www.foe.org/safefood/gefish1018.html

[58] Friends of the Earth Europe, Biotech Mailout, Volume 8 Issue 2, 1st April 2002; www.foeeurope.org/biotechnology/vol8no2.pdf

[59] DG-Environment = Director-General Environment of the European Commission see: http://www.europa.eu.int/comm/dgs/environment/directory.htm

Friends of the Earth argue that such effects should definitely be monitored, since they may clearly imply an environmental risk"[60].

The "Federation of European Aquaculture Producers" (FEAP) was created as an association of trout producer groups in 1969. In 1994 the organisation included all other fish species kept in European aquaculture in its programme. Currently it is composed of 26 national aquaculture producer associations. In July 2000 the member associations of FEAP adopted unanimously the "Code of Conduct for European Aquaculture". "This code addresses those areas that the Federation of European Aquaculture Producers considers to be important and of prime concern." Concerning genetically modified organisms, the Code states: "The FEAP does not endorse the use of genetically modified fish in aquaculture since it is concerned about the maintenance of the natural characteristics of the products, in addition to the environmental qualities of biodiversity"[61].

Industrial lobby groups

At least two years ago "A/F Protein Inc.", a sister company of Aqua Bounty Farms Inc., both based in Waltham, Massachusetts (USA) has applied to the FDA of the United States Department of Health and Human Services for a permission to market its "AquAdvantage™ Salmon". AquAdvantage™ is intended to be produced in net-pen facilities in the Atlantic Ocean (Niiler 2000)[62]. But, according to the vice president of Aqua Bounty Farms Inc., the "FDA is not expected to make its decision on GM salmon until 2004". Aqua Bounty still needs to submit important research data on the fish, such as a time-consuming environmental impact report.

Two other companies stepped back from GM salmon: "Otter Ferry Salmon in Scotland and the New Zealand King Salmon Company scrapped their GM salmon research after unfavourable publicity"[63]. But it is likely, that other companies have transgenic fish or the products thereof "in the pipeline": "Meanwhile, in San Diego, Aquatic Systems, a division of Kent SeaFarms Corp., is developing genetic-engineering techniques for striped bass and hybrid striped bass. Using a U.S.$ 1.8 million grant from the Department of Commerce's

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[60] Friends of the Earth Europe, Biotech Mailout, Volume 8 (2), 1st April 2002. http://www.foeeurope.org/biotechnology/vol8no2.pdf

[61] http://www.feap.org/CodeFinalD.PDF

[62] The AquAdvantage™ Atlantic salmon contains the Chinook salmon growth hormone gene together with a promoter from the ocean pout´s antifreeze gene.

[63] Reichhardt, T. (2000): Will souped salmon think or swim? in: Nature, 406, pp. 10-12 (http://www.centerforfoodsafety.org/gefish/index.html)

Advanced Technology Program, researchers are using genetic engineering to build fish that grow quicker, require less feed and are more disease resistant"[64].

3.5. International science organisations

3.5.1. International Council for the Exploration of the Sea

The International Council for the Exploration of the Sea (ICES) is an international science organisation that is studying and helping to safeguard North Atlantic marine ecosystems. Since its inception in 1902[65] its prime concern has been the environment of the North Atlantic and adjacent seas. International cooperative studies are the main focus of ICES. ICES works with experts from its 19 Member Countries and collaborates with more than 40 international organisations, some of which hold Scientific Observer status[66]. Since the 1970s, a major task for ICES involved the provision of scientific information and advice in response to requests by international and regional regulatory commissions, the European Commission, and the governments of its Member Countries, for purpose of fisheries conservation and the protection of the marine environment[67]. ICES has numerous committees, working and study groups and is organising more than 100 meetings of these groups each year, as well as several symposia and dialogue meetings.

In 1994 ICES drafted a “Code of Practice on the introductions and transfers of marine organisms” (ICES 1995). This Code was drafted by ICES and subsequently finalised by ICES and the "European Inland Fisheries Advisory Commission" (EIFAC) for use by the FAO Regional Fishery Bodies. Section V of the ICES Code provides "a recommended procedure for the consideration of the release of genetically modified organisms. (…) Recognizing that little information exists on the genetic, ecological, and other effects of the release of genetically modified organisms into the natural invironment (…) the Council urges Member

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[64] Source: Under the microscope: We can build super fish, but should we? by Dan McGovern, May 1999, http://www.biotech-info.net/super_fish.html

[65] ICES is the oldest intergovernmental marine science organisation.

[66] For example the Baltic Marine Environment Protection Commission - Helsinki Commission (HELCOM) -, the Oslo and Paris Commissions (OSPAR), the North-East Atlantic Fisheries Commission (NEAFC), the Fisheries Division of the Food and Agriculture Organization of the United Nations (FAO), the Intergovernmental Oceanographic Commission (IOC) of UNESCO, the European Commission, and the World Wildelife Fund (WWF) belong to the organisations that have cooperative relations with ICES (ICES 1995).

[67] http://www.ices.dk/hl/About_ICES.htm , 16th April 2002

Countries to establish strong legal measures to regulate such releases" (ICES 1995, with a reference to Directive 90/220/EEC). Releases should be notified to the Council of ICES before they are made, including a risk assessment[68]. And, it "is recommended that, whenever feasible, initial releases of GMOs be reproductively sterile". A revision of the ICES Code is on the ICES's agenda for 2002[69].

3.5.2. ICLARM – the World Fish Center

ICLARM - the World Fish Center is an autonomous, non-governmental, non-profit, international scientific and technical centre. It was conceived as the World Fish Center in 1973 by the Rockefeller Foundation and became a small programme of the University of Hawaii. In March 1977 the World Fish Center was incorporated as ICLARM – the World Fish Center in Manila (Philippines). At present, there are project offices in the Philippines, Bangladesh, and Malawi. Research is also being carried out in a number of other countries in Asia, Africa, and the Caribbean. ICLARM has been organised to conduct, stimulate and accelerate research on all aspects of fisheries and other living aquatic resources. ICLARM is an operational organisation, not a granting entity.

It has a wide range of research programmes including resource and policy research. Further, ICLARM organised a number of information and training programmes as well as expert consultations. One of these expert consultations took place in Nairobi (Kenya) in February 2002 on the topic of biosafety and environmental impact of genetic enhancement and introduction of improved strains/alien species in Africa[70]. ICLARM, together with partners from Norway and the Philippines, demonstrated that simple selection for faster growing fish can yield significant growth increases in tilapia in Asia. The so called GIFT tilapia – in the sixth generation – has shown an 85 % growth increase as compared to the baseline population. The transfer of the improved strains from Asia to Africa has not been undertaken so far because of concern over the potential adverse impacts on native germ plasm and unknown effects of gene-environment interactions. The key issues – amongst others –

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[68] The ICES Code does not define how a risk assessment should be undertaken.

[69] According to a personal communication of the departmental secretary of the ICES the new Code of Practice is under revision. It has to be accepted by the Advisory Committee on the Marine Environment which will meet in June. After this, it will be placed on the website later this year.

[70] "Expert Consultation on Biosafety and Environmental Impact of Genetic Enhancement and Introduction of Improved Strains and Exotics in Africa", a biosafety workshop organised by ICLARM - The World Fish Center in collaboration with the Technical Center for Agriculture and Rural Cooperation (CTA), FAO and World Conservation Union (IUCN), held on 20-23 February 2002 in Kenya (Africa).

proposed for consultation were: (I) What are the environmental and biodiversity risks of translocation of improved tilapia germplasm/alien species? (II) How can these be evaluated? and (III) What (if any) levels of biological risk from hybridisation can be tolerated? A total of 50 fishery and conservation experts from Africa and the rest of the world, resource managers, geneticists and policy makers had been expected to meet for the consultations. The proceedings will be published. However, the results of this expert consultation are not available as yet[71].

3.5.3. The Asian Fisheries Society

The "**Asian Fisheries Society"** (AFS) is an international, non-government professional body of 3.000 members from 75 countries and territories. Its purpose is to address fisheries issues, promote global cooperation, link fisheries scientists, sponsor and support young scientists, disseminate information through publications and scientific conferences. The participants of the sixth General Assembly adopted on November 28th 2001 "The Kaohsiung Declaration" which has already been submitted to the Johannesburg World Summit. The Assembly declared in a so called Action Plan for the Decade – the first decade of the 21st century – that the AFS will "5. Disseminate and support the implementation of the provisions of the FAO Code of Conduct for Responsible Fisheries (and Aquaculture). (…) (and) 7. Help formulate and implement policies, regulations, and biosafety protocols that safeguard Asia's aquatic biodiversity, including endangered and threatened species and ecosystems."[72] As already noted (see 3.2.2., Food and Agriculture Organisation), the technical guidelines endorsing the FAO's Code of Conduct for Responsible Fisheries state that care should be taken not to use this potential productivity from introduced species (including GMO) as justification for further abuse of habitat or for delaying their restoration.

3.5.4. The European Science Foundation

The **"European Science Foundation"** (ESF) "acts as a catalyst for the development of science by bringing together leading scientists and funding agencies to debate, plan and implement pan-European scientific and science activities." The ESF has currently 70

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[71] http://www.iclarm.org

[72] http://www.nayon.com/afs/

member organisations in 27 countries in Europe[73]. In October 1995 a special Marine Board was set up, which today comprises 24 marine research organisations. In December 2001 this Board published under the title "Marine Biotechnology – A European Strategy for Marine Biotechnology - ESF Marine Board Feasibility Study Group Report" its policy on handling GMO in aquaculture.[74] The report states that under "controlled conditions genetically modified organisms (GMOs) with particularly useful features such as fast growth, resistance to pathogens or low temperature tolerance can be made available for basic research proposals by recombinant technology. Comparable gains can be achieved by conventional or molecular-marker-assisted selection programmes (…). Transgenic technology, while providing a tool for stock improvement for aquaculture purposes, is not widely used because of customer concerns regarding GMOs."

3.6. Approaches and considerations concerning risk governance

> **"In accordance with the precautionary approach contained in Principle 15 of the Rio Declaration on Environment and Development, the objective of this Protocol is to contribute to ensuring an adequate level of protection in the field of the safe transfer, handling and use of living modified organisms resulting from modern biotechnology that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health, and specifically focusing on transboundary movements" (UNEP/CBD 2000).**

Risk assessment is an important tool in the approval process for genetically modified organisms to be the released into the environment or designated for use as food or feedstuff. The precautionary principle could be the basis for such a risk assessment especially in view of the fact that once the GMO have been released at large scale and any hazard would emerge, reversal will not be possible. As a consequence, nothing less than genetic variability will be at risk which "is the foundation of biological diversity" (Kapuscinski & Brister 2001). In biological systems absolute certainty is not reachable but, quoting the Convention on Biological Diversity, "where there is a threat of significant reduction or loss of biological diversity, lack of scientific certainty should not be used as a reason for postponing measures to avoid or minimise such a threat" (UNEP/CBD 1992). A science-based risk

[73] These countries are mainly member states of the European Union.

[74] http://www.esf.org/publication/127/biotech.pdf

assessment should include proper and detailed guidelines to prevent as far as possible the three different kinds of potential threats concerning (I) overall biological diversity, (II) ecological risks in the specific environment of a release, and (III) human health. Risk assessment guidelines should go further than just providing a general description of the items that have to be addressed, as is the case in the ICES's Code of Practice on the Introductions and Transfers of Marine Organisms. General descriptions and policies are helpful at the negotiating stage, but risk assessment needs more advanced tools and instruments to allow for concrete and practical applications. Three projects and initiatives, may be highlighted here, taking into account that their goals are different: (I) Directive 2001/18/EC, (II) the "Manual for Assessing Ecological and Human Health Effects of Genetically Engineered Organisms" (Scientists Working Group on Biosafety 1998), and (III) the "Safety First: Active Governance of Genetic Engineering for Environment and Human Health Worldwide" (ISEES 2001) (referred to here as the Directive, the Manual and the Safety First Initiative).

(I): The **Directive** 2001/18/EC of the European Parliament and the European Council will come into force on 17th October 2002 in the member states of the European Union. Besides current problems regarding the guidance notes on risk assessment and monitoring (see 3.2.4.), the directive provides many commendable elements. For example, "Annex II" of the Directive "describes in general terms the objectives to be achieved, the elements to be considered and the general principles and methodology to be followed to perform the environmental risk assessment" (EU Commission 2001c) and refers to the supplementing guidance notes. Following the Directive the objective of an environmental risk assessment is, "on a case by case basis, to identify and evaluate potential adverse effects of GMO, either direct and indirect, immediate or delayed, on human health and the environment which the deliberate release or the placing on the market of GMOs may have" (EU Commission 2001c). Further, the annexes of the Directive require a huge amount of data to be delivered in the cases of notification for release or the placing on the market, even though in general terms and concepts, to be supplemented again by guidance notes.

(II): The **Manual** was drafted by a group of Scientists which had been invited for two one-week workshops by the Edmonds Institute, a public interest and non-profit organisation, based in Edmonds, Washington (USA). The Manual "offers a framework for systematically evaluating the safety of a planned release of a GEO or introduction of a genetically engineered food" (Scientists Working Group on Biosafety 1998). The evaluation process – proposed by the Manual – follows a set of flowcharts that were modelled after those developed by a group of the Agricultural Biotechnology Research Advisory Committee, the "Performance Standards for Safely Conducting Research with Genetically Modified Fish and

Shellfish" (ABRAC 1995). The Manual aligns with a consistent precautionary approach, and, for example, in the case of the absence of "key information (…) (it) recommends to 'consider disallowing the release' or (…) (in a given case in aquaculture) will probably require relocation of cage aquaculture operations to land-based systems" (Kapuscinski & Brister 2001).

(III): "The Institute for Social, Economic and Ecological Sustainability (ISEES) is pioneering an alternative approach to governing the safety of biotechnology"(ISEES 2001)[75]. The **Safety First** Initiative is in its early stages. To date two workshops have taken place, the first in March 2001 and the second in April 2002, only the final report of the first one is available. Safety First tries to develop a safety programme comparable to those working in other sections of industry branches where complex systems have to be kept under control (aircraft or steel), taking into account of the special traits of animal products. Although the focus is on agricultural biotechnology products – from the lab bench through production to the dinner plate –, the participants of the first workshop recognised "that this approach (of the Safety First initiative) could be useful (…) for evaluating a broader array of biotechnology products" (ISEES 2001). The main advantage of the approach is, that the "legitimate representatives of potentially affected parties" are being brought together in a deliberative process. So the results of the negotiations will be credible. "Two of the major outcomes would be to generate agreement on safety objectives and what is "safe enough" in the products of agricultural biotechnology. Involvement of scientists and safety experts from multiple disciplines will assure that the safety program is also scientifically reliable" (ISEES 2001).

[75] The Institute for Social, Economic & Ecological Sustainability is a program of the Interdisciplinary Center for the Study of Global Change at the University of Minnesota in St. Paul, Minnesota (USA).

4. Basic biological data of *Salmo trutta* L., *Oncorhynchus mykiss* (Wal.) and *Salmo salar* L.

4.1. Salmo trutta L.

4.1.1. Biology of *Salmo trutta* L.

General description and use

General description and morphology

The brown trout has an elongate, somewhat compressed body, especially in larger fish. The caudal peduncle is straight, and the head comparatively large. The shape and size of body vary with habitat, size, and sexual condition.

On the lateral line there are 120-130, and between the adipose fin and the lateral line about 13-19 little scales. The teeth on the vomer shaft are numerous and well-developed (Muus & Dahlström 1978). The brown trout has 3-4 dorsal spines, 11-15 dorsal softrays, 3-4 anal spines, 9-14 anal softrays, 57-59 vertebrae and a caudal fin with 18-19 rays[76].

Brown trout get their name from the brown or golden brown hue on their bodies. The sides are silvery or yellow and the bellies are white or yellowish. Dark spots, sometimes encircled by a pale halo, are plentiful on the back and sides and spotting can also be found on the head and the fins along the back. Rusty-red spots also occur on the sides. The small adipose (or fatty) fin in front of the tail has a reddish hue. The colour pattern of brown trout can vary with their habitat. Sea-run and lake fish have a more silvery coloration and the spotting is less visible[77].

Brown trout closely resemble Atlantic salmon and rainbow trout, but salmon have no red colouration on the adipose fin and rainbow trout have lines of black spots on the tail. Young brown trout (parr) have 9-14 dark narrow parr marks along their sides and some red spotting along the lateral line.

Several subspecies have to be distinguished in brown trout (see chapters taxonomy and evolution). Brown trout in general can grow to be quite large, especially sea-run (*Salmo trutta trutta*) and lake-run (*Salmo trutta lacustris*) fish. Fish seizing up to 140 cm and

[76] source: http://www.fishbase.org

[77] source: http://www.gov.ns.ca

weighing up to 50 kg have been recorded in Europe[78]. *Salmo trutta fario*, the subspecies that is found in fast-flowing streams of mountain and sub-mountainous regions typically range between 2.3 - 3.2 kg. Maximum sizes of about 60.0 cm and maximum weight of 3.5 kg were recorded.

Use and economic importance

No data are available on worldwide or European production of brown trout in aquaculture, since the economic importance of brown trout is quite low as compared to Atlantic salmon or rainbow trout. Most data on trout production refer to the production of rainbow trout, the trout species of foremost economic importance.

In Germany estimated production of brown trout is about 2,500 t per year. This corresponds to about 10 % of the whole trout production in Germany.

Taxonomic situation

In 1758 Linnaeus named brown trout *Salmo trutta*. However, owing to its great morphological and ecological variability, this species has been characterised under several different names since this date. Nowadays several subspecies are distinguished. The most familiar form is the typical river trout of western Europe (Sedgwick 1995).

Classification

Class	Actinopterygii
Order	Salmoniformes
Family	Salmonidae
Genus	Salmo
Species	*Salmo trutta* Linnaeus, 1758

According to Bagliniere & Maisse (1991) and Ladiges & Vogt (1979) the following races and subspecies can be distinguished:

[78] source: http://www.fishbase.org

- Salmo trutta trutta Linnaeus, 1758[79]
- Salmo trutta fario Linnaeus, 1758[80]
- Salmo trutta lacustris Linnaeus, 1758[81]
- Salmo trutta macrostigma Dumeril, 1858[82]
- Salmo trutta marmoratus Cuvier 1817[83]
- Salmo trutta carpio Linnaeus, 1758[84]
- Salmo trutta dentex Heckel, 1851
- Salmo trutta labrax Pallas, 1811[85]
- Salmo trutta letnica Karaman, 1924
- Salmo trutta aralensis Berg, 1908[86]
- Salmo trutta caspius Kessler, 1877[87]

Number of chromosomes, ploidy, genetic variability

Salmonid fish are of autotetraploid origin. Within the Salmonidae there are pronounced interspecific differences in the chromosome complements (Hartley 1987). The karyotype of *Salmo trutta* has been described by numerous authors. It consists of 80 chromosomes (2n = 80) with a chromosome arm number of 100-102 (Woznicki et al. 1997).

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[79] *Salmo trutta trutta*, the sea trout, lives in Europe and Asia and has been widely introduced throughout the Americas and Australia. Fish reaches sizes of about 140.0 cm and a maximum weight of 50 kg.

[80] *Salmo trutta fario,* the brown trout is often found in fast-flowing streams of mountain and sub-mountainous regions. It reaches sizes of about 60.0 cm and a max. weight of 3.5 kg.

[81] *Salmo trutta lacustris*, the lake trout, occurs widely throughout Europe, reaching sizes of about 140.0 cm and a maximum weight of 50 kg.

[82] *Salmo trutta macrostigma* - This subspecies is found around the Mediterranean Sea. It can be classified into 11 regional forms.

[83] *Salmo trutta marmoratus* is an endemic salmonid of the drainage basins of the northern part of the Adriatic Sea and is found in the Pô and the lower and middle section of its left-bank tributaries (Giuffra et al. 1996).

[84] *Salmo trutta carpio* is an endemic species of Lake Garda (northern Italy) (Giuffra et al. 1996).

[85] *Salmo trutta labrax* is endemic to the Black Sea basin (Bernatchez & Osinov 1995).

[86] *Salmo trutta aralensis* - This subspecies is endemic to estuaries of the Aral Sea.

[87] *Salmo trutta caspius* is endemic to the Caspian Sea basin (Bernatchez & Osinov 1995).

With regard to its genetic variability *Salmo trutta* L. is one of the best studied Salmonids. It is composed of numerous distinct geographical forms and shows considerable variability and plasticity in many aspects of its morphology, ecology and behaviour (Apostolidis et al. 1997, Poteaux et al. 1998, Bernatchez & Osinov 1995).

Early studies on its genetic structure were based on the analysis of allozyme variation (reviewed in Ferguson 1989, Guyomard 1989). These studies confirmed that brown trout can be considered to be one of the most polymorphic vertebrates[88]. Considerable genetic differentiation was found between native Mediterranean and Atlantic populations (Apostolidis et al. 1996a, Ferguson 1989, Guyomard 1989). Further important genetic differentiation among natural populations was found on a more regional level as revealed in several studies carried out in different European regions, e.g. in Denmark (Hansen et al. 1993b), Norway (Skaala 1992), Sweden (Ryman 1983), Scotland (McAndrew et al. 1992), Ireland (Ferguson & Mason 1981), France (Krieg & Guyomard 1985), Spain (Bouza et al. 1999, Martinez et al. 1993), and Turkey (Togan et al. 1995). More recently mitochondrial DNA (mtDNA) sequence variation and mtDNA-RFLPs were investigated in several studies (e.g. McVeigh & Ferguson 1988, Bembo et al. 1994). These mtDNA analyses among geographically and phenotypically remote populations from western and central Europe revealed the existence of five major phylogenetic groupings of populations that were geographically disjunct, indicating their possible allopatric origins (Bernatchez et al. 1992, Giuffra et al. 1994). Furthermore, the great genetic diversity in *Salmo trutta* further revealed by allozyme analyses was confirmed in later studies on mtDNA variation (e.g. Apostolidis et al. 1996b, Dunner et al. 2000, Aurelle & Berrebi 2001).

Supplementing natural fish populations (including brown trout) by releasing hatchery-reared fish has become common practice in most countries and is justified for maintaining population density. In general such stocking measures are carried out without regard to wild population gene pools. For brown trout, hatchery stocks originate from the North Atlantic group, which is only one of the numerous groups of this species (Poteaux et al. 1998). Artificial selections for particular traits (e.g. growth) contributes to increase the differentiation between hatchery-reared fish and wild populations.

The effects of stocking hatchery trout into wild populations were studied in different European regions, using different genetic markers (allozymes, microsatellites and mitochondrial DNA markers). Several studies confirmed that interbreeding took place

[88] According to Prodöhl et al. (1997) it has been shown on the basis of protein electrophoretic studies (= analysis of allozyme variation) that 54% of 70 loci examined in brown trout populations throughout their native range have been found to be polymorphic, with individual populations being polymorphic at up to 35% of their loci.

between hatchery and wild brown trout (e.g. Poteaux et al. 1998, Hansen et al. 2000a, Fritzner et al. 2001, Ruzzante et al. 2001). Such interbreeding may result in loss of genetic variability of wild populations. But the presence of sufficient genetic variability is a prerequisite to being able to respond to altered selection enzymes. Therefore the practice of stocking should be carefully monitored using suitable DNA markers. Allozymes have proved useful for detecting loss of variability in hatchery strains. Nevertheless, the low variability at allozyme loci in most salmonid species reduces their sensitivity. In contrast, some nuclear DNA markers, such as mini- and microsatellites, exhibit high levels of polymorphism and many rare alleles. Therefore, these kinds of markers are expected to be useful for detecting loss of variability in hatchery-reared versus wild populations of salmonid fishes (Hansen et al. 2000b).

Genetic and molecular identification

Analysis of phenotypic characters alone may lead to erroneous interpretations of evolutionary history, because their expression is flexible and can be influenced by the environment. Nowadays the application of molecular systematics will help to better understand the evolutionary history of populations and to identify conservation units of biodiversity.

At the very beginnings of studying the genetics of brown trout (*Salmo trutta* L.) the analysis of the variation of allozymes was used as the only molecular tool. At least two major groups of brown trout can be distinguished using this method: the Mediterranean and the Atlantic group. Since the resolution power of allozymes is quite limited, new genetic markers and methods have been developed in recent years. Several studies demonstrated the utility of analysing mitochondrial DNA sequence variation for investigating into the phylogenetic relationships between different brown trout populations[89] (e.g. Bembo et al. 1994, Bernatchez & Osinov 1995, Apostolidis et al. 1996, Hansen et al. 1997b, Hansen et al. 2000a, Bernatchez 2001). This method revealed five major phylogenetic groupings of brown trout populations that were geographically disjunct. Since mitochondrial DNA is inherited maternally, only female gene flow can be studied by analysing mtDNA variation. Additional information can be gained from analysing the variation of nuclear DNA using mini- or microsatellite loci exhibiting high levels of polymorphism (e.g. Prodöhl et al. 1997, Hansen et al. 2000a and 2000b, Fritzner 2001, Mezzera & Largiadèr 2001, Ruzzante et al. 2001), or using random amplified polymorphic DNA (RAPD) (Dunner et al. 2000).

Natural distribution / centres of origin / migration history

Origin and natural distribution

The brown trout (*Salmo trutta* L.) is the most widely distributed freshwater fish native to the Paleoarctic region. It naturally occurs in many different, racially distinct forms throughout Europe, the Middle East, western Asia, and parts of North Africa. From north to south, its range extends from northern Norway and north-eastern Russia, to the Atlas Mountains of North Africa. From West to East, its range spans from Iceland to the headwaters of Aral Sea affluents in Afghanistan. Introduced throughout the world[90], they are found nowadays in rivers, lakes and coastal areas in much of North America. Non-migratory and land-locked relict populations exist south of the British Isles and in central France[91].

Evolution and migration history

The complex evolutionary history of brown trout throughout its native range of distribution was studied by Bernatchez (2001) analysing mtDNA diversity. The comprehensive investigations of Bernatchez (2001) confirmed the existence of five evolutionary lineages that evolved independently in geographic isolation during the Pleistocene and have remained largely allopatric since then[92]. The most ancient separation would have involved allopatric fragmentation between the three major drainage subdivisions: the Atlantic lineage, the Danubian (or Ponto-Caspian) lineage, and the Mediterranean lineage followed by subsequent and possibly simultaneous fragmentation within the Mediterranean basin, which led to the divergence of the Mediterranean, the *marmoratus* and the Adriatic lineages. The most important genetic subdivisions within the brown trout complex are associated with major climatic changes and basin isolations that occurred in Europe between the early to the upper mid-Pleistocene. In addition to physical isolation biological factors must have contributed to limiting their dispersal and introgressive hybridisation among them (Bernatchez 2001).

