GENETICALLY ENGINEERED FISH - NEW THREATS TO THE ENVIRONMENT

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GENETICALLY ENGINEERED FISH - NEW THREATS TO THE ENVIRONMENT PUBLISHED OCTOBER 2004



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### GREENPEACE

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#### summary

This briefing examines the development of genetically engineered (GE) fish, which could soon be produced on a commercial scale.

There are many GE fish under development, often engineered with growth hormones to make the GE fish grow faster enabling them to reach marketable size at an earlier age.

- \* There are numerous concerns over the welfare and environmental impact of these unhealthy, fast growing fish.
- \* The techniques of producing GE fish are crude, and generally involve the random insertion of DNA into the fish genome. This process may disrupt the tightly controlled network of DNA in the fish.

Current understanding of the way in which genes are regulated is extremely limited, and any change to the DNA of an organism at any point may well have effects that are impossible to predict or control. There is already concern regarding environmental effects, human health impacts and welfare of intensive fish farming in aquaculture. Fish are known to escape frequently from aquaculture facilities, which can then interbreed with, or displace, native fish populations. There is a high potential for ecological disruption should GE fish escape from aquaculture facilities. Fast growing GE fish could compete for food, disrupting aquatic food webs and ecosystems.

- Researchers have shown that GE fish with a growth hormone gene could have a mating advantage due to their increased size. An experimental study was developed (called the 'Trojan gene' model), which demonstrated that the release of just 60 GE fish could lead to an extinction of a wild population within only 40 generations.
- \* Contrary to industry claims, sterilisation of GE fish will not be 100 % effective in a commercial situation, and will not prevent all crossbreeding between GE fish and wild fish.

There is widespread opposition to the farming of GE fish in aquaculture facilities, the most economically profitable method of rearing farmed fish. Several governments and intergovernmental organisations have already taken steps to ensure that any GE fish (e.g. for scientific research purposes) are kept in secure, land-based facilities.

However, escaped GE fish will not respect national boundaries, GE fish must be considered as global releases by the multilateral environmental agreement, the UN Cartagena Protocol on Biosafety.

 In a commercial aquaculture situation, the physical containment of these fish can never be guaranteed. Escapes of GE fish into the aquatic environment could have devastating effects on wild fish populations and biodiversity.

Therefore, Greenpeace demands that the genetic engineering of fish for commercial purposes should be prohibited.

GENETICALLY ENGINEERED FISH - NEW THREATS TO THE ENVIRONMENT INTRODUCTION

### introduction

Greenpeace first published a report in 2000 on the hazards of genetically engineered fish.<sup>1</sup> This report is an update, presenting the new scientific findings on threats to the environment of genetically engineered fish.

The first genetically engineered (GE), also called genetically modified (GM) or transgenic, fish was reported in China in 1985. Since then, experiments have been conducted with over 35 different kinds of GE fish in about 50 laboratories around the world.<sup>2</sup> Most of the fish being engineered are varieties important to the aquaculture industry, such as salmon, carp, trout, catfish, and tilapia. Genes introduced into GE fish have come from a wide range of sources, including other fish, chickens, humans, cattle and rats.

Some GE fish are used in laboratory research (e.g. for the study of gene regulation and function) and some have been developed as 'drug factories', their bodies engineered to contain products that are useful to the pharmaceutical industry. **However, much of the research into GE fish has focused on the development of traits such as rapid growth that are valued by companies involved in intensive fish farming.** 



TABLE 1: EXAMPLES OF GE FISH BEING DEVELOPED <sup>3</sup>			
SPECIES*	FOREIGN GENE	DESIRED EFFECT	COUNTRY**
atlantic salmon	Antifreeze promoter gene from ocean pout, growth hormone gene from chinook salmon	Increased growth and feed efficiency	United States, Canada
rainbow trout	Growth hormone gene from sockeye salmon	Increased growth and feed efficiency	United States, Canada
goldfish	Antifreeze gene	Cold tolerance	China, Canada
tilapia	Human gene for clotting factor VII	Production of clotting factor for pharmaceutical production	United States, United Kingdom
tilapia	Tilapia growth hormone gene and promoter sequence from human cytomegalovirus	Increased growth	Cuba
tilapia	Modified tilapia insulin-producing gene	Production of human insulin	Canada
salmon	Lysosome gene from rainbow trout and pleurocidin gene from flounder	Disease resistance	United States, Canada
zebra fish	Green fluorescing protein gene from jellyfish, red fluorescing protein gene taken from Discosoma (Indo Pacific relative of sea anemones and coral)	The GloFish are marketed as pets and designed to glow in fish tanks	Singapore. Sold commercially as pets in United States and Taiwan
channel catfish	Growth hormone gene from salmon, cecropin genes from moth	Increased growth, non-specific bacterial immunity	United States
striped bass	Insect genes	Disease resistance	United States
mud loach	Mud loach growth hormone gene, promoter genes from mud loach and mouse	Increased growth and feed efficiency; 2 to 30-fold increase in growth	China
common carp	Growth hormone genes from salmon and human	150% growth improvement in culture conditions, improved disease resistance, tolerance of low oxygen level	China, United States
grass carp	Human interferon gene	Disease resistance	China
indian major carps	Human growth hormone gene	Increased growth	India
abalone	Growth hormone gene from coho salmon	Increased growth	United States
oyster	Growth hormone gene from coho salmon	Increased growth	United States

\* OTHER FISH THAT HAVE BEEN GE INCLUDE ARCTIC CHAR, AFRICAN CATFISH, INDIAN CATFISH, BLUNTNOSE BREAM, BROWN TROUT, GILTHEAD BREAM, BLACKHEAD BREAM, WUCHANG BREAM, CHINOOK SALMON, COHO SALMON, KILLIFISH, LARGEMOUTH BASS, JAPANESE MEDAKA, MUD CARP, MUMMICHOG, SILVER CRUCIAN CARP, RED CRUCIAN CARP, LOACH, NORTHERN PIKE, PENAEID SHRIMP, SEA BREAM, STRIPED BASS AND WALLEYE.<sup>4</sup> \*\* COUNTRIES WITH LABORATORIES INVOLVED IN GE FISH RESEARCH BETWEEN 1996-2003 INCLUDE THE UNITED STATES, CANADA, CUBA, INDIA, SINGAPORE, SOUTH KOREA, JAPAN, CHINA, ISRAEL, SCOTLAND, ENGLAND, FINLAND, THE NETHERLANDS, HUNGARY, NEW ZEALAND AND TAIWAN.<sup>5</sup>

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### I GENETIC ENGINEERING OF FISH - CRUDE SCIENCE

There are several ways of producing GE fish, all of which are crude and can lead to unexpected and unintended effects. One way to make GE fish is by genetically altering