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[89] Mitochondrial DNA polymorphism in brown trout has been investigated using mainly two different methods: RFLPs (analysis of restriction fragment length polymorphisms) (e.g. Apostolidis et al. 1996, Bernatchez 2001), and DNA sequencing (e.g. Aurelle & Berrebi 2001, Bernatchez 2001).

[90] The species has also been introduced in Eastern and Southern Asia (India, Japan, Sri Lanka, Pakistan, Bhutan), Australia, New Zealand, Africa (Ethiopia, Kenya, South Africa, Tanzania, Zimbabwe), and South America (Argentina, Bolivia, Chile, Peru, Panama, Falkland Islands) – source: http://www.fishbase.org .

[91] source: http://www.fishbase.org

[92] The existence of five major phylogenetic groupings of brown trout populations was already revealed in former studies (Bernatchez et al. 1992, Guiffra et al. 1994).

It is possible to infer hypothetical centres of origins for the five major trout evolutionary lineages, considering the paleo-environmental settings during the Pleistocene. So, it may be assumed, that the ancestral centre of origin of the Atlantic lineages was in the coastal affluents of the Iberian Peninsula or even of North Africa. The ancestral centre of the Danubian lineages is probably located in the drainage basins of the Black Sea. The differential pattern of geographic distribution for the three other lineages (Mediterranean, *marmoratus* and Adriatic) broadly corroborates the established Mediterranean refuge areas: the southwestern (Ibero-Mediterranean), central (Adriatico-Mediterranean or Italian) and eastern (Balkans/Anatolia) refuge areas. The Mediterranean lineage was predominantly associated with affluents of the western basin of the Mediterranean Sea, suggesting that it originated from this region. The *marmoratus* lineage, typically of the phenotypically and ecologically distinct marble trout (*Salmo salar marmoratus*) was mainly confined to the Pô River basin, but included drainages from Croatia and Slovenia. The Adriatic lineage most likely originated from the Balkan/Anatolia refuge (Bernatchez 2001).

The unique evolutionary histories of each lineage have been shaped by highly diverse latitudinal impacts of glaciations on habitat loss and potential for dispersal, as well as climatic impacts and landscape heterogeneity that translated in a longitudinal pattern of genetic diversity and differential population structure at more southern latitudes.

In most European hydrographic basins, a decline in brown trout populations was noted over the past century. The principal causes are industrial uses of water courses, urban and industrial pollution, and habitat loss. Kitamura & Ikuta (2001) showed that spawning brown trout are extremely sensitive to the acidity of ambient water. Nest-digging behaviour is severely inhibited by very slight acidification (pH below 6.4). Field experiments showed that sudden reductions in river flow, produced by waterpower stations, may cause high mortality of juvenile salmonids through stranding (Butz & Rydlo 1996, Hesthagen et al. 2001, Saltveit et al. 2001).

For the purpose of repopulation, American rainbow trout was mainly used. This species is more resistant against water contaminants, increased water temperature and decreased oxygen content. Repopulation has become a commonly used practice in Europe for brown trout[93].

[93] After disappearing in the 1970s some remainder of *Salmo trutta trutta*, the sea trout, were supposed to live again in the European rivers Rhine and Elbe. Finally, the first sea trout was caught in the Sieg at the beginning of the 1980th (Grimm 1993). In 2000, 56 individuals were caught when migrating to their spawning grounds (MUNLV 2001). Nowadays sea trout is found again in the Rhine up to Iffezheim. In autumn/winter 2000/2001, 633 individuals were counted (Degel 2002).

Reproduction biology

Smoltified brown trout change their colour to silvery. As spawning time nears, males undergo conspicuous changes in head shape: the head elongates and a pronounced hook, or kype, develops on the tip of the lower jaw.

Brown trout spawn in winter. They place their nests (redds) on gravelly ground, lake-dwelling brown trout spawn in tributaries, anadromous brown trout in their native waters. Most return to their home streams to spawn, but some straying occurs. The young hatch the following spring. Sea trout can survive to spawn many times in either successive or alternate years, returning to sea to feed in the interim (Sedgwick 1995).

The spawning time of brown trout extents from September to February. Normally the brown trout matures after 3-4 years. Both, female and male may spawn several times. A 2.3 kg (5 lb) female produces about 3,400 eggs, 4 to 5 mm in diameter. The nesting site is chosen by the female, usually a gravel-bottom riffle above a pool (Brumund-Rüther et al. 1996).

The eco-morphological demands to the spawning grounds are: water descend > 0,75%, water depth 10-30 cm, running speed 0,2-0,4 m/s, gravel Ø 10-30 mm, nest size 0,3-0,5 m (MUNLV 2001).

The female digs the nest by flapping strongly with her caudal fin and peduncle while on her side; the redd is formed by the generated water currents. The female rests freely during redd preparation and drives away other males. Females cover their eggs with gravel after spawning and the adults return downstream. The eggs develop slowly over the winter season, hatching in spring. A good flow of clean, well-oxygenated water is necessary for successful egg development. After hatching, the young fish (alevins) remain buried in the gravel and take nourishment from their large yolk-sacs. By the time the yolk-sacs are absorbed, water temperatures have risen to 7 to 12°C. The fish (fry) emerge from the gravel and begin taking natural food. Brown trout fry are aggressive and establish territories soon after they emerge. They are found in quiet pools or shallow, slow flowing waters where older trouts are absent. They grow rapidly. Yearling brown trout move into cobble and riffle areas. Adults are found in still deeper waters and are most active at night.

Lake-run (*Salmo trutta lacustris*) fish are fully migratory and usually spawn in the main river flowing into the lake. Spawning starts in September/October. Maturity is reached at 4-7 years. The young fish spend one to three years of parr life in the river before migrating downstream to the lake, usually at the start of summer. The growth rate is quite comparable to that of the anadromous race and the fish can reach a weight of more than 15 kg.

In sea-run populations (*Salmo trutta trutta*), brown trout spend 2 to 3 years in freshwater then migrate downstream to spend 1 or 2 growing seasons in coastal waters near river mouths and estuaries. Most return to their home streams to spawn, but some straying occurs (Sedgwick 1995). *Salmo trutta trutta* occurs in European rivers from White Sea to the north coast of Spain. The seaward migration of young fish usually takes place in the spring or early summer when they have reached a length of 15-25 cm. They do not range far to sea ,usually remaining well inside the continental shelf during their marine life. Most fish return after having spent one to three years at sea and having reached a weight of 1-2 kg, but some spend up to five years marine feeding and grow to a weight of 7-8 kg before returning on first spawning migration. Sea trout can survive to spawn many times in either successive or alternate years, returning to sea to feed in the interim (Sedgwick 1995).

Crossability

Interspecific hybridisation is widespread in fish taxa. Related species can interbreed within a genus, and even between genera. *Salmo trutta* L. (2n = 80) can hybridise naturally with *Salmo salar* L. (2n = 58) and some species of the genera *Salvelinus* (charr) (Mayer 2001). Natural hybridisation between brown trout and Atlantic salmon has been reported by many authors in different European countries and in Canada in rivers at different latitudes and with differing ecological conditions where the two species occur sympatrically. The proportions of hybrids found in population samples ranged from 0.1% (Sweden) to 18% (England) (Gephard et al. 2000). Normally, the two species are segregated by temporal, spatial, and behavioural patterns of isolation (Heggberget et al. 1988). The benefits of these isolating mechanisms are quite unknown. Hybridisation may be promoted both by environmental factors (e.g. when the environment is physically or biologically disturbed) and by specific characters of the populations (Jansson & Öst 1997). For example, Verspoor (1988) observed widespread hybridisation in Newfoundland rivers and proposed less discriminating behaviour of Atlantic salmon and introduced brown trout as an explanation for higher hybridisation rates. Matthews et al. (2000) observed high incidences of Atlantic salmon x brown trout hybrids in rivers situated near intensive salmon farming in Norway and Scotland, which may be indicative of a breakdown in reproductive isolation between the two species. According to Youngson et al. (1993) escaped farmed female salmon hybridise with brown trout more frequently than their wild con-specifics in western and northern Scotland. A significant increase of Atlantic salmon x brown trout hybrids has also been observed in a Swedish river by Jansson & Öst (1997). According to the authors, massive stockings of hatchery-reared fish and environmental constraint have forced Atlantic salmon and brown trout to common spawning grounds leading to a high level of hybridisation. The direction of the crosses can vary (Moran & Garcia-Vasquez 2000).

4.1.2. Domestication of *Salmo trutta* L.

Hatching and rearing, including health precautions and safety measures

Hatching and rearing methods used for brown trout are similar to those applied for Atlantic salmon (for detailed description see 4.3.2.). Differences exist in stocking density for alevins. Higher densities are possible for brown trout than for of Atlantic salmon. Alevins of salmon may risk adhesion of the yolk sac if they are kept in tanks without gravel bottom or in artificial substrates (Höfer & Riedmüller 2002). The brown trout is very sensitive to UV light. Fish will get sunburn if they have no possibility to shelter (personal information by Hönig 2002).

A French study on the commercial production of brown trout in sea cages showed, that growth in sea water was 15-20% faster than in freshwater, but mortality rates were 10-20% higher, compared with production in freshwater (Tournay 1998).

Pathogenes and diseases

Brown trout, rainbow trout and Atlantic salmon are using similar habitats and may occur together. They largely share the same pathogens and diseases (for detailed description see 4.3.2.). However, there are conspicuous differences concerning their resistance against pathogens and diseases. Hamers (2001) showed, that the native brown trout was less affected by a number of diseases like Viral Haemorrhagic Septicaemia (VHS), Infectious Haematopoietic Necrosis (IHN), Infectious Pancreatic Necrosis (IPN), Furunculosis, Bacterial Kidney Disease (BKD), and Whirling Disease (WD) than rainbow trout.

Conservation of genetic resources

Human activities are rapidly changing the living conditions of many fish species as a result of over-fishing, pollution, alteration and degradation of habitats. In some cases, this may lead to the loss of entire populations, whereas in other cases, populations may, in the long term, be able to adapt to relevant changes in environmental conditions. However, the presence of sufficient genetic variability is a prerequisite for being able to respond to altered selection regimes.

Stocking non-native domesticated fish into wild populations compromises the genetic variability of wild populations, as locally adapted populations exhibiting high levels of genetic variation may be swamped by non-adapted genetically depauparate domesticated fish (Hindar et al. 1991). This is of particular concern also in the case of brown trout (e.g. Poteaux et al. 1998). Several studies carried out in different European countries confirmed

that genetic introgression by hatchery trout had actually occurred (e.g. Largiadèr & Scholl 1995, Guiffra et al. 1996, Machordom 1999, Berrebi et al. 2000, Hansen et al. 2001). Giuffra et al. (1996) reported introgression rates ranging from 0 to 70% within wild brown populations from the Pô basin (northern Italy). Largiadèr & Scholl (1995) found that a major substitution of native *Salmo trutta* stocks from the Adriatic drainages in Switzerland by introduced hatchery trout of Atlantic basin origin has taken place. In central Spain ancestral patterns of genetic variation of native brown trout populations have also been disturbed by the introduction of hatchery reared individuals (Machordom et al. 1999).

Stocking with offspring of local wild fish ("supportive breeding") is often suggested as an alternative to stocking with domesticated fish. However, supportive breeding can also result in inbreeding and loss of genetic variability. Therefore, it has to be ensured that this will be avoided. Hansen et al. (2000b) successfully used microsatellite DNA markers to monitor supportive breeding. According to Hansen et al. (2001) microsatellite analysis also provides a useful tool for distinguishing heavily introgressed populations from those unaffected by stocking. So, microsatellite data can be used to decide which populations should be protected for conservation and which populations should be used as a source for reintroduction.

Biotechnology: Genetic modification/transformation

Salmo trutta L., brown trout, has not been the target of genetic modifications, yet. However, many constructs that were inserted into Atlantic salmon or rainbow trout (see 4.2.2. and 4.3.2.) can be used also in brown trout. For example constructs that contain genetic codes for growth hormones will also function in brown trout, since many growth hormones, including even human growth hormones, can become active in a number of fish species (see e.g. Office of Science and Technology Policy 2001).

4.1.3. Ecology of *Salmo trutta* L.

Survival strategies

Brown trout fry is aggressive and establishing territories shortly after emergence. They are found in quiet pools or shallow, slow flowing waters where older trouts are absent. They grow rapidly and can reach a size of 16,5 cm in their first year. Yearling brown trout move into cobble and riffle areas (Sedgwick 1995).

Adult brown trouts are found in deeper still waters and are most active during the night. Apart from moving upstream to spawn, they tend to stay at the same place in a river with

very little movement to other stream areas. They can be found at these "stations" day after day, even year after year. Brown trouts prefer cool clear rivers and lakes with temperatures of, an average, 12-19°C. They are wary and elusive fishes that look for cover more than any other salmonid. In running waters they hide in undercut banks, instream debris, surface turbulence, rocks, and deep pools. They also take shelter under overhanging vegetation. They return to the stream where they were born, choosing spawning sites that are spring-fed headwaters, the head of a riffle, or the tail of a pool. The gravel-covered sites selected show favourable flow characteristics.

Lagarrigue et al. (2001) reported that the size of trout is correlated to their environments. The mean total length of trout depends on altitude or rather on temperature. The total length is negatively correlated with altitude and total density of brown trout and positively correlated with stream width and summer conductivity. The nature of the flow regime also plays a major role in growth since at equivalent altitude and equivalent mean summer temperature, the mean total length of trout is significantly lower in sites downstream dams and reservoirs with constantly reduced flows than in sites with natural flow.

Salmo trutta fario is often found in fast-flowing streams of mountain and sub-mountainous regions and occasionally also in the valleys. The trout feed in accordance with size on benthic invertebrates, insect larvae, aerial insects, molluscs, small fish and occasional frogs (Elso & Greenberg 2001).

Salmo trutta trutta, the Sea trout, prefers cold, well-oxygenated upland waters and favours large streams in the mountainous areas with adequate cover in the form of submerged rocks, undercut banks, and overhanging vegetation. When at sea, sea trout generally stays close to the shore (100-350 km) (Hartgers et al. 1998).

The Sea trout is an opportunistic feeder, feeding on insects, molluscs, crustaceans and small fish. The food of migratory *Salmo trutta* varies significantly with age, season and habitat of the fish. The main prey categories in terms of frequency of occurrence were fishes followed by crustaceans, surface insects and polychaetes. The main component of the food of sea living trout are the Clupeidae, supplemented by Ammodytidae and *Gasterosteus aculeatus* in the Baltic Sea. An ontogenetic niche shift was observed with post-smolts feeding on inshore and shallow water prey communities, while larger brown trout are mainly feeding on pelagic fishes (Haluch & Skóra 1997, Knutsen et al. 2001).

Sea trout populations exhibit differing behaviour. Long-distance and short-distance migrating stocks can be distinguished. Individuals of short-distance migrating stocks normally rest at the coast. They are comparably small and move to freshwater regions when sea-water temperatures drops in winter (Brumund-Rüther 1996).

Salmo trutta lacustris, the Lake trout, inhabits lakes and fast-flowing rivers, preferring cold, well-oxygenated water. This trout favours large streams in mountainous areas with adequate cover in the form of submerged rocks, undercut banks, and overhanging vegetation. Adult lake trouts are mainly feeding on small fish, insects and zooplankton (Schulz 1997).

Synecology

The results of studies on the predatory effect of fish on invertebrate communities in running waters are highly variable. Several surveys showed only minor or no effects on density and species composition of invertebrates after fish predation (Culp 1986, Reice 1991), while others revealed more severe effects with a loss of a least several invertebrate taxa and a change in community structure (Gilliam at al. 1989, Power 1990, Dudgeon 1993, Dahl 1998).

In addition to direct effects, a reduction in density of certain invertebrate species exposed to fish predation can be caused by behavioural changes in the form of evasive responses and increased prey drift. The presence of predatory fish can lead prey, such as *Baetis* and *Gammarus,* to change foraging strategy, anti-predatory behaviour, change of location, and increase prey drift. Although direct salmonid predation does not seem to have any markedly reductive effect on any major number of invertebrate stocks in running water, many investigations show that salmonid predators will affect the behaviour and history of invertebrates, and may influence community structure and interactions in river ecosystems (McIntosh & Townsend 1996, Crowl et al. 1997).

The choices of food are affected by availability and size of prey, the prey`s digestibility and the predators experience. Periodically grazing on a wide spectrum of prey, enhances the fish`s ability to respond to rapidly changing environmental conditions with respect to the occurrence of different prey. The grazing effects on macro-invertebrates might therefore be different in the winter season as compared to the summer season. Juvenile fish exert low grazing pressure on foraging animals in the winter season (Arnekleiv & Raddum 2001).

Introduced widely throughout the Americas and Australia, several countries report adverse ecological impact after the introduction of brown trout[94]. Young brown trouts and salmons compete for food and cover. Mostly the young trouts are the winners (Symons & Heland 1978, Kennedy & Strange 1987, Vassen 1998).

[94] source: http://www.fishbase.org

Introducing more fish into a system may lead to negative impacts on the wild con-specifics, usually in the form of reduced growth or increased mortality (Berg & Jorgensen 1991, Weiss & Schmutz 1999). Even if a “positive” effect of the stocking measure has been documented, genetic changes may have long-term negative effects (Hindar et al. 1991, Hansen & Loeschcke 1994). Another reason for genetic change, albeit less well documented, is the effect of introducing large numbers of fishes where progeny of one or a few families may dominate the rest (Ryman & Laikre 1991). Both effects may lead to changes in gene frequencies and possibly also to break down of local adaptations (Skaala et al. 1996, Laikre 1999). Stocked fish may harm non-target taxa through various ecological mechanisms, including competition, predation, behaviour anomalies, and pathogenic interactions (Pearson & Hopley 1999). Stocking of fish in small ponds has been regarded as a threat to invertebrates and amphibians (Dolmen 1993). Stocking may influence ecosystem characteristics such as species richness and productivity (Vollestad & Hesthagen 2001). Rösch & Phillipson (1996) ascertained that introduced rainbow trout may suppress native brown trout, because they use the same food.

Interaction with pathogens, diseases, predators

The presence of pathogens and parasites is normal in wild populations of brown trout (see e.g. Dezfuli & De Biaggi 2000, Bernet et al. 2001). Problems of fish health are most frequently related to unfavourable ambient conditions. In a Swiss stream Bernet et al. (2001) found mortality of brown trout caused by furunculosis and proliferate kidney disease, both due to bacterial infections. He found also a higher incidence of *Trichodina sp*. and *Gyrodactylus sp*. in fish from waste water. Bernet et al. (ib.) ascertained also that waste water did not obviously increase the prevalence or abundance of parasite species in fish. Schmidt-Posthaus (2001) reported, that poor water quality can be a major factor causing a decline of brown trout populations. High mortality rates and severe pathological changes of the internal organs were observed in fish kept in river water. Especially gills, liver and kidney of these fish showed significantly more pathological changes than fish from clean water. The bulk of these changes consisted of degenerative and inflammatory responses. In addition, several infectious agents were diagnosed in fish exposed to river water. Brown trout seemed to be more sensitive than rainbow trout to environmental stress and infectious agents.

Another problem is the outspread of diseases from marine cage farms. McKenzie et al. (1998) who investigated infectious diseases in trouts in Scotland stated that the highest incidence of infections of sea trout were recorded in the salmon farming areas of the west and north-west of Scotland. Also Raynard et al. (2001) showed that infectious diseases are spreading to wild salmons from salmon farms in Scotland.

Predators of brown trout are other carnivorous fish species like Millers thumb, pikes and others. Furthermore Vik et al. (2001) and Dannewitz & Petersson (2001) reported of cannibalism in stream populations in the absence of other fish prey. Further predators of brown trout in freshwater areas are birds like heron, Kingfisher and eagle, or mammals like otter. In some freshwater areas the losses by fish-predating birds are very high (Schmidt-Luchs 2001).

Dieperink et al. (2001) reported a 65% mortality of migrating wild and domesticated sea trout smolts due to avian predation (cormorants and herons) in a fjord in the western Baltic Sea. Predation rates were significantly higher among domesticated smolts[95]. The first 2 days after entering the sea, both wild and domesticated smolts suffered severe daily predation rates, ranging from 20 to 34 percent. The results support the hypothesis of a transient period immediately after exposure to full-strength sea water, where smolts experience an elevated risk of predation.

Ecological impact of non-transgenic Salmo trutta individuals

Adverse environmental impacts[96] of brown trout are the risks of interbreeding of escaped hatchery-trouts with wild brown trout populations (including gene introgression into wild stocks), and the risk of hybridisation with Atlantic salmon or several charr species. Furthermore escaped farmed individuals will compete with native populations about resources. And finally they can spread bacteria, viruses, and parasites to wild populations. McIntosh (2000) investigated adverse environmental effects resulting from the introduction of brown and rainbow trout into regions outside of their native range. He studied the influence on several small indigenous fish species of the genus “Galaxias” in New Zealand. The results indicated that predation by adult trouts had likely eliminated small-bodied galaxiids (*Galaxias vulgaris*, *Galaxias brevipinnis*, and *Galaxias paucispondylus*) from many streams but that trout impact is limited by the availability of habitats suitable for large individuals. Data collected of Gillespie (2001) indicate that introduced trout may have played a major role in the decline of *Litoria spenceri*, the spotted tree frog, in Australia. *Litoria spenceri* is one of numerous amphibian species in Australia that suffered dramatic population declines in recent years, and is currently listed nationally as critically endangered.

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[95] According to Mezzera & Largiader (2001) also anglers caught proportionally more introduced hatchery trouts and hybrids than pure wild individuals of native origin.

[96] The practice of commercial aquaculture itself is associated with several adverse ecological impacts like e.g. water pollution and eutrophication through fecal material and excess feed (see 2.5.3., Folke & Kautsky 1989 and Naylor et al. 1998).

Ecological impact of transgenic Salmo trutta individuals

Raising transgenic brown trout in commercial aquaculture facilities would involve the risk that transgenic trouts escape and come in contact with native brown trout populations. Many of the hypothetical adverse environmental effects of such escapes would be similar to those associated with currently used farmed strains of trout (see also 2.5.3.). To reveal further scenarios of the effects of escaped transgenic brown trout, further base data on fitness components would have to be collected.

4.2. Oncorhynchus mykiss (Wal.)

4.2.1. Biology of Oncorhynchus mykiss (Wal.)

General description and use

General description and morphology

The rainbow trout is an elongate, somewhat compressed fish, especially in larger specimens. The caudal peduncle is straight, and the mouth is comparatively small. It has no nuptial tubercles, but minor changes to head, mouth and colour occur, especially in spawning males. Shape and demension of body vary with habitat, size, and sexual condition.

On the lateral line of rainbow trouts are 125-160 scales. It has 3-4 dorsal spines, 10-12 dorsal softrays, 3-4 anal spines, 8-12 anal softrays, 60-66 vertebrae and a caudal fin with 19 rays (Muus & Dahlström 1978).

The colour of rainbow trouts varies. In general the species has black spots on the head, gill covers, back, sides (above and below lateral line), and on the dorsal, adipose and tail fins. The typical rainbow trout is heavily spotted when found in streams and much less so when found in lakes or reservoirs. Some have a dark back, a silvery belly and a brilliant red streak running along the lateral line from below the eye to the tail. Stream fish are generally more highly coloured than lake fish, featuring a pink to bright red lateral stripe, reddish gill covers, white tipped ventral and dorsal fins and a brownish to greenish or bluish back. Lake specimens are more silvery coloured and usually lack the vivid red stripe except in spawners. Hatchery fish are usually less colourful than either lake dwelling or stream

dwelling rainbow trout. Single individuals can reach a size up to 120.0 cm and a maximum weight up to 26 kg[97].

A distinction is made between sea-run populations (irideus-stock), also called “steelheads”, which are most common in the Pacific Northwest, and landlocked populations (shasta-stock) of rainbow trout living in the clean streams and rivers of western North America. The sea-going and freshwater forms are quite distinct in external shape and can be easily recognized even at an early stage of life. The sea-going form is longer and slimmer than the freshwater variety.

Use and economic importance

Rainbow trout is one of the most important fish species raised in worldwide commercial aquaculture production. Furthermore this trout species is often hatched and stocked into rivers and lakes especially to attract recreational fishermen. So it became also a favoured sport`s fish.

Rainbow trout have been domesticated and cultured for the table market since the late 19th century. Nowadays, it is cultivated in aquaculture facilities in practically every country which can provide a suitable fresh or saltwater environment with growing importance (Sedgwick 1995). The fish is utilized fresh, smoked, canned, frozen, fried, broiled, boiled, and baked.

The worldwide trout production amounted from 275,033.0 t in 1990 to 448,142.5 t in 2000 (see Table 6). The most important countries in trout production are Chile (79 566.0 t in 2000), Norway (49 040.0 t in 2000), Italy (44 500.0 t in 2000), France (41 143.0 t in 2000), Denmark (40 681.0 t in 2000), Spain (33 133.0 t in 2000), the USA (26 837.0 t in 2000) and Germany (25 000.0 t in 2000)[98]. In Europe trout production represents more than 50% of total European finfish production[99]. It ranged from 212,759.0 t in 1990 to 290,086 t in 2000[100].

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[97] http://www.fishbase.org

[98] Source: FAO; http://www.fao.org/fi/statist/FISOFT/FISHPLUS.asp# DownloadData - Download Ftp.fao.org/fi/stat/windows/fishplus/aquaq.zip (0.8 Mb)

[99] Source: Federation of European Aquaculture Producers (FEAP) (http://dev.ibicenter.net/feap/default_en.asp)

[100] Aquafeeds make up 5% of the worlds feedstuff produced in 1998. Nearly 27% (440 000 t) of the European aquafeeds were used for trout and other salmonids (New 2001b).

Table 6: Aquaculture production of rainbow trout (Oncorhynchus mykiss Wal.)

Aquaculture production of rainbow trout (*Oncorhynchus mykiss* Wal.) [t][101]					
	worldwide	**Europe***	**Norway**	**USA**	**Canada**
1990	275 033.0	212 459	3 796	26 414	2 990
1991	283 559.0	218 223	5 655	27 428	587
1992	299 503.0	228 646	6 582	26 057	430
1993	312 499.0	233 926	8 351	25 325	403
1994	334 983.5	248 632	14 367	23 887	430
1995	362 611.0	259 628	14 704	25 240	887
1996	384 531.0	270 395	22 966	24 355	1 097
Aquaculture production of rainbow trout (*Oncorhynchus mykiss* Wal.) [t][102]					
	worldwide	**Europe***	**Norway**	**USA**	**Canada**
1997	427 336.0	284 511	33 295	25 719	946
1998	438 635.0	295 710	48 431	24 995	2 354
1999	415 618.5	289 269	48 691	27 344	6 002
2000	448 142.5	289 134	49 040	26 837	5 523

*Figures for rainbow trout production of the following countries were included in the figure given for Europe: Albania, Austria, Belgium, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Faeroe Island, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, United Kingdom and Ukraine.

Taxonomic situation

According to Behnke (1992) the present diversity of rainbow trout evolved in response to different selective factors operating in different geographical regions. Prominent specialisations are associated with anadromy and with fluvial and lacustrine environments. Within each of these broad categories, further adaptations have fine-tuned life histories to favour survival in prevailing local climates, streamflows, temperatures with prevailing predators, prey, and coexisting fish species.

Restriction fragment length polymorphisms (RFLPs) in mitochondrial DNA (mtDNA) have been used to clarify the phylogenetic relationships among salmonid species and have

[101] Source: FAO; http://www.fao.org/fi/statist/FISOFT/FISHPLUS.asp# DownloadData - Download Ftp.fao.org/fi/stat/windows/fishplus/aquaq.zip (0.8 Mb)

[102] Source: FAO; http://www.fao.org/fi/statist/FISOFT/FISHPLUS.asp# DownloadData - Download Ftp.fao.org/fi/stat/windows/fishplus/aquaq.zip (0.8 Mb)

indicated that rainbow trout are more closely related to other Pacific salmonids in the genus *Oncorhynchus* than in the genus *Salmo*. Specifically, rainbow trout are more closely related to coho salmon (*Oncorhynchus kisutch*) and chinook salmon (*Oncorhynchus tshawytscha*) compared with other Pacific salmonids as well as compared with Atlantic salmon (*Salmo salar*). Therefore these molecular data sets support the reclassifaction of rainbow trout into the genus *Oncorhynchus* from its former designation as *Salmo gairdneri*[103].

[103] The name *gairdneri* is still used as a subspecies name for redband trout of the upper north-American Columbia River basin.

Classification

Class:	Actinopterygii
Order:	Salmoniformes
Family:	Salmonidae
Genus:	Oncorhynchus
Species:	*Oncorhynchus mykiss* (Walbaum, 1792)

Number of chromosomes, ploidy, genetic variability

Natural populations of rainbow trout (*Oncorhyncus mykiss*) present different characteristic chromosome numbers within its natural geographical distribution range. Most of these populations present chromosome numbers of fewer than 60 (2n = 60), with 2n = 58 being the most common chromosome number, while some populations (from the coast of California) show 2n = 60-64. Most of the domesticated populations show 2n = 60, which is consistent with that observed in natural populations in the upper part of the Sacramento river (California, USA). Moreover, within the natural geographical distribution range of this species in North America, two types of populations are found with karyotypes of 60 chromosomes; a northern type, with two pairs of subtelocentric chromosomes and a southern type, with one pair of these chromosomes (Colihueque et al. 2001).

Rainbow trout displays a broad variability in their life-history patterns and adaptability to various habitats. Furthermore the species is also characterised by a large genetic heterogeneity and high levels of genetic differentiation among populations (Danzmann et al. 1993, Heath et al. 2001). For example, there exist anadromous forms as well as forms that remain in freshwater throughout their life. Early genetic studies using protein electrophoresis revealed on the one hand that rainbow trout displays a very high allozyme variability and that there is a significant genetic separation between two different main lineages in rainbow trout – the coastal anadromous (steelhead) lineages and the interior freshwater resident (redband) lineages of the north-American Columbia River drainage[104] (see e.g. Danzmann et al. 1993, Williams et al. 1996, Beacham et al. 1999, Nielsen et al. 1999). Studies on mitochondrial DNA variation assessed using restriction fragment length polymorphism (RFLP) analysis confirmed the existence of these two different lineages (McCusker et al. 2000). Furthermore mitochondrial DNA and microsatellite studies of California´s coastal *Oncorhynchus mykiss* populations demonstrated very high levels of genetic diversity in

[104] The crest of the Cascade Mountains separates the two groups (Williams et al. 1997).

populations at the southern extent of this geographical range (Nielsen et al. 1994a, Nielsen 1999).

Nielsen et al. (1994b) studied the differences in genetic diversity for mitochondrial DNA between hatchery and wild populations of *Oncorhynchus mykiss*. Significant differences in mtDNA genotypes were found between hatchery and geographically proximate wild stocks. On average, more mtDNA types were found in hatchery populations than in wild stocks. Danzmann et al. (1993) revealed that rainbow trout from different hatchery sources in New York and Ontario (USA) were characterised by reduced mtDNA diversity relative to western rainbow trout populations.

Genetic and molecular identification

Like in *Salmo trutta* studying the genetics of *Oncorhyncus mykiss* began with the analysis of the variation of allozymes (e.g. Gajardo et al. 1998, Williams et al. 1997, Williams et al. 1996, Krueger et al. 1994). Since the development of DNA technology provided a variety of new tools, numerous studies based on different DNA markers were carried out in the past years. Mitochondrial DNA sequence analysis and the analysis of microsatellites[105] proved to be useful for investigating the phylogenetic relationship between different rainbow trout populations and for studying genetic diversity in this species (e.g. Nielsen et al. 1994b, Palti et al. 1997, Nielsen 1999, Nielsen et al. 1999, Beacham et al. 2000, McCusker et al. 2000, Heath et al. 2001, Rexroad et al. 2002).

Centres of origin, diversity and natural distribution

The natural range of rainbow trout (*Oncorhynchus mykiss*) extends from the Kuskokwim river in Alaska through British Columbia to Baja in California. This species is primarily a native of the coastal rivers of western North America but also occurs on the eastern side of the Great Divide in the headwaters of the Peace river in British Columbia and in the Athabasca in Alberta. Outside this described range there are also native populations in the Rio Casa Grandes in the Mexican province of Chihuahua (Sedgwick 1995), and some parts of Asia (the Russian waters from the Japanese Sea to Kamchatka)[106] (Dussling & Berg 2001).

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[105] In salmonids, microsatellite (SSR) markers are often conserved among closely related species. So SSR markers that were identified in other salmonids like Atlantic salmon or brown trout are often found also in rainbow trout.

[106] There is no longer any reasonable doubt that the rainbow trout of North America and the rainbow trout of Kamchatka (Siberia) belong to the same species.

Rainbow trout was first stocked outside its native range in 1874 in New York. In the following years rainbow trout were spread over most of the U.S. and Canada (except the Northwest Territories) and in other suitable waters over the world (Sterba 1987).

The first successful shipment outside of North America was to Japan in 1877. Other shipments to Germany (1880), to New Zealand (1882), the United Kingdom (1885) and the Netherlands (1898) followed (Gall 1992, Hartgers et al. 1998). The development of a European rainbow trout farming industry began in Denmark in the 1890`s (Laired & Needham 1988).

Rainbow trout is nowadays one of the most widely introduced fishes and may be regarded as global in its present distribution. It was introduced in the whole Europe, Asia (Afghanistan, China, India, Japan, Russia, Korea, Malaysia, Taiwan, Thailand), Africa (Eritrea, Ethiopia, Kenya, Malawi, Madagascar, Morocco, South Africa, Sudan, Tanzania, Zimbabwe), South America (Bolivia, Brazil, Chile, Colombia, Equador, Peru) and Australia[107].

Evolution and migration history

The native range of rainbow trout (*Oncorhynchus mykiss*) has been subject to multiple glaciations over much of the last two million years. Hypotheses on glacial refugia and postglacial recolonisation routes are summarised by McCusker et al. (2000). In rainbow trout two phylogenetically distinct mitochondrial lineages were found. Although the geographical distributions of these lineages overlap extensively, diversity and distribution analyses strongly suggest that rainbow trout survived glaciation in both coastal and inland refugia followed by postglacial gene flow and secondary contact (Beacham et al. 1999, Nielsen et al. 1999, McMusker et al. 2000)[108]. Pure ancestral interior rainbow trout populations retained only in areas isolated by barriers, characterised by significant morphological and genetic differences. Such relic interior populations have been documented in headwater areas of the Kern River, Columbia River, and Sacramento River in California (USA) (Nielsen et al. 1999).

Reproduction biology

The reproduction biology is comparable to the spawning behaviour of the brown trout (Ladiges & Vogt 1979). The female finds a spot and digs a pit. While digging, an attendant male courts her or is busy driving away other males. As soon as the redd is completed, the

[107] Source http://www.fishbase.org

[108] According to Nielsen et al. (1999) relic interior trout populations with significant morphological and/or genetic differentiation from coastal rainbow trout have been documented in headwater areas of different north American rivers.

female drops into it and is immediately followed by the male. The pair are side by side, they open their mouth, quiver and release egg and sperm. Females produce from 700 to 4 000 eggs per spawning event. At this point, a subordinate male moves in and releases sperm into the nest. The female quickly moves to the upstream edge of the nest and starts digging a new redd, covering the eggs. The whole process is repeated for several days until the female deposits all her eggs. Young fish move downstream at night, shortly after emergence (Gall 1992).

The spawning time of rainbow trout is longer than the one of brown trout. It reaches from December to May. Populations of shasta-stock spawn earlier than trout of irideus-stock (Muus & Dahlström 1978).

Crossability

In nature occasional hybridisation occurs with cutthroat trout (*Oncorhynchus clarki*), golden trout (*Oncorhynchus aguabonita*), Gila trout (*Oncorhynchus gilae*) and Arizona trout (*Oncorhynchus apache*) in overlapping ranges (Leary et al. 1984, Fuller 2000). In areas where Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*) is native and rainbow trout have been introduced, Lahontan cutthroat trout were replaced by rainbow trout (McAffee 1966, cited from Fuller 2000). Furthermore rainbow trout is able to cross with a number of other salmonid species, including European brown trout *Salmo trutta* (Sedgwick 1995).

Williams et al. (1996 and 1997) studied hybridisation between hatchery rainbow trout and native interior rainbow trout. They could demonstrate that mtDNA analyses are useful to detect hybridisation events in rainbow populations. Hybrid swarms and pure indigenous populations could be distinguished.

4.2.2. Domestication of *Oncorhynchus mykiss* (Wal.)

Hatching and rearing, including health precautions and safety measures

Hatching and rearing methods used for trout are similar to those applied for Atlantic salmon (for detailed description see 4.3.2.).

Provided good water qualities rainbow trout shows excellent growth at water temperatures between 15-20°C. Under perfect hatchery conditions some male fishes mature at an age of 9 to 12 months. In general stocks tend to mature at an age of 2-3 years depending on water temperature and food availability. The total farming cycle of rainbow trout from production of eyed eggs to harvest of 200 g fish typically varies from 10 to 20 months, depending on water temperature (Shepherd & Bromage 1995).

Selection based on individual merit is the most widely used selection method in fish (including rainbow trout) since it is very simple to practise. An alternative method is family selection. This method is of particular interest for traits like age at maturation, survival and meat quality. Generally, a combination of individual and family selection will be more efficient than using only one of them. Index selection has been shown to be more efficient than other methods of selection when two or more traits are involved. A set of genetic technologies has been very actively applied to rainbow trout over the last two decades (Gall 1992, see also paragraph “Biotechnology”).

The genetic capability of the species is demonstrated by the fact that stocks exist which can produce eggs in almost all months of the year (Gall 1992). Broodstock management and out of season egg production uses management of photoperiod. The development of rainbow trout eggs can be suppressed at water temperatures below 5°C (Ross & Forteath 1992).

Most of the eggs generally obtainable from breeders in Europe or North America come from brood fish which are descendent from a mixture of spring and autumn spawning fish. The spawning times of particular brood stocks have been stabilized and commercial producers can provide eggs from early, middle or late-spawning parent fish (Sedgwick 1995).

Stevenson (1987) reported, that at 15°C, stocking densities can vary between 25 to 45 kg/m^3, depending on fish size. Intense aeration can boost that density to 90 kg/m^3. The food conversion ratio increases with fish density (Gall 1992).

Conservation of genetic resources

Pacific salmonids, including *Oncorhynchus mykiss*, exhibit a wide range of life histories and local adaptation and a high degree of phenotypic plasticity. The determination of patterns and distribution of genetic variation within a species are key steps in developing management plans that aim to conserve biodiversity. The development of molecular tools for studying genetic diversity has made great progress in recent years. Nowadays, quite a lot of genetic markers are available for assessing genetic variations in salmonids. Specially allele size variation at microsatellite DNA has revolutionised the field of conservation genetics (Heath et al. 2001).

A number of studies were carried out to describe the genetic structure of rainbow trout populations for identifying suitable populations for conservation purposes (see e.g. Nielsen et al. 1994, Beacham et al. 1999, Nielsen et al. 1999, Beacham et al. 2000, Heath et al. 2001).

Biotechnology: Genetic modification/transformation

Methods and state of the art in fish biotechnology including gene constructs used were extensively described in 2.1. and 2.2. (including methods used). This chapter summarises the targets of genetic modifications that have been carried out in rainbow trout, the second well-established salmonid species for marine aquaculture.

As mentioned in 2.1. increasing the productivity of fish production by enhanced fish growth is one main target in fish biotechnology[109]. It is also the main target that have been pursued in genetic modifications of rainbow trout until now (see Table 2). "All-fish" gene constructs showed a 3.2 to 17.3-fold increase in growth enhancement in rainbow trout (Devlin 1997, Devlin et al. 2001)[110]. In addition there have been also attempts to improve feed efficiency in rainbow trout by transferring human and rat gene constructs which code for special enzymes (Pitkänen et al. 1999). Due to the high rate of mosaicism commonly observed in the first generation of transgenic fish, any definite conclusion on the efficiency of the used gene constructs could not be drawn.

Experiments on improving rainbow trout´s cold tolerance – a major goal in Atlantic salmon – have not been published yet. Once this target has been realised in Atlantic salmon, it should be quite easy to adapt and transfer the developed methodology to rainbow trout.

Research has been done also in the development of transgenic sterile strains of rainbow trout (Smith et al. 2001). Sexual maturation was hindered by inhibition of gonadotropin-releasing hormone (GnRH) mRNA using antisense technology[111]. The absence of GnRH results in a blockage of the hypothalamo-pituitary-gonad axis. First attempts have been successful. F1 and F2 progeny have been produced of transgenic rainbow trout. However, the problem to obtain fidelity of transgene expression is still unsolved (Smith et al. 2001).

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[109] Several experiments involving genetic modification of rainbow trout have been carried out with regard to improving methodology. These experiments are summarised in 2.1..

[110] However, Devlin et al. (2001) found that the growth of transgenic wild-strain rainbow trout did not surpass that of a fast growing non-transgenic domesticated strain of trout used in aquaculture. Introducing the growth hormone construct into this domestic strain did not cause further growth enhancement. These results indicate that similar alteration of growth can be achieved both by selection and by transgenesis in rainbow trout, but that the effects are not always additive.

[111] The expression of GnRH antisense mRNAs inhibits the biosynthesis of GnRH.

Table 5: Targets of genetic modifications in *Oncorhynchus mykiss* Wal.

Target	structural gene	promoter	reference
Growth enhancement	Ongh1, overexpressing growth hormone gene from *Oncorhynchus*	MT (metallothionein promoter)	Devlin et al. (2001)
Growth enhancement	hgh (humane growth hormone gene)	MT (mouse metallothionein promoter)	Guyomard et al. (1989)
Growth enhancement	hgh (humane growth hormone gene)	SV 40 promoter	Chourrot et al. (1986)
Growth enhancement	rgh (rat growth hormone gene)	MT (mouse metallo-thionein promoter)	Maclean et al. (1987), Guyomard et al. (1989), Penman et al. (1991)
Improvement of the carbohydrate metabolism efficiency of salmonid fish	1) hgluT1 (human glucose transporter type 1 c-DNA)	1) CMV promoter (cytomegalus virus)	Pitkänen et al. (1999)
	2) rhkII (rat hexokinase type II cDNA)	2) OnH3- Histon 3 promoter from sockeye salmon	
		3) OnMT-B (metallothionein-B promoter from sockeye salmon	
Production of L-ascorbic acid	rglo (rat gene for L-gulono-γ-lactone oxidase, the key enzyme of L-ascorbic acid biosynthesis)	OnMT (metallothionein promoter from *Oncorhynchus*)	Krasnov et al. (1998)
Target	**structural gene**	**promoter**	**reference**
Production of sterile strains	salmon gonadotropin-releasing antisense genes	salmon Histone 3 promoter	Smith et al. (2001)

4.2.3. Ecology of *Oncorhynchus mykiss* (Wal.)

Rainbow trout, along with cutthroat, are the only native trouts of the western U.S. Their habitats are cool, clear, clean, well oxygenated waters (e.g. cold, clean mountain lakes) and rivers of moderate to fast flows, which contain an abundance of riffle type waters for

breeding. They live best in waters with only slight alkalinities[112] and a water temperature that ranges between 13-21°C. But it tolerates temperatures from 0 to 20°C (Gall 1992)[113]. It is unclear whether its anadromy is a truly genetic adaptation or simply an opportunistic behaviour. It seems that any stock of rainbow trout is capable of migrating, or at least adapting to sea water, if the need or opportunity arises.

Rainbow trout are known to be highly aggressive and once a feeding territory is occupied by them they will vigorously defend it against invaders, especially other equally sized salmonids. The rainbow trout is an opportunistic feeder but can be very discriminating as well. It is mainly feeding on aquatic insect larvae, like caddis, mayfly, damsel and dragonfly, but many other species were eaten as well (terrestrial insects, snails, drifting organisms like worms and sowbugs, crawfish and small fish). In general, rainbow trout feeding patterns will follow the life history of the organisms they prey upon; spring and early summer feeding is concentrated on aquatic insect larvae and drift organisms, turning more and more to the adults as hatching takes place later in the summer. However, many rainbow trouts, especially larger fish, tend to feed on limited types of food (small fishes, including other trout) and to ignore many other types others are feeding upon. Winter feeding is concentrated mainly on the bottom, but when hatching occurs, the fish will take advantage of them. Primary feeding times are early morning and dusk, but rainbow trout do take advantage of hatches and other feeding opportunities which may arise at any time of the day.

Survival stategies

One of the most important survival strategies is the possibilty to migrate to sea for feeding. The body fluids of rainbow trouts have a salt concentration approximately equivalent to one part sea water and two parts freshwater. In a freshwater environment water diffuses into their tissues. The water surplus will be discharged as urine. The situation is reversed in a more saline environment. In this case rainbow trouts are continuously concentrating a solution of salt in their bodies. The extra salt will be excreted through special cells in the gills. In the migratory “steelhead” race of sea-going rainbow trout the salt-excreting cells increase in number when the fish undergo the change into smolt. This helps them to adapt to live in salty water. The number of salt-excreting cells can be artificially increased by feeding a high salt diet to the fish while they are still in freshwater (Sedgwick 1995).

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[112] The Eagle Lake rainbow trout is an exception.

[113] The rainbow trout is highly adaptable to its environment, which is one of the reasons why it has achieved such a wide distribution (Laird & Needham 1988).

Synecology

The synecology of rainbow trout is comparable to the one of brown trout. Invertebrate communities are affected by predation of rainbow trouts (see 4.1.3. for adverse ecological impact of trouts after their introduction into other areas[114]). Barrow & Peters (2001) found that rainbow trout in lakes preferred areas with abundant food items and water less than 2 m deep. Sixty-nine percent of all trout locations were in shallow water areas where benthic macroinvertebrate densities were significantly higher than in other portions of the lake.

Furthermore the results of Konishi et al. (2001) revealed that predatory fish like rainbow trout had an indirect but significant effect on leaf litter processing and for trophic cascading effects in the stream, through predator-induced lower biomass of detritivore and likely lowered foraging.

Interaction with pathogens, diseases, predators

Rainbow trout is affected by the same pathogens and diseases like brown trout. They are described in 4.1.3. and listed in Table 12. Predators of rainbow trout are also other carnivore fish species like Miller´s thumb and pikes or different species of shark, cod, conger, haddock, pollack, sea lamprey in sea water. Further predators are fish hunting birds like cormorant, fulmar, great skua, seagulls, guillemot or mammals like seals and dolphins.

Ecological impact

Non-transgenic organisms

Chaine & Whoriskey (1992) reported on escaped farmed rainbow trouts in North American lakes outside their native range feeding primarily on zooplankton and insects. Since their ecological niche overlaps partially in depth and in diet with the native lake trout (*Salvelinus namykush*) the two species compete on the existing ecological resources.

Escapes of non-native rainbow trouts were also reported from Europe (Bergheim 2001). According to Hager (1998) rainbow trout is using the same spawning areas like the native species brown trout and grayling (*Thymallus thymallus*). Since rainbow trout is spawning later, they are digging out the eggs of the native species during the spawning process. Reduction in the stocks of brown trout and grayling is the consequence.

[114] According to Rösch & Phillipson (1996) brown trout is affected by the introduction of rainbow trout in Europe, because both species are using the same food sources.

Given the widespread practice of introducing hatchery-reared fishes, Kiesecker et al. (2001) suggested, that fish used in stocking programs could be an important vector for diseases responsible for amphibian losses (for examples see also 4.1.3.).

Transgenic organisms

Concerns with regard to the ecological impacts of transgenic rainbow trouts are quite similar to those evolving from brown trout. Transgenic rainbow trouts could escape from commercial aquaculture facilities and get in contact with native rainbow trout populations. Interbreeding with wild rainbow trout populations and gene introgression into wild stocks cannot be prevented completely since any 100% effective techniques to produce sterile populations do not exist. There is also the risk of hybridisation with other salmonids. It is unknown, whether transgenic rainbow trouts are more likely to hybridise with other species than non-transgenic individuals. Furthermore transgenic individuals would compete with native population about resources. And finally they could spread bacteria, viruses, and parasites to wild populations.

4.3. Salmo salar L.

4.3.1. Biology of *Salmo salar* L.

General description and use

General description and morphology

Atlantic salmon shows a complex development pattern accompanied by changing morphology. The adult Atlantic salmon is a graceful fish, deepening rearward from a small pointed head to the deepest point under the dorsal fin, then tapering to a slender caudal peduncle which supports a spreading and slightly emarginate caudal fin. Atlantic salmon are distinguished from the Pacific salmon (*Oncorhynchus kisutch*) because they have fewer than 13 rays in the anal fin. Their mouth is moderately large. The shape, length of head, and depth of body vary with each stage of sexual maturity. Colour varies with age in this fish. Small "parr," older young salmon, have 8 to 11 pigmented bars, or "parr marks," along each side of their body, alternating with a single row of red spots along the lateral line. These markings are lost when the "smolt" age is reached. Salmon in the sea are silvery on the sides and belly, while the back varies with shades of brown, green, and blue. Atlantic salmon also have numerous black spots, usually "X"-shaped and scattered around the body. When spawning, both sexes take on an overall bronze-purple coloration and may acquire reddish

spots on the head and body. After spawning, the "kelts" are so dark in color that these fish are also called "black salmon".

Designation of development phases of Atlantic salmon:

- “parr”: young salmon (age: from hatching until one year and several months), living in freshwater, coloured with dark bands[115].
- “smolt”: young salmon (age: from one year until two years and several months), migrating to the sea, silvery coloured.
- “grilse”: salmon, returning to freshwater one year after migrating to the sea.
- “kelt”: salmon after spawning, dark coloured

Sea-run Atlantic salmon usually attain a larger size than do landlocked (those living in entirely freshwater) salmon. Sea-run salmon range from 2.3 to 9.1 kg and commercially caught fish average 4.5 to 5.4 kg. The world record rod-caught Atlantic salmon weighed 35.89 kg and was caught in the Tana River of Norway[116].

Economical importance

Salmon farming has boomed during the past decades. Initiated in Norway in the 1960s, it increased steadily in the late 1970s due to technical breakthroughs, high profits, and support from government agencies promoting economic development. Worldwide production has grown rapidly, from 225 643.0 tons in 1990 to 883 558.5 tons in 2000. European salmon production has also increased, rising from 68 105 t in 1987 to 614 964.5 t in 2000[117], thus representing 48% of all fish species reared in Europe. Norway, Scotland and Chile are the major producers, jointly accounting for over 80% of world supply of Atlantic salmon (OSTP 2002).

[115] After one winter only the most rapidly growing juveniles (parr) with a length of 10-15 cm start their seaward migration (Hartgers et al. 1998). The length of the adult fish is not as much dependent on age than it is on the time spent feeding at sea.

[116] Source: http://animaldiversity.ummz.umich.edu/accounts/salmo/s._salar$narrative.html

[117] Source: FAO; http://www.fao.org/fi/statist/FISOFT/FISHPLUS.asp# DownloadData - Download Ftp.fao.org/fi/stat/windows/fishplus/aquaq.zip (0.8 Mb)

Table 7: Aquaculture production of Atlantic salmon (*Salmo salar* L.)

Aquaculture production of Atlantic Salmon (*Salmo salar* L.) [t][116]					
	worldwide	**Europe***	**USA**	**Canada**	**Norway**
1990	225 643.0	201 604.0	3 185.0	9 625.0	145 990.0
1991	266 283.5	228 513.0	6 661.0	13 499.0	154 900.0
1992	247 530.0	193 181.0	10 028.0	17 305.0	124 138.0
1993	305 611.5	238 697.0	10 750.0	23 483.0	155 581.0
1994	374 931.5	298 077.0	10 906.0	27 773.0	202 459.0
1995	465 245,5	357 054.0	14 075.0	33 674.0	261 522.0
1996	551 906.5	416 551.0	13 906.0	36 475.0	297 557.0
1997	646 516.5	473 173.0	18 005.0	51 015.0	332 581.0
1998	688 176.5	510 059.5	14 507.0	49 475.0	360 806.0
1999	803 837.5	611 671.5	17 739.0	61 990.0	425 154.0
2000	883 558.5	614 964.5	22 395.0	68 395.0	436 736.0

* The Atlantic salomon production of the following countries is included in the figures given for Europe: the Faeroe Island, Finland, France, Greece, Iceland, Ireland, Norway, Portugal, Russia, Spain, Sweden, Turkey, and the United Kingdom.

The farming of Atlantic salmon has virtually eliminated seasonal fluctuations in salmon harvesting. Whereas fresh wild salmon is only available for a few months of the year, Atlantic salmon can be harvested daily.

The Atlantic salmon is also very important for angler tourism in Northern Europe and North America, being a highly prized sports fish renowned for its large size and fighting abilities.

The cost of producing 1kg of salmon is the lowest in Norway. In 2000, Norwegian farms expended 1.56 € on average, whereas Scotish farms spent 2.30 € and Canadian farms 2.03 €/kg salmon[118]. From time to time the price of salmon collapses (e.g. down to 1.28 €/kg in 1996).

Taxonomic situation

The Atlantic salmon (*Salmo salar* L. 1758) is a species with deviating genetical potentials of the stocks in the varying spawning rivers. The greatest genetical differences are found between the populations of North America, the European Atlantic and the Baltic Sea.

Based on differences in allele frequencies at single gene loci, Payne et al. (1971) proposed the designation of European and North American salmon as distinct subspecies, *Salmo salar europaeus* and *S. s. americanus*. Further studies confirmed the phylogenetic distinctiveness of the two continental population groups, but they are not distinguished as subspecies.

Classification

Class:	Actinopterygii
Order:	Salmoniformes
Family:	Salmonidae
Genus:	Salmo
Species	*Salmo salar* L..

A list of non-valid synonyms of *Salmo salar* L. is compiled in Table 8.

[118] Source: Fischmagazin 7/2001

Table 8: List of synonyms of *Salmo salar* L.

Synonyms of *Salmo salar*

[n=22]

Synonym	Author	Status	Valid
Trutta salar	Linnaeus, 1758	new combination	No
Salmo salar	Linnaeus, 1758	original combination	Yes
Salmo nobilis	Olafsen, 1772	junior synonym	No
Salmo goedenii	Bloch, 1784	junior synonym	No
Salmo salmulus	Walbaum, 1792	junior synonym	No
Salmo caerulescens	Schmidt, 1795	junior synonym	No
Salmo renatus	Lacepède, 1803	junior synonym	No
Salmo rilla	Lacepède, 1803	junior synonym	No
Salmo nobilis	Pallas, 1814	other	No
Salmo hamatus	Cuvier, 1829	junior synonym	No
Salmo ocla	Nilsson, 1832	junior synonym	No
Salmo salmo	Valenciennes, 1848	junior synonym	No
Salmo salar lacustris	Hardin, 1862	other	No
Trutta relicta	Malmgren, 1863	junior synonym	No
Salmo gracilis	Couch, 1865	other	No
Salmo hardinii	Günther, 1866	junior synonym	No
Salmo brevipes	Smitt, 1882	junior synonym	No
Salmo salar brevipes	Smitt, 1882	junior synonym	No
Salmo salar biennis	Berg, 1912	other	No
Salmo salar brevipes relictus	Berg, 1932	other	No
Salmo salar saimensis	Seppovaara, 1962	junior synonym	No
Salmo salar europaeus	Payne, Child & Forrest, 1971	junior synonym	No

Number of chromosomes, ploidy, genetic variability

Regarding its number of chromosomes, the Atlantic salmon (*Salmo salar*) is somewhat unusual in having a variable number of chromosomes, even within offspring from a single female. The number will vary between 54 and 60 (2n = 54-60)[119].

[119] Source: (http://www.fishbase.org/Genetics/FishGeneticsList.cfm?ID=236&GenusName=Salmo&SpeciesName=salar)

Anadromous and “land-locked” populations of Atlantic salmon demonstrate extensive population subdivision across the species range. Despite the fact that Atlantic salmon undergo extended ocean migrations, they exhibit a high homing fidelity to their natal river or tributary. This is a behaviour it has in common with several other salmonid species. The substantial reproductive isolation between populations has facilitated the evolution and persistence of local adaptation.

Atlantic salmon populations exhibit diverse physiological, anatomical and behavioural characteristics and it is assumed that these population differences are genetically based on local adaptation (Fontaine et al. 1997, McConnel et al. 1997). The genetic variability in Atlantic salmon has been extensively studied using different approaches. The first investigations were based on the analysis of allozymes (Ståhl 1987, Elo et al. 1994, Skaala et al. 1994, Bourke et al. 1997). Ståhl (1987) demonstrated that Atlantic salmon populations from throughout the range form three distinct clusters, corresponding to Western Atlantic, Eastern Atlantic and Baltic Sea drainages. These findings were confirmed by Bermingham et al. (1991), McConnell et al. (1995), Taggart et al. (1995) and Bourke et al. (1997) using different molecular approaches. The Baltic populations show quite low levels of variation. Probably these populations have undergone some population bottleneck during the last glaciations (Nilsson et al. 2001).

Recent microsatellite studies have revealed a higher genetic diversity in Atlantic salmon populations than other approaches applied before (McConnel et al. 1997, King et al. 2001). King et al. (2001) genotyped 29 populations from the western (= North American populations) and eastern North Atlantic region (= European populations) at 12 microsatellite DNA loci. In total, they could find 266 alleles at the 12 investigated loci. The data collected by King et al. (2001) confirmed the large genetic distances between populations of the western and eastern North Atlantic region. Furthermore, microsatellite analyses revealed a high number of alleles unique to each region. Within each region there existed a strongly significant relationship between genetic distance and geographical distance. Less genetic differentiation was observed within North American populations than within European populations. The authors hypothesised that this difference probably resulted from different histories of postglacial colonisation of the two continents rather than differing management histories. The North American range of Atlantic salmon was glaciated more recently and more uniformly than the European range.

In European Atlantic salmon the microsatellite data of King et al. (2001) suggest three geographical groupings: Iceland, Finland and Atlantic Europe (western Norway, Ireland, Scotland and Spain). Since only one population of the Baltic Sea region was analysed, the data collected cannot be used to confirm or reject any geographical grouping corresponding

to the Baltic Sea drainages suggested by data of Bourke et al. (1997), Bermingham et al. (1991) and Ståhl (1987). The discreteness of the Icelandic populations is consistent with the findings based on allozyme data of Bourke et al. (1997).

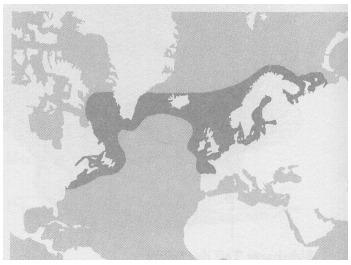
Genetic and molecular identification

As in the case of *Salmo trutta* and *Oncorhyncus mykiss*, studying the genetics of *Salmo salar* began with the analysis of the variation of allozymes (e.g. Ståhl 1987, Elo et al. 1994, Skaala et al. 1994, Bourke et al. 1997). However, *Salmo salar* is characterised by low levels of protein variation in comparison with other species of salmonids (Bourke et al. 1997). Therefore the use of protein electrophoresis to determine stock structure in Atlantic salmon has certain limitations. Several studies demonstrated that the analysis of microsatellite DNA markers is a very suitable method to study the genetic structure of Atlantic salmon populations and to determine the extent of genetic variation within and among Atlantic salmon populations (e.g. Fontaine et al. 1997, McConnell et al. 1997, Stone et al. 1997, Martinez et al. 2000, King et al. 2001).

Centres of origin/diversity

Origin, natural distribution

The Atlantic salmon is native to the basin of the North Atlantic Ocean, from the Arctic Circle to Portugal in the eastern Atlantic, from Iceland and southern Greenland, and from the Ungava region of northern Quebec southward to the Connecticut River (Kendall 1935, Scott & Crossman 1973).



The native area of *Salmo salar* (according to Muus & Dahlström 1978, revised by Pätzold).

Migration history

The Atlantic salmon colonised its native areas 15 000 years ago, after the last ice decade. Atlantic salmon is introduced only in some countries, primarily for salmon farming but also stocked out for angling. The species was imported to British Columbia (Canada), to

the west-coast of the United States of America (1897), to South America (Argentina, Chile), Australia (1880), New Zealand (1892) and South Africa[120].

Evolution

Three major phylogenetic groups can be distinguished in *Salmo salar* L.: a west and an east Atlantic group, and a Baltic group. The deep genetic divergence between the North American and European populations was demonstrated by allozyme studies (e.g. Bourke et al. 1997), studies on mitochondrial DNA variation (Bermingham et al. 1991) and studies on the variation of microsatellites (McConnell et al. 1995). Nilsson et al. (2001) were able to proof the split between Eastern Atlantic and Baltic salmon by investigating mtDNA variation. All haplotypes found in the Baltic populations were also common in the Atlantic populations, suggesting that the division occurred when these haplotypes were already widespread and common in salmon, but the distribution of frequencies differed markedly. Compared to the Atlantic populations, Baltic populations show low levels of variation, indicating that Baltic populations have undergone some population bottleneck (Nilsson et al. 2001)[121].

In most of European and North American hydrographic basins systems, a sharp drop in the salmon populations was noted over the past century. Declining numbers and loss of whole stocks in some rivers are causing increasing concern. The principal causes are habitat loss (destruction, fragmentation or degradation of the habitats), denial of access to spawning grounds by dams and other obstructions, pollution, and, in certain cases, over-fishing.

Till the 20th century the river Rhine was one of the most important European salmon rivers. It accommodated one of the greatest salmon stocks (Schmidt 2000). In 1885 more than 130 000 salmon were caught in the Rhine, in 1945 less than 2 000 (Grimm 1993). The species was extinct in the 60s of the past century. The Atlantic Salmon is threatened also at the coasts of North America, so for example the salmon stocks in the bay of Fundy rivers declined because of habitat loss from about 40 000 in the mid-1980s to a few hundred in 1999 (Musick et al. 2000).

Many Baltic salmon rivers have lost their natural juvenile production due to human activities blocking or reducing access to spawning grounds, e.g. dams, power generation, partial hindrances (Rivinoja et al. 2001). A significant decrease in the level of natural reproduction of the salmon was noted in the Northern Dvina basin (Russia) in comparison with the beginning of the 20th century. The primary causes of this development are the

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[120] Source: http://www.fishbase.org

[121] Hypothesis of phyleogeographic colonisation lineages of Atlantic salmon is discussed by Koljonen et al. (1999).

wasteful over-fishing of the area for the salmon processing industry and uncontrolled poaching (Studenov et al. 2001).

Another cause of declining salmon stocks in the northern area is acid rain. Due to acidification, 18 Norwegian stocks of Atlantic salmon are extinct and an additional eight are threatened. In the two southernmost counties, salmon is eradicated. Due to its high sensitivity to acidification, salmon was greatly reduced as early as in 1920 (Kroglund et al. 2001, Sandoy & Langaker 2001).

In 1978, the first co-ordinated operations for the restoration of migratory fish got under way in the Garonne and Dordogne basins with a "Salmon Plan" (France). At that period, seven of the eight large migratory species were still present; only the Atlantic salmon (*Salmo salar*) had completely disappeared (Boyer et al. 2001). Other restoration plans, like the European “Aktionsprogramm Rhein” [122], in 1987, or the program “Elbelachs 2000”, in 1994[123] (Schmidt 2000), had followed. Since the native salmon stock of the river Rhine was extinct in the 60s of the past century (Grimm 1993), re-introduction into the Rhine is a naturalizing process. Relevant measures are taken in several rivers of the Rhine system (Sieg, Wupper, Lahn, Ruhr, Sauer-Mosel, Murg, Breusch-Ill, Rench, Kinzig). The most serious problems encountered in the development of a self supporting salmon population are dams and other structural hindrances for migration to and from the spawning grounds, availability of appropriate spawning places (water body and interstitials oxygen-saturated, adequate pool riffle compartments). Strenuous efforts are made to achieve this aim, especially by means of "fish passes", and systematical re-stocking. The salmon eggs for these activities are obtained from Ireland, Sweden and Norway. The first returning salmon in the German part of the Rhine system was sighted in 1988 near Karlsruhe (Weibel 1990) and in 1990 in the Bröl river, a tributary of the Sieg (Steinberg et al. 1991)[124].

International agreements on reduced atmospheric emissions will hopefully reduce acidification effects substantially during the coming 20 to 50 years. However, the extreme acid sensitivity of salmon makes the destiny of this species in Southern Norway uncertain. Liming is an effective measure to protect and restore fish populations in acidified waters, which in combination with reduced emissions will be an important contribution to the protection of the Atlantic salmon species (Sandoy & Langaker 2001, Walseng et al. 2001).

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[122] This program is also called “Programm Lachs 2000”.

[123] The program “Elbelachs 2000” started in 1994, the first parr were placed in the river in 1995. The first returning salmon was observed in 1998. In the same year 28 salmon were caught (Steffens 2000).

[124] There are comparable activities for other German rivers, like the Weser, Ems and Elbe (IKSR 1999).

In the River Otra (southern Norway) the Atlantic salmon population was lost during the 1960s due to acid rain and industrial and municipal pollution. Emissions from industrial and municipal sources were curbed by 1995. A concurrent reduction in acid deposition during the last 10 years has raised pH from 5.2 to 5.7 and reduced inorganic monomeric aluminium from 71 to 28 mg Al/l measured in the air above the industrial area. The water quality improvement resulted in salmon fry again being caught from 1995. The quality of smolt caught in 1999 suggests that the river is able again to support a native salmon population, provided there will be no negative change in water quality. Specific winter episodes and acid tributaries within the watershed can, however, disturb and offset the recovery process (Kroglund et al. 2001).

The restoration plans normally resulted in the following improvements: On the statutory level, protection of part of the spawning habitats was assured, and fishing was banned for threatened species. On the water management level, freedom of passage will be re-established over large stretches of the rivers, thus giving migratory fish access to the breeding grounds in the upper stretches of these waters. On the biological level, efforts to restore Atlantic salmon stocks have started, with structural and organisational backing, including reconditioning centres and fish farms, give rise to the hope for full re-establishment in the long term. For population monitoring, "check points" were set up at several strategic sites within the river basins, providing information about the colonisation process.

The first population figures showed a progressive re-establishment of salmon stocks in European rivers (Schmidt 2000, Boyer et al. 2001). However, despite the monitoring efforts made, there are still many unresolved questions regarding evaluation, and the data are still insufficient to appraise the dynamics of each individual population.

Reproduction biology

The spawning season of the Atlantic salmon is winter. The migrating salmon return to the tributary or growth area they left as smolts. Homing behaviour is more or less the same for wild and reared salmon (Insulander 2001).

Atlantic salmon spawn in October to February, the peak of spawning usually occurring in late October and November. As spawning time nears, males undergo conspicuous changes in head shape: the head elongates and a pronounced hook, or kype, develops on the tip of the lower jaw. The nesting site is chosen by the female, usually a gravel-bottom riffle above a pool (Bigelow 1963, Scott & Crossman 1973). The ecomorphological demands to the spawning grounds are: water descent 0.2- max. 3%, water depth 50-90 cm, running speed 0.3-0.7 m/s, gravel Ø 30-50 mm, nest size 1-2 m (MUNLV 2001).

The female digs the nest, called the "redd," by flapping strongly with her caudal fin and peduncle while on her side; the redd is formed by the generated water currents. When the redd is finished, the male aligns himself next to the female, the eggs and sperm are released, and the eggs are fertilised during the intermingling of the gametes. On average, a female deposits 700-800 eggs per pound of her body weight. The eggs are pale orange in colour, large and spherical, and somewhat adhesive for a short time. The female then covers the eggs with gravel, using the same method used to create the redd. The eggs are buried in gravel at a depth of about 12 to 25 cm (Bigelow 1963, Scott & Crossman 1973).

The female rests after spawning and then repeats the operation, creating a new redd, depositing more eggs, and resting again until spawning is complete. The male continues to court and drive off intruders. Up to six redds for a single female and seven for a single male were detected. Both sexes ranged extensively. Distance between redds involving the same parent varied from a few metres to > 5 km. Distances > 1 km were common. Both males and females ranged to a similar extent. Range limit was not correlated to fish size. Pairs were not monogamous, both males and females mating with different partners at different sites. Redd superimposition was found to be common, although it was not correlated to the number of anadromous spawners present. High levels of nonanadromous mature parr mating success were recorded. Although reproductive success by mature male parr increases the effective number of males, this increase seems likely to be most pronounced in natural populations when the number of anadromous males is low (Taggart et al. 2001). Complete spawning by individuals may take a week or more, by which time the spawners are exhausted. Some Atlantic salmon die after spawning but many survive to spawn a second time[125]; a very few salmon spawn three or more times. Spawning completed, the fish, now called "kelts," may drop downriver to a pool and rest for a few weeks, or they may return at once to the ocean. Some may also remain in the river over winter and return to sea in the spring.

Egg hatching usually occurs in April but the young remain in the gravel until the yolk sac is absorbed and finally emerge in May or June of the year following egg deposition. The newly hatched salmon, called "alevins", remain in rapid water until they are about 65 mm long. These fish are now called "parr," and their growth is slow. Parr are called "smolts" when they reach a length of 12 to 15 cm and are ready to go to sea. Salmon grow rapidly while at sea. Some may return to the river to spawn after one year at sea, as "grilse," or may spend 2 years at sea, as "2-sea-year salmon" (Bigelow 1963, Scott & Crossman 1973).

[125] Only 5% of adult fish (mostly females) spawn a second time.

Crossability

Hybridisation is known to occur between Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) (see also 4.1.1.) (e.g. Matthews et al. 2000). Natural hybrids are found in areas, where Atlantic salmon and brown trout (*Salmo trutta*) are spawning together. The rate of natural hybrids normally is less than 1%, indicating that efficient reproductive isolating mechanisms normally exist between these closely related species (Leaniz & Verspoor 1989, Jordan & Verspoor 1993). The low frequency of occurrence of Atlantic salmon x brown trout hybrids has been attributed to a combination of temporal, spatial and behavioural differences during spawning (Heggberget et al. 1988). Higher rates (up to over 20%) of hybridisation have been reported (Jansson & Öst 1997). Breakdown of reproductive isolation between the two species is normally associated with a range of factors, including competition for spawning habitat[126], disparity in sex ratio, decline in overall numbers of either salmon or trout, human or environmental disturbance such as stocking or introduction of new species, or "sneak" fertilisation by mature male parr[127] (Hubbs 1955, Hindar & Balstad 1994, Jansson & Öst 1997, Gephard et al. 2000, Matthews et al. 2000, Garcia-Vazquez et al. 2001). The hybrids are, with few exceptions, sterile (Chevassus 1979, Hindar & Balstad 1994) and normally show a higher morphological similarity to brown trout (Hedenskog et al. 1997).

According to Youngson et al. (1993) and Jansson & Öst (1997) escaped farmed salmon hybridise with brown trout more frequently than their wild con-specifics[128]. Farmed salmons strains are generally genetically different from local wild populations. For example, many farmed strains used in Ireland and Scotland are of Norwegian origin (McGinnity et al. 1997). Numerous studies demonstrated that escaped farmed salmon interbreed with native wild populations resulting in genetic changes in wild populations (e.g. Fleming et al. 1996, McGinnity et al. 1997, Clifford et al. 1998, Martinez et al. 2001).

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[126] Massive stockings of hatchery-reared fish and environmental constraint have forced Atlantic salmon and brown trout to common spawning grounds leading to a high level of hybridisation.

[127] Sexually mature parr may be less discriminating than adult spawners. This behaviour, also called as "sneak" fertilisation, could be one of the factors enhancing the frequency of interspecific crosses.

[128] According to Lura & Sægrov (1993) who studied the timing of spawning in cultured and wild Atlantic salmon and brown trout in the Norwegian River Vosso, the peak spawning of cultured immigrant Atlantic salmon occurred 21 and 26 days earlier relative to wild salmon in 1991 and 1992, respectively. In the River Vosso the spawning time of cultured Atlantic salmon overlapped with that of brown trout.

4.3.2. Domestication of *Salmo salar* L.

Hatching and rearing, including health precautions and safety measures

A freshwater fish farm using surface water subject to temperature fluctuations typical of the temperate zone must have an available water supply of 3-5 l/s per tonne of fish. This presupposes that the water is fully oxygenated. A neutral or mildly alkaline water is to be preferred with a pH of 7.0-7.5. A pH of less than 6.0 should be avoided. The ideal water temperature for salmonid production is one that does not rise too high in summer nor fall too low in winter. A temperature of 15-18°C has been found experimentally to be the optimum for salmonid metabolism.

Freshwater culture is normally practised in earth ponds (Danish-type), fish tanks or (mostly concrete) raceways (Sedgwick 1995).

The cultivation cycle of salmonids comprises several distinct phases such as spawning, egg fertilisation, larval development, and the subsequent growth of juvenile fish up to adulthood.

In the case of the anadromous Atlantic salmon, seasonal influences impact both on spawning and smoltification. Salmon fry during their first summer already show signs of a “bimodal” distribution of unit weight with two distinct weight bands of fish emerging. The larger fry will become “S1” smolts the following May or June, whereas the smaller fish will take another year to smoltify as “S2” smolts.

The smolts now become adapted to seawater life.

Salmons typically weigh 3-4 kg. The total growth cycle from egg to marketable fish normally lasts around 4 1/2 years for “S2” salmon (Shepherd & Bromage 1995). Sea migrating stocks of rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) show a comparable development.

There are obvious limiting factors to the intensification of salmonid farming, starting with the need to increase the level of artificial feeding. Oxygen levels decline and the concentration of waste products from fish rise with increasing stocking density. If water is scarce it becomes necessary to install recirculation and aeration systems to an extent where costs become prohibitive. As fish are crowded together the risk of mass mortality due to system failures grows.

Fish farming is concerned with the transformation of inputs, such as eggs and juvenile stocks, feed ingredients and oxygen into valuable outputs (marketable fish). Small fish need

more water per kilogram of body weight than large fish, and the demand for oxygen, and hence water, increases with increasing water temperature.

In intensive fish culture great care must be taken to prevent system failures causing asphyxia, poisoning, sickness, mass mortality or even escapes (Shepherd & Bromage 1995).

Stripping and egg incubation

Salmonid eggs and sperms are usually produced by stripping. In preparation of harvesting, all fish should be starved for 24 or preferably 48 hours. After stripping from the brood female or henfish, salmonid eggs are soft. At this stage and before fertilisation occurs, they are referred to as “green” eggs. Green eggs can be transferred between farms for 24 hours after stripping provided they are kept cool and do not come into contact with water. Most hatcheries fertilise their eggs immediately, or at most after 4 or 5 hours (Shepherd & Bromage 1995).

Bacteria in water sources are responsible for high losses during egg hatching. The appearance of fungus colonies is a result of bacterial attack (Nieslony 2001). Therefore water inflow to the hatcheries is often disinfected through irradiation with UV-light (Adam 2002).

Following fertilisation and water hardening (eggs become hard after contact with water), the eggs are transferred to a suitable incubator where they remain at least until they become “eyed”. This is the stage of development when the eye of the fish embryo within in the egg becomes darkly pigmented.

At this point eggs should under no circumstances be disturbed for the next 10 or 15 days of incubation. During this stage it is very important to remove dead and fungal colonised eggs from the cases because they damage adjacent healthy eggs. Nowadays most hatcheries treat all their eggs every day or on alternate days with suitable medicaments. Recommended disinfectants are iodophors (Sedgwick 1995). Jodun & Millard (2001) suggested that to optimise egg survival, contact with iodophor during water hardening should be no more than 30 min. If a greater disinfection efficacy is desired, an increase in iodophor concentration may be preferable to an increase in contact time.

This minimises fungal attack and infection of neighbouring healthy eggs. Water temperature should be less than 6 °C for egg development (Adam 2002). Eggs should also be shielded from light by black covers.

There are two major types of incubation systems. The first is the hatchery tray, which looks like a bottle, and the second is the vertical incubator (hatchery jar), a kind of flat case.

Generally, most trays take about a litre of eggs, i.e. 6 000-8 000 salmon or 10 000-20 000 trout eggs.

The outside dimensions of each tray are such that they can be placed in rows along the length of a fry trough or a raceway. Water enters at one end of the trough and leaves at the other after passing the eggs in each of the arranged hatchery trays. Generally an inflow of 3-5 l/min per litre of eggs is required.

Whatever system of incubation is used, at eying the eggs should be “shocked” by pouring or siphoning the eggs from one container to another. Unfertilised or damaged eggs are killed by this shock treatment. Their yolk proteins turn white or opaque. In contrast, healthy eggs are quite resilient at this stage and remain undamaged by the shock treatment. The dead eggs must be removed by manual picking or by salt or sugar flotation. At a certain concentration the dead eggs float and the viable ones remain at the bottom.

Harvesting and breeding of salmon eggs normally works without any problems. The average of loss is about 2-3% (Schwevers & Adam 1998).

Fry systems

After hatching in conventional hatchery trays, the yolk-sac fry or alevins fall through elongated perforations in the base of the tray into the trough below, leaving behind any dead eggs, discarded eggshells, and deformed fry. The tanks of fry should also be covered because exposure to strong sunlight may produce abnormalities and additional mortality up to 30% if this coincides with high water temperatures up to 10-12 °C (Schwevers & Adam 1998).

Yolk-sac fry also grow faster if they are maintained in dim light or darkness. They have to be removed as soon as the first of the batch show any signs of rising to the surface to take food. Transfer of the fry from incubator to tank should always be done by floating or immersing the tray and fry in a new tank and allowing the alevins to swim out on their own volition. On no account should the fry be tipped or netted (Shepherd & Bromage 1995).

After hatching probably the most crucial point in the development of young salmonids is the time of first feeding. It is essential to find the right timing, formulation and frequency of feeding. Errors at this stage let alevins quickly loose weight and die. The best time to start feeding is when fry are willing to consume food; this point can easily be established by spreading a little food on the water surface. Feeding must start before the yolk-sac is completely consumed. As soon as feeding commences, the fry should be fed for 20 hours a day using automatic feeders. As the number of fry which are feeding increases the inflow of water should be increased to 0.2-0.3 m/s because only then do salmon alevin react to food particles and catch them. The particles must be smaller than those given to trout alevins at

first feeding. It is possible to use commercial trade food or zooplankton (Schwevers & Adam 1998). At this stage of development the fry will be consuming 5-10% of their body weight per day and doubling their weight every week. By continually monitoring growth and performance and feeding optimally, farms will get their fry up to 4-5 g 120-130 days after fertilisation. Faster growth can be achieved by artificially warming the water, and for many salmonids 14-16° C is considered the optimum temperature for growth. This is the stage when many hatcheries sell on their stocks to production farms.

After fry have reached 500 fish/kg they can be stocked in any form of tank or raceway. By this stage any potential risks of infection with pancreatic necrosis virus (IPN) ought to have passed, whereas fry which are to be moved to earth ponds should remain in fibre-glass tanks or raceways until 16-18 weeks post-fertilisation to reduce the chance of infection with the protozoan parasite *Myxosoma cerebralis*, which causes the whirling disease. Predominant diseases of alevins are also fungal (*Saprolegina sp*.) and bacterial (Myxobacteria) attacks. In France these diseases are treated with “Chloramin T“ and antibiotics (Schwevers & Adam 1998).

Towards the end of the fry stage the tanks should be covered with nets because fish of such small size will be eaten by many different predators.

Fingerling and smolt production

Fingerling production relies on very much the same principles and techniques used in culture of fry and with on-growing fish. Adjustments of feed rates and pellet sizes and the use of larger tanks and other enclosures are often the only alterations in methods employed by farms. The breeding of fingerlings takes place in covered raceways with a water depth of 30 cm. The intensity of stocking must be as low as possible to avoid diseases (Schwevers & Adam 1998).

In contrast to other salmonids, for the Atlantic salmon the period of development between fry and production-sized fish is arguably the most crucial in its life cycle. During this period the young fish, which is known as parr, becomes a smolt and is able to move from freshwater to a seawater existence. Atlantic salmon smolts are produced approximately one or two years after hatching. Growth to a body length of 8-12 cm by September/October appears to be a prerequisite for smoltification the following spring. Parr which fail to reach this size invariably become "S2". Farms are continually looking for improved diets and feeding methods and use earlier spawning strains, culture of fry and parr in heated waters, to improve growth and hence the overall percentage of "S1s" produced.

One of the major difficulties of smolt production is finding the optimum time for the transfer of smolts into salt water. Smoltification involves marked changes in behaviour, body

shape and colour and the development of a tolerance for seawater. The condition factor decreases progressively for a month before smoltification, while at the same time the characteristic silvery colour of smolts appears.

The direction of swimming is also reversed from being against the current to being with the current. Smolts tend to swim less actively than parr, a trait which in the wild would tend to carry them downstream, i.e. towards the sea. Consequently farms with supplies of both fresh and saltwater adapt their prospective smolts to saltwater by adding increasing levels of seawater to the freshwater inflow to the tanks. Increased levels of salt in the diet may also help adaptation. Similar techniques are also used to acclimate trout destined for stocking in sea cages. Young fish weighing 30-70 g can be transferred successfully. Smolts should also be adapted to lighter conditions before transfer to sea cages (Shepherd & Bromage 1995).

On-growing

On-growing salmonids to market weight traditionally takes place in excavated earthen ponds, concrete troughs, or in cages.

Because of the strong market demand, on-growing of salmon is practised in an active aquaculture industry, involving sea ranching with net-pen and cage-rearing. For regional production there is also the option of stocking landlocked salmon. It is recommended that landlocked salmon stocking should be carried out in lakes with relatively low fishing pressure (Hyvarinen et al. 2000).

Salmonid stocks exhibit hierarchical or peck-order patterns of feeding behaviour. This means that some fish regularly receive more food than others in a population. These differences are reflected by differential growth. This spread in size has important implications for commercial practice. Firstly, populations of divergent growth rate cannot be fed on pellets of uniform size. Secondly, divergent growth leads to bullying, tail and fish nipping by the larger fish and sometimes to cannibalism. To avoid this aggressive behaviour and enable optimum rations and pellet sizes, ideally all fish should be the same size. This is achieved by sizing and grading the stock. Stocking densities at which salmonid are kept in raceways vary in relationship to water temperature and available flow. A density of 4-5 kg is standard (Sedgwick 1995).

Salmonids which are to be sold with pigmented flesh should have been on an appropriate diet (trout for at least 6 weeks before harvest). Generally Atlantic salmon are fed on a pigmented diet throughout the period of salt water growth.

Harvesting salmon is labour-intensive and made much easier by the use of automatic hoists, particularly in the case of cage farms. Much improvement in production has recently

been achieved by reducing the handling and netting of fish. The most cost-effective farms now use central grading areas to which the fish are transported by fish pumps or a gravity-fed piped system.

Major difficulties of transporting fish relate mainly to the provision of an adequate supply of oxygen, the removal of ammonia and carbon dioxide, and the maintenance of an acceptable temperature. Oxygen must never be allowed to fall below 6 mg/l. In the event of a shortage, oxygen can easily be provided by pumps, gas bottles, by spraying the water during its circulation or passing it through a venturi device. Suitable aeration and circulation also serve to blow off excess carbon dioxide. This does not constitute a problem to salmonids until levels reach 15 mg/l. Provided the pH of the water is not too high (less than 7.5 mg/l), ammonia, which is only toxic in its nonionised (NH_3) form, should not be a problem (Shepherd & Bromage 1995).

Selection

Efforts to domesticate salmon have been aimed at establishing desirable characteristics such as rapid growth, good food conversion, uniform rate of sexual maturation and stable spawning times. The initial step is simple selection. The best fish are graded out. The basic selection objectives can be summarised as follows:

- resistance to specific diseases.
- the ability to continue to feed effectively over a wide temperature range.
- the ability to achieve satisfactory growth on a diet low in animal protein.

This kind of selection has been used by hatcheries to breed a variety of different races. Many of these races are easily distinguishable. One problem with these varieties is their small genetic variability, which makes them unsuitable for restocking projects. However, restocking programmes require artificially bred fish with wide genetic variability.

The use of induced triploidy has led to a new "species" for aquaculture development. Ideally, research on this new species should first be aimed at determining its optimum rearing requirements (Benfey 2001). Induced triploidy is the only effective method currently available for mass production of reproductively sterile salmonids for aquaculture. At present triploids have reduced survival rates and high rates of skeletal deformity. Up to 60% of triploids suffer from the absence of primary gill filaments (gill filament deformity syndrome - GFD) during development (Sadler et al. 2001). Triploids grow more slowly initially, are easily frightened and have a higher oxygen demand (Rösch 1998).

Cage cultivation

Fish cages are used in lakes and the sea. A great advantage of cage culture is that there is only little risk of failure of the water supply or lack of oxygen in comparison to freshwater farming in ponds or raceways (Sedgwick 1995). Burgov (1992) describes submersible cages in deep sea waters protecting fish from overheating. The position of the cage depends on vertical thermostratification, making it possible to farm salmonid fish with a preference for cool water during the hot summer in southern seas.

Atlantic salmon and other salmonids are typically reared in intensive marine farms in two-year production cycles. Large-scale production units are in operation in Northern Europe, Canada, the United States, and Chile. Typical input rates per unit range 100 000-200 000 smolts per year (Shepherd & Bromage 1995, Varadi 2001).

The salmon spend their first year in freshwater ponds. Fish are then transferred to floating net pens anchored in coastal bays for another 1 to 2 years of growth (Morkore & Rorvik 2001). Stocking density ranges between 30-40 kg/m^3 for on-growing fish. The largest fish can be stocked at the greatest density. The main reason for sea farming is the unrestricted water space. In the colder, northern countries there is the added advantage that the sea is usually relatively warm in winter. The fish will continue feeding through the winter months and achieve much more rapid growth than in freshwater. European coastal waters warmed by the Gulf Stream seldom fall below +5 °C (Sedgwick 1995).

Intensive fish farming has given rise to various problems. Being carnivorous in the wild, farmed salmon depend on a diet that is 45% fishmeal and 25% fish oil. In 1997 some 1.8 million tons of wild fish for feed were required to produce 644 000 tons of Atlantic Salmon – a ratio of 2.8 : 1. The European salmon farming industries require a marine support area for feed equal to about 90% of the fishing area used for primary production in the North Sea. Consequently, they depend heavily on fishmeal imported from South America (Naylor et al. 1998).

A second problem is the pollution of farm areas with production waste. Heath (1992) has shown that water quality varies seasonally as a function of feeding intensity and water temperature. The highest total phosphorus content was found under the cages. The water quality of the impoundments deteriorated over time, resulting in toxic algal blooms and diurnal and seasonal dissolved oxygen fluctuations. Cornel & Whoriskey (1992) found a 5.5 times higher sedimentation rate directly below the cages.

Water quality and high stocking densities have facilitated outbreaks of salmon diseases and parasite attacks that have caused large losses to salmon farms. These problems have led salmon farmers to use antibiotics and pesticides, which also end up in

coastal waters. European research aimed at reducing antibiotic use has led to the development of salmon vaccines (Naylor et al. 1998).

Ernst et al. (2001) reported that pesticides are used extensively in the finfish aquaculture industry to control sea lice infestations of farmed salmon. The most prevalent method of use is to enclose a net pen with an impervious tarpaulin and mix a pesticide solution within that enclosure. After treatment the pesticide solution is released to the environment. Concerns have been raised that there is a potential risk to non-target aquatic organisms from those releases. Most samples taken after the releases of azamethiphos were not toxic. By contrast, almost all samples taken after the release of cypermethrin were toxic. Data suggest that a single release of cypermethrin can cause toxic effects over many hectares. The drastic increase of sea louse in the vicinity of salmon farms has also caused a decrease of sea trout parr.

Nutrition and feeding

The feeding of salmonids depends on fish size, water temperature, oxygen level, quality of food and also stocking intensity. An important parameter to monitor in this connection is the total weight of the stock.

Feeding influences the growth and flesh quality of fish, their susceptibility to diseases or parasites as well as waste outputs and the total costs of fish rearing. Feeding of salmonids therefore needs to be controlled with a mind to economy as well as ecology.

One important factor of feeding is its intensity, which is decisively determined by its frequency and the technique employed. Manual feed distribution is widely used. It has the advantage that fish behaviour can be observed during feeding, especially where this is facilitated by spatial confinement. The cost of this method is often high in relation to the margins earned by fish farming. Arndt et al. (1998) showed that salmonids fed with a floating extruded diet via demand feeder had significantly better feed conversion rates than fish fed a floating diet or a sinking diet administered by hand. Alanärä (1992) describes the advantage of demand-feeding systems compared to timer-controlled feeding. The fish can determine the proper time of day for feeding, and therefore, lower the energy loss due to high metabolism and swimming activity. Studies by Ladu & Ross (1992) showed that it is important to wait with feeding of trout about 5 hours after stressful operations, because any increase in oxygen consumption due to stress will tend to reduce metabolic scope.

A second important factor is the food and its quality. Proper food composition is a decisive prerequisite for obtaining healthy and well-growing fish. Proteins and amino acids, lipids and essential fatty acids, carbohydrates, vitamins, minerals and also carotinoids are the most important ingredients of fish nutrition.

Proteins and amino acids

Fish meal is the most common supplier of proteins. However, the protein demand of European fish farms is enormous (Naylor et al. 1998), and natural fish resources are declining worldwide. Soybean, rapeseed, and maize gluten have the greatest potential for replacing fish meal as a protein source. However, diets consisting only of vegetable protein are less efficient in terms of reproductive indices than diets based on animal protein. The amino acid profile of fish meal seems to be the most balanced for a trout brood stock diet (Pereira et al. 1998).

Data of Bransden et al. (2001) suggest that Atlantic salmon could be fed diets with the fish meal component reduced to supply approximately 600 g/kg of the total protein and the remaining 400 g/kg supplied by dehulled lupin meal or a combined dehulled lupin and hydrolysed poultry feather meal without any adverse effects on growth, immune function or blood chemistry.

Lipids

The most important classes of lipids in fish nutrition are triglycerides and phospholipids. Lipid supply in the feeding of fish is vital firstly to satisfy essential fatty acid requirements. Fish oils are very rich in fatty acids, and fish meals also contain lipids which are rich in fatty acids. Lipids are usually well digested. Diets containing more than 30% fat give excellent results for trout and Atlantic salmon, implying a good digestive utilisation.

Diets deficient in essential fatty acids lead to slowed-down growth and decreased feeding efficiency. After a certain period pathological signs appear such as hepatic degeneration, fin erosions or gill lesions. In trout a deficiency lasting several months can lead to a loss of movement in response to stress. In spawning fish it causes a significant reduction in egg production. In addition, the majority of alevins show various morphological deformities and have limited survival rates.

The current trend in feeding salmonids in particular is to increase the lipid content of the feed. Increasing the lipid content from 14 to 20% improves growth and feeding efficiency without altering growth performance even when protein content is decreased by about 35% to 48%. The effect of lipids on feeding efficiency results mainly from their high energy content. Reftsie et al. (2001) reported that salmon fed high-fat diets on average reached a higher final weight. Together with the saving made on protein, this improvement in feeding efficiency contributes to a decrease in aquaculture pollution as well as to maintaining the quality of aquatic environments.

Carbohydrates

Carbohydrates are the most widespread organic compounds in the biosphere. Although carbohydrates are not indispensable in fish feed, they constitute an inexpensive source of energy. In the absence of carbohydrates there is increased utilisation of proteins and lipids as an energy source.

One problem in feeding carbohydrates to fish is their low rate of digestion of complex carbohydrates. The digestibility of carbohydrates is linked to the complexity of the molecule. Simple sugars such as glucose are more readily digestible than dextrin.

For all fish, previous hydrothermal treatment improves the digestibility of complex carbohydrates, thus increasing the dietary digestible energy supply. The use of cereals or pulses as an energy source in fish feeds thus requires technological pre-treatment. A level of gelatinisation above 70% appears to be required to maximise carbohydrate digestibility. In this way, pulses can be incorporated up to a level of 255 g/kg in salmonid feeds. Economic results are only achieved with complex carbohydrates (Guillaume et al. 2000).

Vitamins

Vitamins are an essential feed constituent because most animals are unable to synthesise them. Young or stressed fish generally have an elevated demand. Demand also varies between vitamins and from species to species. Bohl (1999) reported that salmonids normally need 100 mg ascorbic acid per 1 kg dried food (Cyprinids, 30-50 mg/kg). Absence of vitamins leads to reduced feeding, unspecific depression in growth and deficiency diseases. Hypervitaminosis may also cause damage, however. Licek (1999) describes that the feeding of vitamins and glucane prevents diseases through stimulating the immune system. The use of vitamin additives is common practice. Special recommendations are given by Bohl (1999).

Carotinoids

Carotinoids are a class of natural fat-soluble pigments found principally in plants, algae, and photosynthetic bacteria, where they play a critical role in the photosynthetic process. They also occur in some non-photosynthetic bacteria, yeasts, and moulds, where they may carry out a protective function against damage by light and oxygen. Although animals appear to be incapable of synthesizing carotenoids, many animals incorporate carotinoids from their diet. Within animals, carotenoids provide bright coloration, serve as antioxidants, and can be a source for vitamin A activity.

An important aspect for fish farmers and feed manufacturers is the pigmentation efficiency of carotinoids. This is determined by their structure, specific colour, digestibility, metabolic conversion and specific affinity for a tissue. Natural sources of carotinoids always contain a mixture of different pigments (Guillaume et al. 2000).

Minerals

Fish require minerals as constituents of certain tissues (mainly skeletal) or for molecules that serve as enzyme co-factors. In an aquatic environment, management of dietary supply is closely linked to fishes' capacity to absorb material (via gills, skin and mouth) from the environment. In freshwater, the external environment is very hypotonic in relation to the internal environment, and the difference in osmotic pressure leads to a loss of minerals. In salt water, the environment is hypertonic in relation to the internal environment, and minerals are taken up far more readily than in freshwater. This makes it difficult to define exact requirements. In general it is agreed that "requirements" are the dietary levels that allow fish to achieve optimal deposition in their tissues in the absence of waterborne minerals.

Effluent treatment

During growth fish produce a number of waste products which are released into the water. Waste products include ammonia, suspended solids, organic phosphates and nitrates. These organic materials have a biochemical oxygen demand (BOD) which removes oxygen from the water. A high BOD can produce anaerobic conditions. Under anaerobic conditions nitrates may also be converted into nitrites which are extremely toxic. Ammonia, suspended solids and BOD are toxic to fish and other organisms, and high concentration levels of organic phosphate leads to eutrophication.

All these waste products including uneaten feed, are discharged from fish farms into rivers and other water courses. In many countries the discharge of agricultural wastes is subject to strict emission regulations. Most discharge permits are based on limit values for ammonia, suspended solids and BOD. Less commonly regularised but also important are pH, phosphates, temperature, and the presence of sewage fungi, formaldehyde, free chlorine, total phenols, copper, antibiotics and oils. Certain countries (e.g. Sweden) place limits on the tonnage of fish which farms are allowed to produce, mainly because of the correlation between food fed and the amounts of waste produced. Limits on total production are often also imposed on cage farms because of the difficulty of measuring effluent.

While waste production levels vary with environmental and farm conditions, the following figures give a rough idea of the waste arising from one kg of dry pelleted food fed

to salmonids: 25-50 g of ammonia, 200-300 g suspended solids, 100-200 g BOD, 5-15 g phosphates and 30-60 g of nitrate; moist and trash fish-based diets produce much higher levels of waste products.

If the amount of water flowing through a farm is known, then the levels of waste products in the effluent may be calculated. If the permitted levels are exceeded, some form of treatment of the water must be carried out. A low oxygen level can be remedied by aeration or splash boards. High levels of suspended solids and, to a lesser extent, high effluent BOD can be remedied by settling in specific sedimenting ponds. Reductions in suspended solids and BOD levels can also be achieved by modifications in feeding.

Ammonia and phosphate constitute the most difficult effluent components as far as treatment is concerned because of their solubility. Less than 10% of the total ammonia excreted is settleable. The remainder can only be removed by biological filtration which, because of the often large volumes of effluent to be treated, is uneconomic and impracticable for salmonid production farms. Biological filtration can be used advantageously with high value stocks maintained in relatively small or recirculated water flows.

At present there is no practical method of removing phosphate from the effluent of production farms with high volumes of water flow. Phosphate stripping by ion-exchange is possible but expensive. Currently, the reduction of phosphate by dietary means is the only means of control available to the farmer. At present most commercial diets contain 10-20 g of phosphate in every kg of feed. The formulation of these diets includes a large safety margin because experimentally the ionic demands of the fish are fully met with diets which contain only 6-8 g phosphate/kg feed (Shepherd & Bromage 1995).

The most significant effect of aquaculture waste is increasing the nutrient concentrations in natural waters (hyper-nutrition). The source of these nutrients are mainly wasted fish feed and solid faecal waste[129]. In addition to organic wastes, residues of chemicals (biocides) or drugs (pharmaceuticals) used for fish farming are liable to enter the water column or the sediment. A factor that is often overlooked is that of temporal variations in waste loading. These are related to feeding periodicity, tank cleaning, pond harvesting operations, and seasonal changes in stock biomass (Midlen & Redding 1998). Naylor et al. (1998) reported that salmon net pens allow faeces and uneaten feed to flow directly into coastal waters, resulting in substantial discharges of nutrients. The Nordic salmon farming industry discharges quantities of nitrogen and phosphorous equivalent to the amount of untreated sewage produced by about 2 million people.

Pathogens and Diseases

Nearly all diseases which occur epidemically among salmonid in fish farms are indirectly attributable to fish domestication, and fish density during rearing. Wild fish stocks in rivers and lakes only rarely suffer massive losses such as occurring in fish farms. Diseases in fish may result from any of the following conditions:

- Bacterial or viral infection[130],
- Infestation by internal or external parasites,
- Environmental conditions (lack of oxygen, entrained gases, or physical damage), and
- Toxic algal blooms and deficiencies or toxins in the diet.

Viral diseases occur primarily in cold water and bacterial diseases in warmer waters (Hamers 2001). Some pathogens are only found in freshwater, some in the sea and others in both fresh and salt water. Sea-going salmonids, like the Atlantic salmon, bear a double risk. Diseases can be transferred from fresh to salt water by sea-bound young fish, or the pathogenic effects of a disease which infected the fish while in freshwater may become manifest under the stress of migration to the sea. Fish pathogens can be classified into two main groups. Those which are termed "obligate" are normally absent from water in which there are no diseased fish or carriers of disease. Many of the common bacterial and viral diseases in fish belong to this group. The second group is termed "facultative". These are pathogens which are naturally present in the water and may infect fish and cause symptoms of disease when they are stressed or in the event of physical changes in their environment such as abnormal fluctuations in temperature or salinity.

Many different fish diseases produce symptoms of confusing similarity. For this reason it is particularly important that diagnosis is confirmed as soon as possible by appropriate tests carried out in a laboratory so that the correct treatment can be applied before it is too late (Sedgwick 1995).

For example spring water should be tested for the presence of metal salts which can be toxic to fish and it also should be tested to make sure that it is not supersaturated with air, as this can give rise to a condition known as “gas-bubble disease”, to which young fish are particularly susceptible (Sedgwick 1995).

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[129] The development of high-energy feed by manufacturers has, to some extent, reduced pollution from fish farms through products of fish metabolism.

[130] Infections with the fungus S*aprolegina* sp. are normally secondary.

The majority of fish health problems are caused by disease processes involving living agents, such as bacteria, fungi, parasites and viruses. According to Bergheim (2001) most salmonid loss in Norwegian fish farms is caused by diseases (45%) and lack of smoltification (12%). The scale of mortality in certain bacterial or viral infections may reach 30% or more within several days.

There are three methods available for administering therapeutic compounds: oral administration (by adding the drug to the food), bath treatment (immersion of the stock in a chemical solution), and mass injection.

Environmental concerns over the use of chemicals in the open aquatic environment relate to the direct toxicity of the compounds and the development of resistance to compounds by pathogenic organisms.

Bacterial or viral infection[131]

Virulent fish diseases are the most dangerous because there is almost no way of combating them nor of curing affected fish. Important viral diseases are:

- Viral Haemorrhagic Septicaemia (VHS), a disease that causes mortality in rainbow trout and Atlantic salmon (other salmonids and pike), seldom in brown trout. The only prevention is to buy young salmonids from VHS-free farms.
- Infectious Haematopoietic Necrosis (IHN), a disease that causes mortality in rainbow trout and Atlantic salmon; brown trout normally are not affected, but can be carriers. The only prevention is to buy young salmonids from IHN-free farms (Schlodtfeldt & Aldermann 1995).
- Infectious pancreatic necrosis (IPN), a disease that primarily affects fry and parr of all salmonids (pike, carrier can be eel and cyprinids). Mortality 10-90%. The only prevention is to buy young salmonids from IPN-free farms.
- Infectious Salmon Anaemia (ISA) first observed in Norway in 1984, observed also in Scotland in 1998. All salmonids are susceptible, while sea trout and rainbow trout are carriers. The only prevention is to buy young salmonids from ISA-free farms.
- Sleeping disease (SD), a disease that occurs without or with low mortality in rainbow trout. The disease could be transmitted to Atlantic salmon and brown trout under

[131] For the purpose of preventing epidemics Directive 91/67/EEC (1994) has declared the following diseases to be notifiable: ISA, VHS, IHN, IPN, ERM, Furunculosis, BKD, PKD, Whirling disease.

experimental conditions. The only prevention is to buy young salmonids from SD-free farms.

Important bacterial diseases are:

- Bacterial Kidney Disease (BKD), pathogen: *Renibacterium salmoninarum;* primarily affects salmonids (Coregones and grayling are only carriers). Mortality ranges from single fish to total loss. Prevention is possible through buying young salmonids from BKD-free farms and quarantine. Injecting the parent stock 6-8 weeks before stripping with a high dose of erythromycin can ensure BKD-free eggs.
- Furunculosis, pathogen: *Aeromonas salmonicida;* affects salmonids of all age groups. Furunculosis was originally a freshwater disease, but due to the intensification of marine salmon farming is now also a very significant disease in the marine environment. Mortality up to 30%, for fry up to 50%. Prevention is possible through minimising stress and overcrowding. Vaccines are available, but these require intraperitoneal injection, so that the labour costs of vaccination are high.
- Enteric Redmouth Disease (ERM), pathogen: Y*ersinia ruckeri*; primarily affects rainbow trout and Atlantic salmon, but also brown trout (other salmonids, cyprinids, pike, eel and percides can be carriers). Mortality 10-60%. Prevention is possible through quarantine, and avoidance of stress and high densities. Vaccination is very successful and must be carried out on fry weighing more than 3 g.
- Rainbow Trout Fry Syndrome (RTFS) and Bacterial Cold Water Disease (CWD), pathogen: *Cytophaga psychrophila;* mainly affects fry and fingerlings of salmonids. Mortality up to 50%. Prevention is still not understood. Possibilities are egg disinfection with iodophores, formalin bath of fry after yolk sac resorption and salt baths (1-1.5%, 30 min.). Therapies with Oxytetracycline, Amoxycillin and Enrofloxacin may be recommended. No vaccine is as yet available (Schlodtfeldt & Aldermann 1995).

Infestation by internal or external parasites

Healthy fish living in favourable environmental conditions can survive infestation quite well in the case of many of these parasite species. Treatment to control such parasites is only to be recommended in cases of massive invasion. External parasites of salmonids are:

- Costia, *Ichthyobodo necator* (Flagellates) primarily affects salmonid (trout) fry. The best prevention is hygiene in the hatchery. Treatment is possible with salt water or formalin bathing.

- Hexamita[132], *Octomitus salmonis* (Flagellates) affects salmonids of all age groups. Mortality mainly in fry. The best prevention is hygiene in the hatchery. Treatment is possible with Dimetridazol or magnesium sulphate-medicated feed (Schlodtfeldt & Aldermann 1995).
- *Ichthyophthirius multifiliis* (Ciliates) affects all fishes. Mortality depends on fish size and infection intensity. Infected fish should be kept at reduced stocking density. The best treatment is malachite green (nowadays forbidden) or alternatively formalin bath.
- *Chilodonella sp*. (Ciliates) affects all fishes and age groups. Mortality depends on fish size and infection intensity. Prevention is possible through low stocking densities and increasing water flow. Treatment is possible with salt water or formalin bath.
- *Trichodina sp*. (Ciliates) affects all fishes and age groups. Mortality depends on fish size and infection intensity. Prevention is possible through hygiene and quarantine for ornamental fish. Treatment is possible with formalin and sodium chloride bath. Similar damage is caused by *Trichodinella sp., Tripartiella sp., Foliella sp.* and other related ciliates.
- *Glosatella sp. /Apiosoma sp.* (Ciliates) affects all fishes and age groups. Mortality occurs seldom in fry. Prevention is possible through hygiene, quarantine immersion bath before stocking. Treatment is possible with formalin bath (Schlodtfeldt & Aldermann 1995).
- Proliferate Kidney disease (PKD) (Myxozoa), a severe clinical disease in rainbow trout. The same or similar parasites are known to occur in other salmonids. PKD is water-system linked and only appears in fish in infected water systems, not in fish in well or spring water. Mortality depends on water temperature during the summer season. Fish which have been exposed to PKD and recovered become immune.
- Whirling disease (WD), *Myxobolus cerebralis*, (Myxozoa); affects most fry and fingerlings of salmonids. On infected farms, young fish should be kept in concrete or plastic tanks until they are large enough (6-7 cm) to resist infection. There is no therapy.
- *Dactylogyrus sp.* (Metazoa); affects all fishes and age groups. Mortality occurs in fry of rainbow trout. Prevention is possible through hygiene and quarantine. Treatment is

[132] Wedekind & Schlotfedt (1999) recorded that *Hexamita* and *Ichthyophithirius* are a cause of concern in Germany because there are no legal and effective treatments available for these pathogens.

possible with Trichlorfon, but permission from the appropriate authorities has to be obtained for it.

- *Gyrodactylus sp.* (Metazoa), affects all fishes and age groups. Mortality occurs seldom, i.e. in the case of massive infection of fry and ornamental fish. Prevention is possible through hygiene, quarantine and routine bath treatment. Treatment is possible with Trichlorfon and Hydrogen peroxide (Rach 2000), permission has to be obtained from the appropriate authorities.
- Eye Fluke, larvae of different Diplostomidae (Trematoda); affects all fishes and age groups. Mortality is very rare. There is no effective treatment other than to break the parasite's passage between snails, water bird and fish (Schlodtfeldt & Aldermann 1995).
- Tape worms *Caryophyllaeus sp., Proteocephalus sap., Eubothrium sp.* (Cestodes); affects all salmonids. Morality is very rare. Prevention is possible through hygiene and yearly pond liming.
- *Acanthocephalus sp.* (Metazoa); affects all fishes. Morality is very rare. Prevention is possible through hygiene and yearly pond liming.
- Fish louse *Argulus sp.* (Crustacea); affects all fishes. Acute mortality only with heavily infected young fish. Fish lice can transmit viral and bacterial pathogens. Prevention is possible in landlocked systems through hygiene and yearly pond liming.
- Sea louse *Lepeophtheirus sp., Caligus sp.* (Crustacea). Ectoparasitic sea lice cause stress and increase the susceptibility of fish to secondary infections. In extreme infestations, fish can suffer from osmoregulatory failure and death. The most immediate treatment is the use of chemotherapeutics such as Ivermectin, either by bath or oral administration. Ivermectin is a neurotoxin poorly absorbed by fish, with a high percentage of the administered dose being excreted largely unchanged with the faeces. Ivermectin can reach the marine environment via excretion from the bile, unabsorbed via the fish faeces and by uneaten food pellets and has a strong affinity to lipid, soil and organic matter. Risk assessments by Davies & Rodger (2000) have shown that Ivermectin is likely to accumulate in sediments and that sediment-dwelling species would be more at risk than species living in the pelagic environment. Ivermectin has been shown to be toxic to some benthic infaunal species. Boxaspen & Holm (2001) reported success in removing sea louse from Atlantic salmon in sea cages by an oil-based pyrethrum treatment mixture. Overall delousing efficiency with this large-scale method was 85%.

- *Ergasilus sp.* (Crustacea); frequently found on brown trout from extensive water systems. Mortality is rare. Treatment is possible with Trichlorfon, but only if permission from the appropriate authorities has been obtained.
- Leeche, *Piscicola sp.* (Metazoa); affects all fishes and age groups. Mortality can occur in the event of massive outbreaks on fry and ornamental fish. Prevention is possible through hygiene and yearly pond liming. Treatment is possible with Trichlorfon, but only if permission from the appropriate authorities has been obtained (Schlodtfeldt & Aldermann 1995).

In intensive fish culture great care must be taken to prevent system failures causing asphyxia, poisoning, sickness, mass mortality or even fish escapes. Typical diseases are:

- Gill inflammation caused by poor water quality or incorrect use of chemotherapeutics followed by bacterial growth on the gill surface. Mortality ranges from single fish to total loss.
- Gas bubble disease caused by a supersaturation of the water by gases. Nitrogen (N_2) is the main cause of this disease, because supersaturation with nitrogen can rapidly lead to severe problems and start at as low as 103-104% (Schlodtfeldt & Aldermann 1995).

Farmed fish are continually subjected to environmental change as well as husbandry practices, such as grading and transport. These factors can impose considerable stress. Stress causes reduced inflammatory and immune responses, this in turn resulting in lower resistance to microbial invasion. For example, salmonids become stressed at spawning and frequently succumb to fungal and bacterial infection. Sandodden et al. (2001) showed that metomidate anaesthesia combined with a 48 h recovery period lessens the stress burden imposed by hauling and transport.

High mortalitiy rates in salmonids aquaculture can also be caused by toxic algal blooms and deficiencies or toxins in the diet:

- Algae blooms of *Cochlodinium sp*. were monitored by Whyte et al. (2001) on the west coast of Vancouver Island where they caused substantial mortality to farmed salmon, accounting for economic losses of about CAN $ 2 million.
- Deficiencies in amino acids can lead to impairment of growth and erosion of fins. There are also known diseases arising from deficiencies of lipids, vitamins and minerals. Excessive carbohydrate levels can result in hepatocyte degeneration and excessive glycogen deposition (Bailliere & Saunders 1989).

Conservation of genetic resources

Escaped farmed Atlantic salmons invade rivers throughout the native range of this species. This fact has generated growing concern about their impacts on native populations. Competition for natural resources, such as food, space, and mates, altered predation regimes and transfer of diseases and parasites are besides interbreeding and disruption of local adaptation and genetic homogenisation the main adverse impacts resulting from escapes of farmed salmons. Fleming et al. (2000) studied the interactions of farm salmon invading a native population. The farm fishes were competitively and reproductively inferior. However, evidence of resource competition and competitive displacement existed as the productivity of the native population was depressed by more than 30%. In summary, the results indicated that annual invasions have the potential for impacting on population productivity, disrupting local adaptations and reducing the genetic diversity of wild salmon populations. Similar conclusions were drawn by other researchers, like e.g. by Einum & Fleming (1997).

To preserve the genetic diversity of Atlantic salmon significant efforts have to be made. Local adaptation and differentiation of gene structure among geographically distinct lineages need to be explored further across the full range of the species. Finally considerations of evolutionary significant units for the conservation of the Atlantic salmon are to be made based on the collected data (Nielsen 1998). Restoration efforts should take into account inter- and intra-river diversity and utilise supplementation only in a manner that does not significantly perturb the recipient population by shifting gene frequencies, influencing demographic and physiological parameters, or introducing disease (King et al. 2001).

Biotechnology: Genetic modification/transformation

Methods and state of the art in fish biotechnology including the gene constructs used were extensively described in chapters 2.1. and 2.2.. This chapter summarises the targets of genetic modifications that have been carried out in Atlantic salmon, the most important salmonid species for marine aquaculture.

As in the case of rainbow trout and other fish species increasing the productivity of fish farming by enhancing fish growth is one main target in attempts of genetic modification in Atlantic salmon (see also chapter 2.1.). One well-known example of such a transgenic Atlantic salmon is the AquAdvantage variety being developed by Aqua Bounty. The AquAdvantage gene construct uses a Chinook salmon growth hormone gene and a promoter sequence derived from a different species, the ocean pout. The AquAdvantage construct was inserted into Atlantic salmon of Canadian origin. These GM salmons grow from egg to about 3.6 kg in only 14 to 18 months, half the normal time. At one year, they are

4 to 6-times larger than a conventionally bred salmon of the same age (Niiler 2000). However, it is known that there are many growth hormones active in Atlantic salmon and other fish, therefore a lot of other constructs are possible. The trend is going to use “all-fish” gene constructs developed from the genome of other fish species. Saunders et al. (1998) and Cook et al.(2000a) reported 2 to 3-fold growth enhancement in Atlantic salmon when using "all-fish" gene constructs, while 3 to 5-fold growth enhancement has also been recorded, with some individual fish being 20 to 30-times larger in the early phase of growth. Furthermore growth-accelerated transgenic salmons undergo precocious smoltification up to two years before their natural transformation (Devlin 1997).

In Atlantic salmon, much research has also been done with regard to cold tolerance improvement. Relevant trials involve the transfer of antifreeze protein genes identified and isolated from other fish species that inhabit waters at sub-zero temperatures. These antifreeze proteins (AFPs)[133] are produced in the liver and are secreted into the blood. They serve to reduce the freezing point by interacting with ice cristals (Hew & Fletcher 2001b). But up to now, these experiments have only been successful in part. The antifreeze protein genes were successfully integrated and expressed, but the cold tolerance of the fish could not be significantly improved. The transgenic salmon lacks the processing enzymes necessary for the maturation of the AFPs, it only generates the pro-AFP with an approximately 70% activity as compared to the mature polypeptides (Hew & Fletcher 2001a).

Experiments on the development of disease-resistant strains of Atlantic salmon – a further target in fish biotechnology – are underway but no data have been published as yet (Hew & Fletcher 2001a). For example, Hew & Fletcher (2001) generated transgenic salmon by inserting a gene construct that consisted of the rainbow trout lysozyme gene and the ocean pout AFP promoter. Lysozyme is a non-specific antibacterial enzyme important in fish defense. More specifically, the rainbow trout lysozyme is a potent antibacterial agent against many Gram-positive bacteria such as *Vibrio anguilliarum*, *Aeromonas salmonicida*, *Yersinia ruckeri* and *Flavobacterium ssp*..

Another potential application of biotechnology that would be of interest for Atlantic salmon aquaculture is the production of transgenic sterile fish strains. First attempts have been made in rainbow trout, but further research is still necessary. Once this target has been realised in other fish species, it should be quite easy to adapt and transfer the developed methodology to Atlantic salmon.

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[133] The protein chemistry of these proteins has been investigated extensively by many laboratories and can be grouped into at least four types of AFPs and one type of AFGP (antifreeze glycoproteins). Among all these proteins the Type I AFP from the winter flounder is the best characterised one (Hew & Fletcher 2001a).

Table 9: Genetic modification/transformation

Target	structural gene ("gene of interest")	promoter	reference
Growth enhancement	csgh (growth hormone gene from *Oncorhynchus tshawytscha* - chinook salmon)	antifreeze gene promoter (AFP) from *Macrozoarces americanus* (ocean pout)	Saunders et al. (1998), Stevens et al. (1999), Cook et al. (2000a)
Growth enhancement	csgh (growth hormone gene from *Oncorhynchus tshawytscha* - chinook salmon)	antifreeze gene promoter	Du et al. (1992)
Growth enhancement	hgh (human growth hormone gene)	MT (metallothionein promoter from mouse)	Rokkones et al. (1989)
Enhancement of cold tolerance	wflafp-6 (antifreeze protein gene from *Pleuronectes americanus* - winter flounder)	no details given	Shears et al. (1991), Hew et al. (1999)
Enhancement of cold tolerance	afp (antifreeze protein gene) from winter flunder (*Pleuronectes americanus*)	antifreeze gene promoter	Hew et al. (1991), Hew & Fletcher (1997 and (2001)
Resistance to bacterial pathogens	lysozyme gene from rainbow trout	antifreeze gene promoter (AFP) from *Macrozoarces americanus* (ocean pout)	work in progress (see Hew & Fletcher 2001a)

4.3.3. Ecology of *Salmo salar* L.

Survival stategies

The spawning migration of salmon is considered very precise, with adult fish normally returning to their river of origin, to the tributary or growth area they left as smolts. Through imprinting, young salmon (from fry to smolt) memorize details about their home streams, and they use this knowledge as adult spawners to find their way back (Maynor 1996). The homing behaviour is more or less the same for wild and reared salmon (Insulander & Ragnarsson 2001). About 2% of wild salmon seem to stray (data suggest straying for salmon of hatchery origin is much greater, up to 10%). For reaching their spawning grounds salmon are able to overcome hindrances. Attainable jump level of Atlantic salmon is about 3 m, depending on water temperature and possible swim speed. The best results are obtained at awater temperature of 15 °C and a swim speed of about 5-8 m/s (Höfer & Riedmüller 2002).

The distribution of fry leaving the redd is strongly peaked. It is about 80% within a 2-week period. Fry leaving the redd during the first half of the dispersal period tended to settle in different first feeding sites than those dispersing later (De Leaniz et al. 2001). Baby salmon swim in schools. Salmon from many rivers swim together in the same areas through much of their ocean-going life. Salmon have a great sense of smell, hearing, and taste which helps them find food and sense danger. Salmon are also able to sense danger by feeling the waves on their body. Atlantic salmon also use their senses to find and return to their home river.

The salmon is able to adapt to different rivers. Those which are fierce, with waterfalls and cascades, have strong, sleek, muscular salmon. Where the rivers flow more graciously, the salmon is adapted to a different kind of river.

The choices of food are affected by availability and size of prey, the prey`s digestibility and the predators experience. According to optimal foraging, the predator at all times will choose the most profitable prey providing it occurs in sufficiently large quantities. If the density of the prey decreases, the diet will be supplemented by less profitable prey. In the stomach of juvenile salmon one often finds most of the available kinds of prey in the environment. This type of opportunistic grazing behaviour is presumed to be common when the density of prey is low or preferable prey items are difficult to find. A periodically grazing on a wide spectrum of prey, enhances the fish`s ability to respond to quick changes in the environment with respect to the occurrence of different prey. During the summer, drifting invertebrates in the water are the main food source for salmon. During the warmer periods of the year the parr keeps its position above, but close to substrate in river stretches with riffles, while spreading throughout pools. In the colder periods the parr takes cover in the substrate. The fish also have a distinct change of behaviour between day and night at low temperatures during the winter. The fish reappears from its daytime hiding place and may stay active all night. This changes in behaviour occurs when the temperature falls below 8-10 °C, and is regulated by light. Simultaneously, physiological changes in the fish`s retinae occur, enhancing its vision and the possibility to catch a prey in the dark. This type of behaviour may cause salmon to shift from being primarily drift-feeders to benthic-feeders. Benthic feeding appear to be a particularly important in winter and in subarctic rivers, when drift rates appear to be low (Arnekleiv & Raddum 2001).

Atlantic salmon may withstand exposure to temperatures of -0.7°C (lower lethal limit) and 27.8 °C (upper lethal limit), but only for a short period of time (Bigelow 1963).

Synecology

Young salmon and trout are living in competition for food and cover. The winners mostly are the young trout (Symons & Heland 1978, Kennedy & Strange 1987, Vassen 1998). Similar observations have been made in waters where salmon and charr are living together (Gibson 1993). Young salmon and trout do not occupy exactly the same ecological niche (Heggenes & Saltveit 1990). At the population level, both salmon and trout are multi-prey feeders with a broad diet, but at the individual level, both species are specialized on a single or a few prey categories (Jorgensen et al. 2000), so the two species can live together quite well.

Young brown trout are more aggressive and growing faster than the Atlantic salmon pushing little salmon away. The salmon draws back to faster and deeper areas of the stream, which the trout is not able to use in the same way (Vassen 1998).

In streams with a more widespread spectrum of fish species the young salmon compete with different non-salmonids like dace (*Leuciscus leuciscus*), chub (*Leuciscus cephalus*), barbell (*Barbus barbus*) and gudgeon (*Gobio gobio*). Scientific studies showed that the young salmon are able to hold the field in this situation (Mann & Blackburn 1991, Schmidt & Feldhaus 1999).

Young Atlantic salmon in streams eat mainly the larvae of aquatic insects such as black flies, stone flies, caddis flies, and chironomids. Terrestrial insects may also be important, especially in late summer (Bigelow 1963). The results of studies on the predatory effects of fish on invertebrate communities in running water are highly variable. Several surveys show only minor or no effect on density and species composition of invertebrates after fish predation (Culp 1986, Reice 1991), while others have shown stronger effects with the reduction of at least some invertebrate taxa and a change in community structure (Gilliam at al. 1989, Power 1990, Dudgeon 1993, Dahl 1998). Reduction in biomass may also occur as a direct effect of fish predation.

When temperatures fall below +8 - 10 °C salmon will shift from primarily drift-feeding to benthic feeding. Grazing pressure vis-à-vis macroinvertebrates might therefore be differing between the cold and the warm seasons. Juvenile fish exert low grazing pressure on foraging animals in the cold season (Arnekleiv & Raddum 2001).

In addition to direct effects, a reduction in the density of certain invertebrate species exposed to fish predation may be due to behavioural changes in the form of evasive reactions and increased prey drift. The presence of predatory fish can lead prey, such as *Baetis* and *Gammarus* to change foraging strategy, provoke anti-predatory behaviour, change of location, and increase prey drift. Although direct salmonid predation does not

seem to reduce many invertebrate stocks to a great extent in running water, many investigations show that salmonid predators influence the behaviour and the history of invertebrates, and may influence community structure and interactions in river ecosystems (McIntosh & Townsend 1996, Crowl et al. 1997).

When at sea, salmon eat a variety of marine organisms. Plankton such as *Euphausiids* (popularly known as krill) are important food for pre-grilse, but amphipods and decapods are also consumed. Larger salmon eat a variety of fishes such as herring, alewife, smelt, capelin, small mackerel, sand lace, sand eel, small cod, squids and shrimps (Bigelow 1963).

Until July, post-smolt salmon in the Northern Baltic Sea largely relies on surface fauna (mainly terrestrial insects). From August onwards, fish is the principal type of food used. The smallest piscivorous post-smolts measured were <200 mm, but the main shift to piscivory occurred at sizes of 240-320 mm. Piscivory was observed to be enhanced by large smolt size. Almost all one-sea-winter salmon were piscivorous. Over 70% of the post-smolt and 96% of the one-sea-winter salmon with identifiable fish species in their stomachs had preyed on herring *Clupea harengus*. Other fish prey included the ten-spined *Pungitius pungitius* and three-spined sticklebacks *Gasterosteus aculeatus*. The results support earlier observations of a close relationship between recruitment of herring and production of salmon in the Bothnian Sea, and of the crucial role of smolt size in determining the ability of feeding salmon to utilise the food resources of the area (Salminen et al. 2001)

Interaction with pathogens, diseases, predators

For a description of pathogens and diseases, see sub-chapter "Pathogens and diseases" in 4.3.2..

As regards interactions with pathogens and diseases, a distinction must be drawn between wild and farmed fish. In wild salmonid populations pathogens are found very often, but an outbreak of diseases is rarely seen. The most well-known, predominantly freshwater external parasites of Atlantic salmon are the freshwater louse, *Argulus foliaceus,* and the leech, *Piscicola geometra.* Atlantic salmon lose their freshwater parasites but acquire others from the marine environment. The variety of parasites may increase for sea dwelling Atlantic salmon.

Adverse effects in wild salmon populations are reported to be caused by the parasite *Gyrodactylus salaris*, and the ectoparasitic sea lice, *Lepeophtheirus salmonis*, and furunculosis.

Some diseases are spread to rivers by fish escaping from fish farms. Furunculosis has had particularly serious effects in recent years in Norway, but bacterial kidney disease (BKD)

and infectious pancreatic necrosis (IPN) have also caused problems. Furunculosis was first introduced to Norway in 1964 with rainbow trout imported from Denmark. The disease has since been discovered in fish imported from Scotland. Farmed fish have spread the disease to numerous rivers. Furunculosis is the only documented source of mortality in wild Atlantic salmon stocks in North America (ASF 1999).

Davies & Rodger (2000) investigated ectoparasitic sea lice, *Lepeophtheirus salmonis* (Kroyer) and *Caligus elongatus* (Nordmann). The sea lice browse on the skin of Atlantic salmon, *Salmo salar* L., the resulting lesions causing stress and increasing the susceptibility of the fish to secondary infections. In extreme infestations, mostly seen in salmon farms, fish can suffer from osmoregulatory failure and death. Although originally an innocuous parasite, Morton (2002) reported that sea lice infections of wild salmon have become a critical issue in areas where wild and farm salmon share marine habitat. It is generally believed that farm salmon initially acquire sea lice (*Lepeophtheirus salmonis*) from adult wild salmon stocks returning from the sea to spawn. Under natural conditions, this species of sea louse dies when wild salmon enter freshwater to spawn, depriving this salmon-specific parasite of a host. Now it would appear salmon farms could offer sea lice artificial, inshore, over wintering habitat. In Norway, the level of sea lice infestation in wild fish in some areas where Atlantic salmon farming is concentrated has been found to be ten times greater than in areas where there are no farms.

Gyrodactylus salaris has, in the past decade, resulted in serious problems for Atlantic salmon populations in Norway. This parasite is a major disease problem in Norwegian salmon rivers, and has caused almost total eradication of young salmonids in some rivers. It does no harm to Baltic salmon or rainbow trout. Once *G. salaris* has spread into a river it takes only a few years to kill all the salmon fry, so destroying its entire stock of salmon. *G. salaris* can also live on the skin of other fish of the salmonidae family, apart from salmon fry, without causing much visible damage. It multiplies in the salmon, rainbow trout, grayling and char but can also survive for short periods in sea trout, eels and flounders. *G. salaris* cannot survive for more than a few days without finding a living fish to attach itself to. If the fish are killed using rotenon herbicide, the parasite is indirectly killed too (EELA 1999).

Fry, especially during dispersal (De Leaniz et al. 2001), as well as young parr and migrating smolts are eaten by adult trouts, pikes and other carnivorous fish species. Further predators of salmon in freshwater are birds like heron, kingfisher and eagle or mammals like otter. Losses caused by fish-predating birds are very high in some areas (Schmidt-Luchs 2001). Salmon migrating over long river stretches are subject to increased predatory pressure.

In the sea, carnivorous fish (shark, cod, sea lamprey), mammals (seals, dolphins) and fish-hunting birds (seagull, cormorant, gannet, skua) are also feeding on small and adult salmon[134].

In Scottish estuaries seals were seen to eat salmonids, mostly *Salmo salar* L. and *S. trutta* L.. However, seal predation on large salmonids in these estuaries was lower than mortality caused by angling along the river (Carter et al. 2001). On the other hand, anglers invested millions of Euros for stocking fish. In Germany anglers stocked angling waters with about 60 million trouts and several 100 000 young salmons per year (IKSR 1999, Schmidt-Luchs 2001, Höfer & Riedmüller 2002).

Ecological impact

Non-transgenic organisms

Resident fish normally outperform challengers, regardless of species. Volpe et al. (2001) suggested that Atlantic salmon may be capable of colonising and persisting in coastal British Columbia river systems that are underutilised by native species such as the steelhead.

Atlantic salmon escaped from farms have become so common (see 2.7.) that they sometimes dominate catches in Norway (between 30-40% of the coastal catch in Norway may be farmed fish), and they are frequently caught in Pacific waters in North and South America. Since 1994, over 9 000 Atlantic salmon have been recovered from coastal waters between Washington and Alaska, and recent evidence from Volpe et al. (2001) suggests that this species is now naturally reproducing in Vancouver Island rivers in western Canada.

Escaped fish interbreed with wild salmon stocks (and sea trout) in the sea, along coasts and in rivers. Occasionally, well-reputed salmon rivers are totally dominated by escaped fish. Salmon strains which have been brought into aquaculture are a mixture of salmon from different rivers (Naylor et al. 1998). Recent studies have proved genetic interactions between wild and farmed salmon stocks in rivers. Consequently, interbreeding with escaped farmed salmon may lead to genetic degradation of wild salmon populations, especially since wild populations have genetic characteristics specific to the rivers where they spawn. If new diseases break out, escaped fish could have a major negative impact (Bergheim 2001).

[134] Source: http://www.fishbase.org

As for competition between wild and farmed salmon, Jacobsen & Hansen (2001) reported that there was no difference in condition factor, number and weight proportions of prey, nor in diet between wild and escaped farmed salmon.

Transgenic organisms

Many of the potential adverse environmental effects of transgenic Atlantic salmon are similar to those of currently used farmed strains of Atlantic salmon. Different types of adverse effects will result from escapes and conventional aquacultural practice.

As a result of escapes, transgenic individuals can interbreed with wild atlantic salmon or hybridise with wild brown trout. The consequence would be introgression of transgenes into wild stocks. Furthermore escaped transgenic individuals will compete for resources (food, spawning areas and mates). Since there exists a great lack of knowledge with regard to the competitive abilities of transgenic strains, it is not possible to predict the ecological impact of such escapes (see also 2.5.3.).

Adverse effects due to conventional aquaculture practices are eutrophication through fecal material and excess feed, the spread of bacteria, viruses, and parasites to wild Atlantic salmon and other fauna, and the introduction of chemicals, e.g. those used for the treatment and prophylaxis of fish diseases.

5. Summary

The worldwide demand for fish as a protein source for human nutrition has grown continuously during the past century. To meet this demand large-scale production in aquaculture has been started as early as in the mid-eighties of the past century. Nowadays about 26% of all dietary fish is produced in aquaculture. Since genetic manipulations in fish can be carried out quite easily as compared to other vertebrates, gene technology was proposed as a solution to make aquaculture even more productive and to remove the pressure on wild aquatic resources. During the past two decades intensive research has been done in the field of fish biotechnology. Considerable improvements in gene transfer and gene constructs have been made since the first report on a successful gene transfer in fish was published in 1985. Up to now 35 different fish species have been target of genetic modifications. The development of transgenic fish has proceeded to the extent that commercial utilisation appears possible.

In the nineties of the past century the development of commercially useful transgenic fish strains was focused on growth enhancement. In 2001, the European Patent Office already granted its first patent for transgenic growth-enhanced fish. The Canadian company

Seabright obtained patent EP 0578 653 B1 for Atlantic salmon and all other fish species carrying an additional gene, opAFPghc, for faster growth in July 2001. A private US-Canadian company has applied for the commercial use of these fast-growing *salmons* in the United States of America, Canada, and Chile.

Other targets of genetic engineering in fish are the development of disease-resistant fish strains, improved cold tolerance, impoved tolerance to pollution, transgenic sterility, improved meat quality (e.g. higher protein content), and the development of monitor organisms for detecting mutagens and other pollution factors in aquatic environments. A lot of basic research, like gene identification and characterisation, improvement of gene transfer methods, and enhancement of gene expression is done by a large number of different Chinese and Japanese research groups or e.g. by the U.S.-American research group around Thomas Chen (University of Conneticut). Several research groups in Canada, the United States of America, and Cuba are doing more applied research. Choy Hew (University of Toronto, Canada) and Garth Fletcher (Memorial University of Newfoundland, Canada) are for example working on the enhancement of cold tolerance and on growth enhancement. Robert Devlin (West Vancouver Laboratory, Canada) is mainly working on transgenic growth-altered fish and disease resistant strains. Thomas Chen (University of Conneticut) is also working on the development of disease-resistant strains. In Cuba, José de la Fuente and Isabel Guillén (both Centro de Ingenieria Genética y Biotecnologia, Havanna) are working on transgenic growth-altered tilapia. Several European research groups, including Norman Maclean (University of Southampton, United Kingdom), P. Aleström (Norwegian College of Veterinary Medecine, Oslo, Norway), Bernard Breton (INRA Rennes, France), and Manfred Schartl (University of Würzburg, Germany) are working on transgenic sterile fish strains. Research on biosafety aspects of transgenic fish is done by Anne Kapuscinski (University of Minnesota, U.S.A.), William Muir and Richard Howard (both Purdue University, U.S.A.).

With regard to world fish production in aquaculture the two species Atlantic salmon (*Salmo salar* L.) and rainbow trout (*Oncorhynchus mykiss* Wal.) are playing a very important role. In Europe, marine finfish aquaculture is currently dominated by the production of these two species. Developing commercially valuable transgenic strains of these two salmonids have been one main target in fish biotechnology research. More than half of the research on transgenic salmonids has been conducted with gene constructs designed to influence growth, and first strains are ready for the market. Enhanced fish growth has been achieved by transferring an additional growth hormone gene construct to fertilised fish eggs. Nowadays such growth hormone gene constructs are developed from the genome of other fish species like the Pacific salmon (*Oncorhynchus kisutch*) or chinook salmon

(*Oncorhynchus tshawytscha*). In rainbow trout there have also been attempts to improve the feed efficiency by transferring human and rat gene constructs coding for special metabolic enzymes.

To improve cold-tolerance of salmonid species antifreeze protein genes from the winter flounder (*Pleuronectes americanus*) have been identified, analysed and transferred to Atlantic salmon. Up to now, the results of this research have not yet reached commercial stage. The enhancement of **disease resistance** in salmon and trout and the development of **transgenic sterile populations** are also on the agenda of these emerging transgenic strains of commercial interest. First attempts concerning the development of such strains have been made. The ulitimate realisation of these targets still needs a lot of basic research to do.

Scientific biosafety studies concerning transgenic fish have just started. Preliminary data on environmental impacts of transgenic fish releases and related questions of animal health are available. Adverse effects have been shown at the level of individuals, however there are many indications that other organisational levels (populations, ecosystems) are likely to be impaired, too. Three major aspects have to be taken into consideration:

First, genetic modifications can entail unintended effects like skull and body deformities, tumours, abnormal gill growth or altered feeding behaviour. These side effects have all been observed with transgenic salmon and trout.

Secondly, the stable expression of transferred genes cannot be guaranteed yet. Even though great progress has been made in the methodology of fish biotechnology, low frequency rates of genome integration and non-stability of transgene expression are still unsolved problems. In certain cases, e.g. the production of transgenic sterile populations as a containment method to avoid interbreeding with wild individuals, this can become a biosafety problem.

Finally, escaped salmon or trout from aquaculture are able to crossbreed with wild stocks of these species. They are also able to hybridise with brown trout (*Salmo trutta* L.). As a consequence of crossbreeding and hybridisation transgenes might spread into natural populations. This phenomenon may be accompanied by adverse effects on natural communities and may impair the whole ecosystem. For example, a transgene for cold tolerance would allow fish with that gene to invade waters in colder climates. This situation is comparable to the introduction of exotic species which can even lead to the elimination of entire populations of native species. Another example of ecological relevance is the modified sexual behaviour resulting from altered growth hormone production in fish. Quite often larger

male have a mating advantage over small males. Such a size advantage has been confirmed for Atlantic salmon.

Scientific investigations have not yet identified all the possible mechanisms by which transgenic fishes might influence ecosystems. One new methodology for estimating the risk of gene flow from escapees to wild relatives has been developed by William Muir and Richard Howard (University of Purdue, USA). This model integrates data on several “fitness components” into a single prediction on gene flow from escapees to wild relatives.

Escapes of aqua-cultured fish to the wild are a fact and pose a major problem. In recent years numerous escapes of farmed salmon occurred. Total **physical containment** of fish farmed in sea-based facilities is an unrealistic option for technical reasons. As an alternative, land-based facilities were proposed by the *Bergen Declaration*, a Ministerial Declaration of the “Conference on the Protection of the North Sea”, in March 2002 in order to prevent releases into the marine environment. In both cases **possible environmental impacts and costs should be thoroughly evaluated.**

Another possibility is the “biological containment” of transgenic fish that involves the production of sterile lines of fish to avoid possible gene transfer from escaped farmed fish. Up to now this has been achieved mainly by polyploidisation of the genome. But this method is not 100% effective. A new approach involves the inhibition of sexual maturation using genetic engineering methods. This strategy is based on the fact that the production of the sexual hormone gonadotropin can be inhibited by transferring an antisense gene construct into the organism. First attempts have been partly successful, but 100% sterility cannot be guaranteed by this method, either. The problem of instability of gene expression is still unsolved and has to be improved with a view to practical application.

Regarding the commercialisation of transgenic fish with all its unclear side effects, expertise and consensus on ecological risk assessment and risk management is needed. Further research is also necessary with regard to the **evaluation of potential adverse effects** of the escape or introduction of transgenic fish strains into natural fish communities. Concepts for **monitoring adverse effects** of transgenic fish have to be developed as well.

Facing the benefits and concerns connected with the putative commercial use of transgenic fish strains, some intergovernmental organisations and fora (like FAO, OECD, EU, the Network of Aquaculture Centres in Asia-Pacific or the "Conference on the Protection of the North Sea" (Bergen Declaration of the Fifth Conference)), a number of national governments, an increasing number of scientists, and different industrial, consumer and environmental lobby groups are now discussing the potentials and biosafety aspects of this technology. Reflecting on the consequences of the use of genetic engineering in aquaculture

it emerging ever more clearly that there is a great need for international harmonisation of regulation, including international trade regulations and the question of operating aquaculture facilities in international waters. Another important aspect that has to be discussed is the fact that escaped transgenic fish can easily pass borders. Facing the above described potential ecological impacts of escaped transgenic fish, international agreements are needed on how to proceed in conflicts concerning biosafety hazards

Decisions during regulatory processes on transgenic organisms should be based on a broad base of technical information on the product, including biological base data for the organism concerned. This study provides biological base data on the three commercially interesting salmonids *Salmo salar* L. (Atlantic salmon), *Oncorhynchus mykiss* Wal. (rainbow trout), and *Salmo trutta* L. (brown trout). The data compiled include information on morphology, taxonomic status, reproduction biology, ecology, genetic structure and genetic variation, crossability, centres of origin and evolutionary history, natural distribution, genetic conservation, domestication, breeding and cultivation practices, pathogenes and diseases, use and economic importance, and genetic modifications.

Molecular data collected in numerous scientific studies of different research groups suggest, that all three salmonid species are characterised by great genetic variability. Significant subdivisions have been found in the population genetics of each of these species. Differences have been found over broad geographic regions, as well as among tributaries within individual river basins, or even within specific rivers. Populations of these three salmonids exhibit diverse physiological, anatomical and behavioural characteristics, and it is assumed that these population differences are genetically based on local adaptation. Facing the problem of genetic conservation of these three species, it seemed to be important to identify suitable populations throughout their geographic range that can serve as gene reservoirs. Further research, including the identification of such populations must be integral part of the ongoing management of these species.

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7. Appendices

7.1. Abbreviations

AFS	Asian Fisheries Society
CAC	Codex Alimentarius Commission (WHO/FAO)
CBD	Convention on Biological Diversity (UN)
CCRF	Code of Conduct For Responsible Fisheries (FAO)
COFI	Committee on Fisheries (FAO)
COP	Conference of the Parties (CBD)
EEA	European Environmental Agency (EU)
EIFAC	European Inland Fisheries Advisory Commission (FAO)
ESF	European Science Foundation
EU	European Union
FAO	Food and Agriculture Organization (UN)
FEAP	Federation of European Aquaculture Producers
GM	genetically modified
GMO	genetically modified organism
GTA	Gene Technology Act (Norway)
ICES	International Council for the Exploration of the Sea
ISEES	The Institute for Social, Economic and Ecological Sustainability
NADA	New Animal Drug Application (USA)
NACA	Network of Aquaculture Centres in Asia-Pacific (FAO)
NASCO	North Atlantic Salmon Conservation Organization
SPS	Agreement on Sanitary and Phytosanitary Measures (WTO)
TBT	Agreement on Technical Barriers to Trade (WTO)
TRIPS	Agreement on Trade Related Aspects of Intellectual Property (WTO)
UN	United Nations
USA	United States of America
USC	United States Code (USA)
WHO	World Health Organization
WTO	World Trade Organization

7.2. Tables

Table 10: Selection of relevant intergovernmental organisations working on GM fish

Organisation	Most Important Activities	Statements/Results	Date
OECD	Research programme: "Biological Resource Management for Sustainable Agriculture Systems."	One focus is the quality of animal products and safety of food.	2002-2004
	Conference: "Living Modified Organisms (LMO) and the Environment"	The problem of interbreeeding of GM fish with wild fish population was discussed.	2001
	Conference: "New Biotechnology Food and Crops: Science, Safety and Society"	Recommondation for greater transparency on GMO and demand for independent scientific research into the risks and benefits of GM foods	2001
	Workshop: "Environmental Impacts of Aquaculture using Aquatic Organisms derived from Modern Biotechnology" organised by the OECD in co-operation with the Norwegian Ministry of Environment	OECD publication (1995)	9-11 June 1993
FAO	COFI[1] Sub-Committee on Aquaculture (1st meeting in Beijing, China) [1]FAO´s Committee on Fisheries (COFI)	Genetic engineering should be used with due protection of aquatic diversity.	18-22 April 2002
	"Conference on Aquaculture in the Third Millenium" organised by FAO and NACA (Network of Aquaculture Centres in Asia-Pacific)	Potential implications for aquaculture of GMO should be addressed in a precautionary, safe and practical way.	2000
	Publication: Third issue of "The State of the World Fisheries and Aquaculture"	-	2000
	Code of Conduct for Responsible Fisheries (CCRF)	Efforts should be undertaken to minimize the harmful effects of genetically altered stocks	1995
Conference on the Protection of the North Sea	Fifth International Conference on the Protection of the North Sea	Ministerial Declaration (Bergen-Declaration): all possible actions should be taken, in accordance with the EU-Directive 2001/18/EC, to ensure that the culture of GMO is confined to secure, self-contained, land-based facilities in order to prevent their release to the marine environment	20-21 March 2002

Organisation	Most Important Activities	Statements/Results	Date
UNEP/Convention on Biological Diversity	Convention on Biological Diversity	See article 8 and article 19	1992
	Cartagena Protocol on Biosafety	Regarding GMO the protocol is intended to safeguard the safe handling, transfer and use of GMO by assesssing the impact of GMO on biodiversity, and exchanging information through a Biosafety Clearing House.	2000
Nordic Council of Ministers	Conference “Genetically modified organisms in Nordic habitats – sustainable use or loss of diversity?"	-	1998
	Conference “Research and Regulation with regard to GM fish”	-	21-22 September 1996
North Atlantic Salmon Conservation Organization (NASCO)	NASCO Guidelines for Action on Transgenic Salmon (NASCO document CNL(97)48)	NASCO Parties should advise the Council of any proposal to rear transgenic salmon, including proposed measures for containment. The use of transgenic fish should be confined to secure, self-contained, land-based facilities.	1997
International Council for the Exploration of the Sea (ICES)	ICES drafted a “Code of Practice on the introductions and transfers of marine organisms” (This code is currently under revision. The update is expected for autum 2002)	The Council urges Member Countries to establish strong legal measures to regulate the release of GMO.	1994 (update)

Table 11: Targets of genetic modification in fish since 1997

Fish species	target of genetic modification	gene construct			reference
		reporter gene	structural gene ("gene of interest")	promoter	
Zebrafish (*Brachydanio rerio*)	development of a monitor organism for detecting mutagens in aquatic environments	kanamycin-resistance gene	rpsL gene (strA) of *Escherichia coli* (375 bp long) – a gene that shows a high mutation rate	-	Amanuma et al. (2000)
Rainbow trout (*Oncorhyn-chus mykiss*)	growth enhancement (reduction of production time)	no detail given	Ongh1 (overexpressing growth hormone gene from *Oncorhynchus nerka*)	MT (metallothionein promoter)	Devlin et al. (2001)
Zebrafish (*Brachydanio rerio*)	comparison of transformation techniques		"enhanced“ green fluorescent protein gene (eGFP)	*Xenopus* enhancer/pro-moter region 1-α (Ef1 α)	Linney et al. (1999)
Zebrafish (*Brachydanio rerio*)	enhancing uniform expression of transgenes by using inverted repeats of Adeno-associated virus (AAV-ITRs)	green fluorescent protein gene (GFP)	eGFP (enhanced green fluorescent protein gene) - promoter and eGFP gene were flanked by inverted terminal repeats	α-actin (a skeletal muscle-specific promoter from zebrafish) and β-actin (a ubiquitous promoter from medaka)	Hsiao et al. (2001)
Zebrafish (*Brachydanio rerio*)	study of skeletal muscle formation during myogenesis	eGFP (enhanced green fluorescent protein gene)	myf-5 – a gene that is involved in the myogenesis of zebrafish	no detail given	Chen et al. (2001)
Zebrafish (*Brachydanio rerio*)	development of a monitor organism for detecting vari-ous contaminants	PGL3-basic luciferase gene construct	EPRE (electrophile response element) from the mouse Gsta1 region	mMT1 mouse metallothionein promoter	Carvan et al. (2001)
Zebrafish (*Brachydanio rerio*)	study of hematopoiesis	modified green fluorescent protein gene (GM2)	-	GATA-1 promoter cloned from the zebrafish genome	Long et al. (1997)

Fish species	target of genetic modification	gene construct			reference
		reporter gene	structural gene ("gene of interest")	promoter	
Zebrafish (*Brachydanio rerio*)	study of the regulatory mechanisms of the gata1 gene	eGFP (enhanced green fluorescent protein gene)	gata1	HRD (hematopoietic regulatory domain)	Kobayashi et al. (2001)
Coho salmon (*Oncorhyn-chus kisutch*)	study of feeding behaviour and competitive ability of transgenic strains	no detail given	type 1 of the gh region of *Oncorhyn-chus kisutch*	metallothionein-B from sockeye salmon (*Oncorhynchus nerka*)	Devlin et al. (1999)
Zebrafish (*Brachydanio rerio*)	growth enhancement	LacZ gene of *Escherichia coli* (a gene that encodes for the enzyme β-galactosi-dase)	pgh growth hormone gene of yellow porgy (*Acanthopagrys latus*) and rtgh growth hormone gene of rainbow trout (*Oncorhynchus mykiss*)	Zp promoter (regulatory sequence from *Pseudopleu-conectes americanus*, winter flounder)	Sheela et al. (1998)
Tilapia – (different sub-genera of African Cichliden)	safety evaluation (study of different selected environmental impacts of transgenic tilapia and food safety assessment)	-	tigh (growth hormone gene cloned from *Oreochromis hornorum* – tilapia)	1) CMV promoter (cytomegalo-virus); 2) SV40 promoter	Guillén et al. (1999)
Rainbow trout (*Oncorhyn-chus mykiss*)	study for testing the utility of different GFP gene constructs as cell-labelling tools and reporters of gene expression in transgenic rainbow trout	GFP (two variants: S65T and eGFP)	-	1) CMV promoter; 2) EF1 (1α-enhanced promoter from the frog *Xenopus laevis*)	Takeuchi et al. (1999)

Fish species	target of genetic modification	gene construct			reference
		reporter gene	structural gene ("gene of interest")	promoter	
Rainbow trout (*Oncorhyn-chus mykiss*)	study of the developmental expression of the grf/pacap gene, that encodes for the two hormones GRF (growth hormone-releasing hormone) and PCAP (pituitary adenylate cyclase-activating polypeptide) – both hormones are involved in the growth hormone release from the pituitary	-	1) hypothalamic (hyp)-grf/pacap gene construct from sockeye salmon (*Oncorhynchus nerka*) cloned into pbluescript II KS +/-	645 base pair long promoter region of the grf/pacap gene	Krueckl & Sherwood (2001)
			2) pituitary (pit)-grf/pacap gene construct from sockeye salmon (*Oncorhynchus nerka*) engineered in a pUC19 vector	gh promoter from chinook salmon (*Oncorhynchus tshawytscha*)	
Rainbow trout (*Oncorhyn-chus mykiss*) and arctic charr (*Salvelinus alpinus* L)	improvement of the carbohydrate metabolism efficiency of salmonid fish	-	1) hgluT1 (human glucose transporter type 1 c-DNA)	1) CMV promoter (cytomegalus virus)	Pitkänen et al. (1999)
			2) rhkII (rat hexokinase type II cDNA)	2) OnH3-Histon 3 promoter from sockeye salmon (*Oncorhynchus nerka*)	
				3) OnMT-B (metallothio-nein-B promoter from sockeye salmon (*Oncorhynchus nerka*)	
Rainbow trout (*Oncorhyn-chus mykiss*)	study of the inhibitory effect of antisense mRNA	-	antisense-sGnRH-cDNA cloned from the genome of Atlantic salmon (*Salmo salar*), GnRH: gonadotropin releasing hormone	Pab promoter of the GnRH region of Atlantic salmon (*Salmo salar*)	Uzbekova et al. (2000)

Fish species	target of genetic modification	gene construct			reference
		reporter gene	structural gene ("gene of interest")	promoter	
Atlantic salmon (*Salmo salar*)	study of growth rate, feed intake, feed digestibility, feed conversion and body composition of transgenic Atlantic salmon in comparison to non-transgenic Atlantic salmon	-	csgh, growth hormone gene cloned from the genome of chinook salmon (*Oncorhynchus tshawytscha*)	antifreeze gene promoter (AFP) cloned from the genome of ocean pout (*Macrozoarces americanus*)	Cook et al. (2000a)
Atlantic salmon (*Salmo salar*)	study of the effect of food deprivation on oxygen consumption, metabolic rate and body composition of transgenic Atlantic salmon in comparison to non-transgenic Atlantic salmon	-	csgh, growth hormone gene cloned from the genome of chinook salmon (*Oncorhynchus tshawytscha*)	antifreeze gene promoter (AFP) cloned from the genome of ocean pout (*Macrozoarces americanus*)	Cook et al. (2000b)
Atlantic salmon (*Salmo salar*)	comparison of oxygen consumption and metabolic rate of transgenic Atlantic salmon in comparison to non-transgenic Atlantic salmon	-	csgh, growth hormone gene cloned from the genome of chinook salmon (*Oncorhynchus tshawytscha*)	antifreeze gene promoter (AFP) cloned from the genome of ocean pout (*Macrozoarces americanus*)	Cook et al. (2000c)
Atlantic salmon (*Salmo salar*)	study of the smolt development in growth hormone transgenic Atlantic salmon	-	csgh, growth hormone gene cloned from the genome of chinook salmon (*Oncorhynchus tshawytscha*)	antifreeze gene promoter (AFP) cloned from the genome of ocean pout (*Macrozoarces americanus*)	Saunders et al. (1998)

Fish species	target of genetic modification	gene construct			reference
		reporter gene	structural gene ("gene of interest")	promoter	
Atlantic salmon (*Salmo salar*)	study of respiratory metabolism and swimming performance in growth hormone transgenic Atlantic salmon under various specified conditions, in comparison to non-transgenic Atlantic salmon	-	csgh, growth hormone gene cloned from the genome of chinook salmon (*Oncorhynchus tshawytscha*)	antifreeze gene promoter (AFP) cloned from the genome of ocean pout (*Macrozoarces americanus*)	Stevens et al. (1999)
Coho salmon (*Oncorhyn-chus kisutch*)	study of morphological alterations in transgenic salmon	-	pOngh1 growth hormone gene from sockeye salmon (*Oncorhynchus nerka*)	MT (metallo-thionein promoter)	Ostenfeld et al. (1998)
Coho salmon (*Oncorhyn-chus kisutch*)	study of seawater adaptability and hormone levels of transgenic coho salmon	no detail given	gh (no further details given)	antifreeze gene promoter (AFP) cloned from the genome of ocean pout (*Macrozoarces americanus*)	Devlin et al. (2000)
Tilapia (*Oreochromis niloticus* L.)	long-term study of growth development in transgenic tilapia	β-actin/lacZ carp gene	csgh, growth hormone gene cloned from the genome of chinook salmon (*Oncorhynchus tshawytscha*)	antifreeze gene promoter (AFP) cloned from the genome of ocean pout (*Macrozoarces americanus*)	Rahman et al. (2001)
Mud loach (*Misgurnus mizolepis*)	generation of transgenic homozygous lines	CAT reporter gene construct (pFV4CAT) – chloram-phenicol-acetyl-transferase	-	β-actin carp promoter	Nam et al. (2000)

Fish species	target of genetic modification	gene construct			reference
		reporter gene	structural gene ("gene of interest")	promoter	
Tilapia (*Oreochromis niloticus* L.)	growth enhancement	βA/lacZ carp gene construct	csgh, growth hormone gene cloned from the genome of chinook salmon (*Oncorhynchus tshawytscha*)	antifreeze gene promoter (AFP) cloned from the genome of ocean pout (*Macrozoarces americanus*)	Rahman & MacLean (1999):
Three different Indian carp species: *Labeo rohita*, *Cirrhinus mrigala*, *Catla catla*	growth enhancement	-	rtgh growth hormone gene cloned from the genome of rainbow trout (*Oncorhynchus mykiss*)	Rous sarcoma-Virus promoter	Venugopal et al. (1998)
Mud loach (*Misgurnus mizolepis*)	growth enhancement	-	mlgh growth hormone gene cloned from the genome of mud loach (*Misgurnus mizolepis*)	mud loach β-actin promotor	Nam et al. (2001)
Atlantic salmon (*Salmo salar*)	studying the inheritance of the antifreeze protein gene wflafp-6	-	wflafp-6 antifreeze protein gene cloned from the winter flounder (*Pleuronectes americanus*)	no details given	Hew et al. (1999)
Zebrafish (*Brachydanio rerio*)	analysis of pancreatic development in embryos	GFP (green fluorescent protein gene)	Pdx-1 gene and insulin gene(two pancreatic genes) cloned from the zebrafish genome	no detail given	Huang et al. (2001)
Carp (*Cyprinus carpio*)	study of growth and feed utilisation of transgenic carp	-	hgh (human growth hormone gene)	MT (metallo-thionein promoter)	Fu et al. (1998)

Fish species	target of genetic modification	gene construct			reference
		reporter gene	structural gene ("gene of interest")	promoter	
Zebrafish (*Brachydanio rerio*)	analysis of pancreatic development in embryos	GFP (green fluorescent protein gene)	Pdx-1 gene and insulin gene(two pancreatic genes) cloned from the zebrafish genome	no detail given	Huang et al. (2001)
Carp (*Cyprinus carpio*)	study of growth and feed utilisation of transgenic carp	-	hgh (human growth hormone gene)	MT (metallo-thionein promoter)	Fu et al. (1998)
Tilapia (*Oreochromis niloticus* L.)	growth enhancement	-	tigh (growth hormone gene cloned from the Tilapia genome)	CMV promoter (human cytomegalovirus)	Martínez et al. (1996)
Tilapia (*Oreochromis niloticus* L.)	growth enhancement	-	tigh (growth hormone gene cloned from the tilapia genome)	1) CMV promoter (human cytomegalovirus) 2) RSV (rous sarcoma virus) 3) SV40 4) INT (the first intron of the rainbow trout growth hormone gene)	Hernández et al. (1997)
Japanese medaka (*Oryzias latipes*)	growth enhancement	-	hgh (human growth hormone gene)	1) chicken β-actin promoter 2) Atlantic salmon growth hormone promoter	Muir et al. (1994 and 1995)

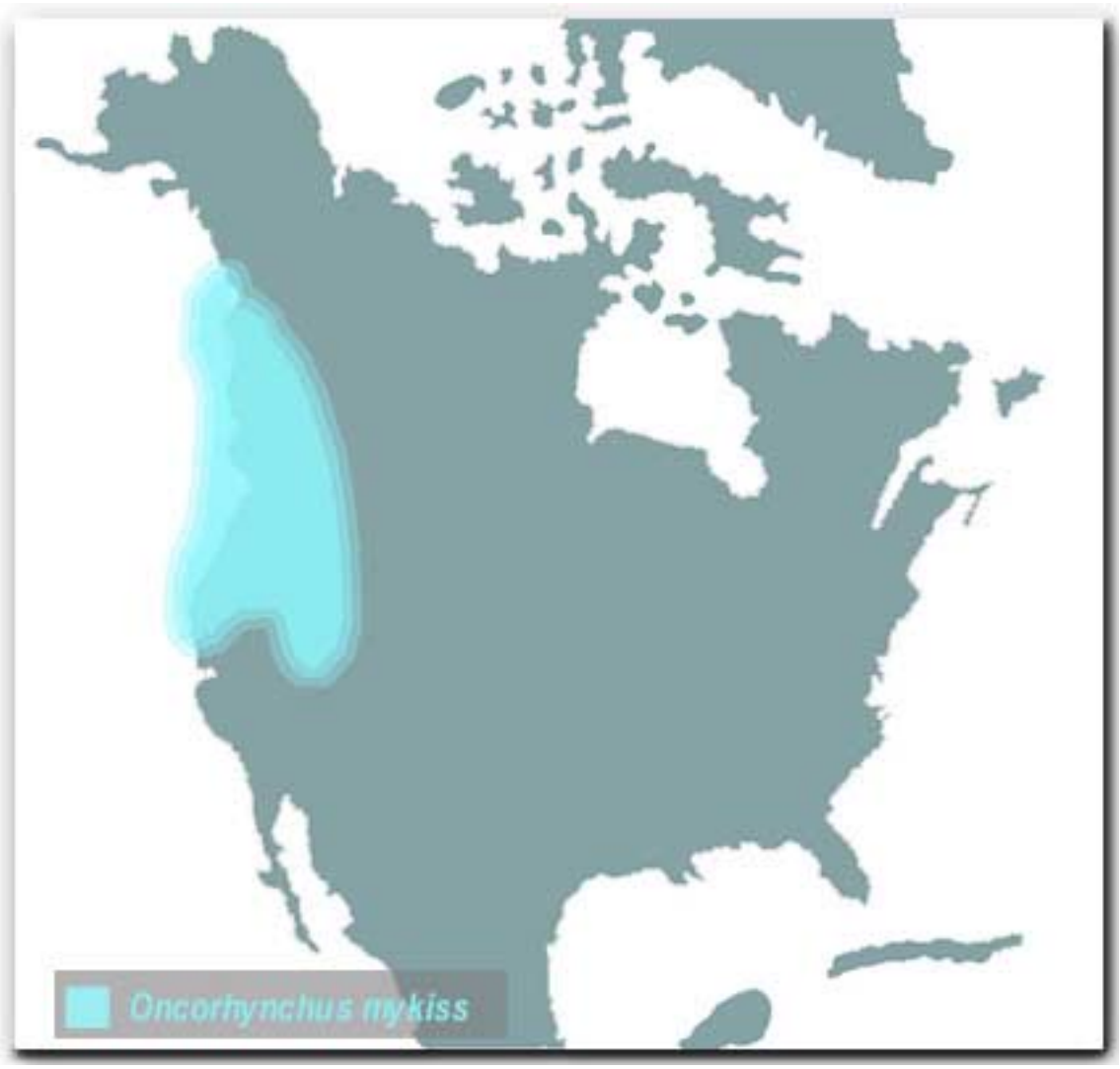
Table 12: List of predators of *Salmo salar*, *Oncorhynchus mykiss* and *Salmo trutta*

	Freshwater environment	**Marine environment**
Leech	(*Piscicola* sp.)	
Insects	Larvae of Dragon-fly	
Crayfish	several species of Crayfish	
Carnivorous fish	Salmonids	Salmonids
	Millers thumb (*Cottus gobio*)	Sharks
	Burbot (*Lota lota*)	Conger (*Conger conger*)
	Eel (*Anguilla anguilla*)	Cod (*Gadus morhua*)
	Perch (*Perca fluviatilis*)	Haddock (*Melanogrammus* sp.)
	Pike (*Esox lucius*)	Pollack (*Pollachius virens*)
	Pike-perch (*Sander lucioperca*)	*Lophius piscatoris*
	carnivorous Cyprinids	Sea lamprey (*Petromyzon marinus*)
	Sea lamprey (*Petromyzon marinus*)	
Birds	Heron	Gannet
	Cormorant	Cormorant
	Black kite	Fulmar
	Osprey	Great Skua
	White-tailed eagle	Seagulls
	Goosander, Merganser	Guillemot
	Kingfisher	
Mammals	Otter	Seals
	(Racoon)	Dolphins

Table 13: List of pathogenes and diseases of *Salmo salar*, *Oncorhynchus mykiss* and *Salmo trutta*

	Causative organism
Viral diseases	
Infectious Pancreatic Necrosis (IPN)	virus
Infectious Haematopoietic Necrosis (IHN)	virus
Viral Haemorrhagic Septicaemia (VHS)	virus
Infectious Salmon Anaemia (ISA)	virus
Sleeping Disease (SD)	virus
Bacterial diseases	
Furunculosis	Aeromonas salmonicida
Furunculosis	Aeromonas liquifaciens
Enteric Redmouth Disease (ERM)	Yersinia ruckeri
Vibrosis	Vibrio anguillarum
Bacterial Kidney Disease/BKD	Corynebacterium ssp.
Rainbow Trout Fry Syndrome (RTFS)	Cytophaga psychrophila
Bacterial Cold Water Disease (CWD)	Cytophaga psychrophila
Bacterial Gill Diesase	Myxobacterium
Proliferate Kidney Disease (PKD)	Tetracapsula bryosalmonae
Whirling Disease (WD)	Myxobolus cerebralis
Costiasis	Costia necatrix
Hexamitaisis	Hexamita truttae
White Spot Disease	Ichthyophthirius multifilis
Costia	Ichthyobodo necator
Hexamita	Octomitus salmonis
	Chilodonella sp.
	Trichodina sp.
	Glosatella sp. /Apiosoma sp.
	Gyrodactylus ssp.
	Dactylogyrus sp.
Eyefluke	Diplostomum spataceum
	Acanthocephalus sp.
Sea louse	Lepeophtheirus sp.
Sea louse	Caligus sp.
Fish louse	Argulus sp.
	Ergasilus sp.
Leech	Piscicola sp.
Fungus	Saprolegnia ssp.
Algae/Dinoflagellates	several species

Figure 1: Native range of *Oncorhynchus mykiss* Wal.

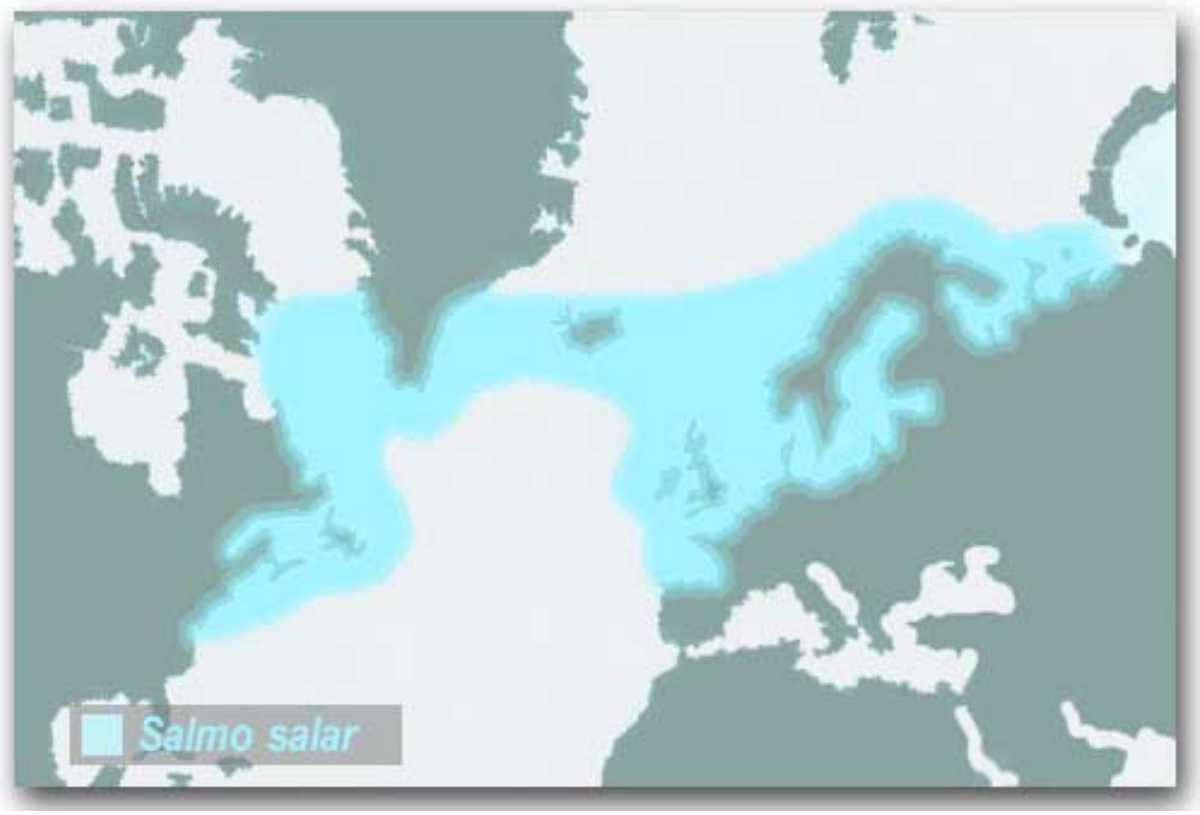


(according to Muus & Dahlström 1978, revised by Pätzold)

Figure 2: Native range of *Salmo trutta* L. in freshwater

(according to Muus & Dahlström 1978, revised by Pätzold)

Figure 3: Native range of *Salmo salar* L.



(according to Muus & Dahlström 1978, revised by Pätzold)

8. Zusammenfassung

Im Laufe des letzten Jahrhunderts ist der weltweite Bedarf an Fisch als wichtige Eiweißquelle für den Menschen stetig gestiegen. Seit Mitte der achtziger Jahre des letzten Jahrhunderts wird deshalb versucht, diesen Bedarf durch eine intensive Fischproduktion in Aquakulturen zu befriedigen. Heutzutage stammen bereits rund 26% der jährlich verzehrten Speisefischmenge aus Aquakulturen. Laut Schätzungen der Welternährungsorganisation FAO sind 60% der weltweit wertvollsten Fischbestände überfischt oder werden bis an die Grenzen überfischt. Es ist abzusehen, dass die Fischfangmengen künftig sinken werden. Eine Möglichkeit die prognostizierten sinkenden Fischfangmengen auszugleichen und die Ausbeutung von wilden Fischpopulationen einzuschränken, besteht darin die Produktivität von Aquakulturen zu steigern.

Da gentechnische Manipulationen an Fischen im Vergleich zu anderen Vertebraten vergleichsweise einfach durchzuführen sind, besteht schon seit über zehn Jahren die Idee, die Produktivität von Aquakulturen durch den Einsatz von gentechnisch veränderten Fischlinien zu steigern.

In den letzten zwanzig Jahren ist intensiv auf dem Gebiet der gentechnischen Forschung an Fischen gearbeitet worden. Seit der ersten Publikation über die erfolgreiche gentechnische Veränderung von Fischen, die 1985 veröffentlicht wurde, sind beachtliche Fortschritte im Bereich der Methodenentwicklung für den Transfer und hinsichtlich der Herstellung von Genkonstrukten gemacht worden. 35 verschiedene Fischarten waren bereits bis heute das Ziel gentechnischer Modifikationen. Die Entwicklung bestimmter transgener Fischlinien hat mittlerweile ein Stadium erreicht, in dem eine kommerzielle Nutzung dieser Linien Möglichkeit geworden scheint.

In den neunziger Jahren des letzten Jahrhunderts lag der Schwerpunkt in der Entwicklung von kommerziell bedeutenden transgenen Fischlinien darauf, Linien zu entwickeln, die ein gesteigertes Größenwachstum besitzen. Im Jahr 2001 hat das Europäische Patentamt schließlich das erste Patent für eine transgene Fischlinie vergeben, die um ein Vielfaches schneller als ihre nicht-transgenen Artgenossen wächst. Die kanadische Firma Seabright erhielt das Patent EP 0578 653 B1 für ein Verfahren, Atlantischen Lachsen und anderen Fischarten das Genkonstrukt opAFPghc einzubauen - ein Genkonstrukt, das für ein zusätzliches Wachstumshormon kodiert. Eine private US-kanadische Firma hat bereits eine Zulassung für die kommerzielle Züchtung und Vermarktung dieser schnellwachsenden Lachse in den USA, Kanada und Chile beantragt.

Weitere Zielsetzungen, die in der biotechnologischen Forschung bei Fischen verfolgt werden, sind die Entwicklung von transgenen krankheitsresistenten oder kältetoleranten

Linien, Linien mit einer erhöhten Toleranz gegenüber Schadstoffen oder mit einer veränderten Fleischqualität (z.B. einem höheren Proteingehalt), sterile Linien und die Entwicklung von Linien, die sich als Monitororganismen für das Vorhandensein bestimmter Mutagene oder Schadstoffe in Gewässern eignen. Umfangreiche Grundlagenforschung auf dem Gebiet der Biotechnologie, wie die Identifizierung und Charakterisierung von Genen, die Verbesserung von Gentransfermethoden und Genexpression wird von sehr vielen verschiedenen chinesischen, japanischen und einzelnen US-amerikanischen Arbeitsgruppen betrieben, wie z.B. von der Arbeitsgruppe um Thomas Chen (University of Conneticut, USA). Verschiedene Arbeitsgruppen in Kanada, den USA und Kuba betreiben verstärkt angewandte Forschung. Choy Hew (University of Toronto, Kanada) und Garth Fletcher (Memorial University of Newfoundland, Kanada) arbeiten an der Entwicklung von kältetoleranten Linien und an Linien mit einem verbesserten Größenwachstum. Robert Devlin (West Vancouver Laboratory, Kanada) arbeitet hauptsächlich ebenfalls an Linien mit einem verbesserten Größenwachstum sowie an der Entwicklung von krankheits-resistenten Linien. Auch die Arbeitsgruppe um Thomas Chen (University of Conneticut, USA) beschäftigt sich mit der Entwicklung von transgenen Linien, die gegenüber bestimmten Krankheiten resistent sind. In Kuba arbeitet eine Arbeitsgruppe um José de la Fuente und Isabel Guillén (beide am Centro de Ingenieria Genética y Biotecnologia, Havanna, Kuba) an transgenen Tilapien, die ein verbessertes Größenwachstum aufweisen. Verschiedene europäische Arbeitsgruppen, u.a. die Arbeitsgruppen um Norman Maclean (University of Southampton, Großbritannien), P. Aleström (Norwegian College of Veterinary Medecine, Oslo, Norwegen), Bernard Breton (INRA Rennes, Frankreich) und Manfred Schartl (Universität Würzburg, Deutschland), arbeiten an der Entwicklung von sterilen Fischlinien. Forschung zu potentiellen ökologischen Risiken der Nutzung von transgenen Fischen wird vor allem in den USA durch die Arbeitsgruppen um Anne Kapuscinski (University of Minnesota, USA), William Muir und Richard Howard (beide Purdue University, USA) betrieben.

Weltweit betrachtet spielen in der Aquakultur-Fischproduktion vor allem die beiden Arten Atlantischer Lachs (*Salmo salar* L.) und Regenbogenforelle (*Oncorhynchus mykiss* Wal.) eine bedeutende Rolle. Diese beiden Arten dominieren derzeit die Produktion in marinen Aquakulturen. Die Entwicklung transgener Linien dieser beiden Salmoniden-Arten ist deshalb bislang immer eines der Hauptziele in der angewandten biotechnologischen Forschung an Fischen gewesen. Über die Hälfte der Forschung in diesem Bereich befasste sich damit, dass Wachstum dieser beiden Arten zu manipulieren. Die ersten transgenen Linien sind nun marktreif. Wachstumssteigerungen sind in der Regel erzielt worden, indem Genkonstrukte, die für ein zusätzliches Wachstumshormon kodieren in befruchtete Fischeier transferiert wurden. Heutzutage werden für solche gentechnischen Manipulationen an

Fischen Gene und Regulationselemente verwendet, die aus dem Genom von anderen Fischarten, wie z.B. aus dem Genom des Pazifischen Lachs (*Oncorhynchus kisutch*) oder aus dem Quinnat (*Oncorhynchus tshawytscha*), stammen. Bei der Regenbogenforelle ist auch versucht worden, die Effizienz der Futterverwertung zu steigern, indem Gene transferiert wurden, die aus dem menschlichen und Ratten-Genom stammen. Diese Gene kodierten für bestimmte Stoffwechselenzyme.

Im Rahmen der Forschung zur Erhöhung der Kältetoleranz bestimmter Salmoniden-Arten hat man bislang versucht, Gene, die man aus der Amerikanischen Winterflunder (*Pleuronectes americanus*) isoliert hat und die für bestimmte Antifrostproteine kodieren, in den Atlantischen Lachs zu transferieren. Die Entwicklung von solchen kältetoleranten Lachslinien hat allerdings noch nicht das Stadium einer kommerziellen Nutzung erreicht.

Weitere Zielsetzungen, die bei der Entwicklung von transgenen Lachs- und Forellenlinien verfolgt werden, ist die Erhöhung der Krankheitsresistenz und die Etablierung von transgenen sterilen Populationen. Erste Versuche auf dem Gebiet der Entwicklung solcher Linien sind unternommen worden, allerdings ist im Hinblick auf die Realisierung dieser Ziele noch viel Grundlagenforschung zu leisten.

Die wissenschaftliche biologische Sicherheitsforschung zur potentiellen kommerziellen Nutzung von transgenen Fischen steht noch in ihren Anfängen. Die ersten Daten über potentielle negative Umwelteinflüsse, die eine Freisetzung von transgenen Fischen mit sich bringen könnte, liegen vor. Ebenfalls liegen eine Reihe von Daten vor, die die Gesundheit transgener Tiere betrifft. Nachteilige Auswirkungen, die von der Freisetzung von transgenen Fischen ausgehen, konnten auf der Ebene einzelner Individuen festgestellt werden. Es gibt jedoch zahlreiche Hinweise dafür, dass auch andere Organisationsebenen (Populationen, Ökosysteme) durch die Freisetzung von transgenen Fischen beeinträchtigt werden. Hinsichtlich der potentiellen Risiken, die mit der Freisetzung von transgenen Fischen verbunden sind, müssen folgende drei Hauptaspekte beachtet werden:

Erstens, genetische Modifikationen können mit einer Reihe von ungewollten Nebenwirkungen, wie z.B. Schädel- und Körperdeformationen, abnormen Kiemenwachstum oder verändertes Fraßverhalten, verbunden sein. Alle diese Nebenwirkungen sind bei transgenen Lachsen und Forellen beobachtet worden.

Zweitens, bislang lässt sich noch nicht garantieren, dass die Expression der transferierten Genkonstrukte stabil bleibt. Obwohl in den letzten Jahren zahlreiche Fortschritte in der Methodenentwicklungen gemacht wurden, gehören niedrige Integrationsraten und die Instabilität der Genexpression immer noch zu den ungelösten Problemen auf dem Gebiet der Fischbiotechnologie. In bestimmten Fällen, wie z.B. der Produktion von transgenen sterilen Populationen als eine Sicherheitsmaßnahme, um die

Hybridisierung mit Individuen aus Wildpopulationen zu verhindern, stellt eine solche Instabilität der Genexpression ein erhebliches Sicherheitsrisiko dar.

Als letzten Punkt muss im Zusammenhang mit den potentiellen Risiken einer Freisetzung von transgenen Fischen die Auskreuzung genannt werden. Lachse oder Regenbogenforellen, die aus Aquakulturen entweichen, sind in der Lage sich mit Individuen aus Wildpopulationen derselben Art zu paaren. Der Atlantische Lachs (*Salmo salar* L.) ist außerdem in der Lage, sich mit der Bachforelle (*Salmo trutta* L.) zu paaren. Auskreuzung und Hybridisierung können schließlich zu einer genetischen Kontamination von Wildpopulationen mit transgenen Genkonstrukten führen. Eine solche genetische Kontamination kann wiederum zahlreiche nachteilige Effekte auf die betroffenen Wildpopulationen haben und dem gesamten Ökosystem Schaden zuführen. Als prägnantes Beispiel lassen sich die potentiellen Auswirkungen der Ausbreitung von Kältetoleranzgenen in Wildpopulationen nennen. Solche Gene können es ermöglichen, dass Fischarten, in die diese Gene eingekreuzt wurden, in kältere Klimazonen vordringen, in denen sie bislang nicht heimisch waren. Eine solche Situation ist vergleichbar mit der Einführung einer exotischen Art in ein bestimmtes Gebiet, die unter Umständen die Auslöschung einer anderen, in dem Gebiet heimischen Art nach sich ziehen kann, wenn die exotische Art z.B. die gleiche Nahrungsnische besetzt, aber konkurrenzkräftiger als die im Gebiet heimische Art ist. Ein weiteres, ebenfalls ökologisch bedeutendes Beispiel ist das veränderte Sexualverhalten von transgenen Fischen, deren Hormonproduktion durch den Transfer eines zusätzlichen Wachstumshormongens verändert worden ist. Größere männliche Tiere besitzen bei manchen Fischarten häufig einen Paarungsvorteil gegenüber kleineren. Dies ist zum Beispiel auch beim Atlantischen Lachs der Fall.

Insgesamt betrachtet sind die potentiellen ökologischen Risiken, die mit der Freisetzung von transgenen Fischen verbunden sind, bislang nur wenig untersucht worden. In den USA haben hierzu in den letzten sieben Jahren zwei Wissenschaftler, William Muir und Richard Howard, von der Purdue Universität eine Methode entwickelt, mit Hilfe derer das Risiko des Genflusses von aus Aquakulturen entkommenen Fischen und Individuen aus nah verwandten Wildpopulationen abgeschätzt werden kann. Mit Hilfe dieser Methode werden die populationsgenetischen Veränderungen, die durch die Auskreuzung von entwichenen Fischen in Gang gesetzt werden, modelliert. Das Model integriert Daten zu bestimmten sogenannten “Fitnesskomponenten“, so dass eine Aussage zum zu erwartenden Genfluss gemacht werden kann.

Dass Fische aus ihren Aquakultur-Anlagen entweichen können, ist Realität. In den letzten Jahren konnten weltweit betrachtet zahlreiche Kultur-Lachse in freie Gewässer entkommen. Immer wieder wird von Massenausbrüchen berichtet. Diese entkommenen

Kultur-Lachse stellen ein erhebliches ökologisches Risiko dar, da sie die genetische Diversität von Wildlachspopulationen gefährden. Aus technischen Gründen ist es nicht möglich, marine Aquakultur-Halterungsanlagen so zu gestalten, dass sie 100% ausbruchsicher sind. Deshalb wurde im Rahmen der sogenannten *Bergen Deklaration*, einer Deklaration der Umweltminister der Nordseeanrainerstaaten, die im Rahmen der 5. Internationalen Nordseeschutzkonferenz im März 2002 verabschiedet wurde, vorgeschlagen, dass transgene Fische nur in ausbruchssicheren, nicht-marinen, auf dem Land gelegenen Aquakultur-Anlagen gehalten werden sollten. Diese Maßnahme soll verhindern, dass transgene Fische in das freie Meer gelangen. Weder die Kosten, noch die Umweltauswirkungen solcher auf dem Land gelegenen Anlagen sind bisher evaluiert worden, weshalb keine Aussagen über die Wirtschaftlichkeit derartiger Anlagen gemacht werden können.

Eine weitere Möglichkeit, die Ausbreitung von transgenen Genkonstrukten in Wildpopulationen zu vermeiden, wird im sogenannten biologischen "Containment“ gesehen. Das heißt mit Hilfe biologischer Maßnahmen soll eine potentielle genetische Kontamination verhindert werden. Im Rahmen eines biologischen Containments ist vorgesehen, dass in der Aquakultur-Produktion nur sterile Populationen herangezogen werden. Bislang werden sterile Populationen mit Hilfe der Polyploidisierung des Genoms aufgebaut. Allerdings kann die Sicherheit dieser Methode bislang nicht gewährleistet werden. Ein neuer Ansatz zur Herstellung steriler Populationen beinhaltet, dass das Heranreifen der Tiere zur Geschlechtsreife mit Hilfe gentechnischer Methoden verhindert wird. Diese Strategie beruht auf der Tatsache, dass mit Hilfe eines Antisense-Genkonstruktes die Produktion des Sexualhormons Gonadotropin verhindert wird. Erste Versuche waren zum Teil erfolgreich. Das Problem der Instabilität der Genexpression ist aber bislang noch nicht erfolgreich gelöst worden, so dass die Methode im Hinblick auf ihre praktische Anwendung noch verbessert werden muss.

Bezüglich einer kommerziellen Nutzung von transgenen Fischen ist angesichts der mit ihnen verbundenen potentiellen und nicht ausreichend erfassten Risiken festzustellen, dass zum einen ein großer Bedarf an weiterer Sicherheitsforschung besteht, und zum anderen eine staatenübergreifende Einigung hinsichtlich des Umgangs mit den vorhanden Risiken erfolgen sollte. Weiterer Forschungsbedarf besteht auch in Hinsicht auf die potentiellen nachteiligen Auswirkungen von aus Aquakulturen entwichenen transgenen Fischen auf Wildpopulationen. Außerdem besteht angesichts einer bevorstehenden kommerziellen Nutzung der dringende Bedarf, Konzepte für ein Monitoring der potentiellen nachteiligen Auswirkungen von transgenen Fischen zu entwickeln.

Angesichts der Tatsache, dass eine potentielle kommerzielle Nutzung transgener Fischlinien sowohl eine Reihe von Vorteilen besitzen kann, andererseits aber auch große Bedenken hinsichtlich der Risiken einer solchen Nutzung bestehen, haben sich inzwischen eine ganze Reihe von internationalen Organisationen (wie z.B. die FAO, die OECD, die EU, das "Network of Aquaculture Centres in Asia-Pacific“ oder die Internationale Nordseeschutzkonferenz), eine Reihe von einzelnen Nationalstaaten, eine stetig wachsende Zahl von Wissenschaftlern sowie verschiedene Umwelt- und Verbraucherorganisationen und einige industrielle Interessenverbände in die Diskussion um die Anwendung dieser Technologie eingeschaltet.

Angesichts der potentiellen Konsequenzen, die aus der Nutzung von gentechnisch veränderten Fischlinien in Aquakulturen folgen können, ergibt sich zwangsläufig, dass ein großer Bedarf darin besteht, dass auf internationaler Ebene eine Abgleichung von einzelnen nationalen und multinationalen Regelungen hinsichtlich dieser Nutzung stattfindet. Dabei müssen auch internationale Handelsregelungen und die Frage, wie das Betreiben von Aquakulturen in internationalen Gewässern geregelt werden soll, miteinbezogen werden. Nationale Grenzen stellen für Fische, auch für transgene Fische, häufig kein großes Hindernis dar. Auch dieser Punkt muss in internationalen Diskussionen berücksichtigt werden. Angesichts der potentiellen negativen Auswirkungen von transgenen Fischen müssen internationale Vereinbarungen über das Vorgehen in einem Schadensfall getroffen werden.

Entscheidungen, die im Rahmen laufender Zulassungsverfahren von transgenen Organismen getroffen werden, sollten auf einer möglichst breiten Basis an technischen Informationen - inklusive einer breiten Auswahl von biologischen Basisdaten – beruhen.

Dieses Gutachten liefert biologische Basisdaten für die drei kommerziell besonders interessanten Salmoniden *Salmo salar* L. (Atlantischer Lachs), *Oncorhynchus mykiss* Wal. (Regenbogenforelle) und *Salmo trutta* L. (Bachforelle). Die zusammengestellten Daten beinhalten Informationen zur Morphologie, taxonomischen Status, Reproduktionsbiologie, Ökologie, genetische Struktur und Variabilität, Maßnahmen zur Erhaltung der genetischen Vielfalt, Kreuzbarkeit, Ursprungszentren der Arten und Evolutionsgeschichte, natürliche Verbreitung, Domestizierung, Züchtung, Haltungspraktiken, Parasiten, Krankheiten, Verwendung, wirtschaftliche Bedeutung und vorgenommene genetische Modifikationen.

Die drei im Rahmen dieses Gutachtens behandelten Salmoniden-Arten zeichnen sich durch eine erstaunlich hohe große genetische Variabilität aus. Dieser Befund wird durch zahlreiche molekulare Daten aus verschiedenen wissenschaftlichen Studien gestützt. Alle drei Arten zeichnen sich durch signifikante genetische Unterschiede innerhalb der eigenen Art aus. Genetische Unterschiede bestehen sowohl zwischen Populationen aus

verschiedenen Regionen als auch zwischen Populationen aus verschiedenen Nebenflüssen eines individuellen Flusseinzugsgebietes, als auch sogar zwischen unterschiedlichen Flussabschnitten eines Flusses. Innerhalb einer Art bestehen zum Teil auch deutlich erkennbare morphologische und verhaltensökologische Unterschiede, die sich vermutlich aufgrund der Anpassung an spezifische lokale Bedingungen herausgebildet haben. Angesichts der Problematik der Erhaltung der genetischen Vielfalt dieser drei Arten, erscheint es notwendig, dass über das gesamte jeweilige Verbreitungsgebiet stabile Populationen der jeweiligen Art identifiziert werden, die als Genreservoir für die jeweilige Art dienen können. Im Rahmen von weiteren Maßnahmen, die dem Erhalt der drei Arten dienen, sollte dieser Aspekt unbedingt berücksichtigt werden und auch in weitere Forschungsaktivitäten miteinbezogen werden.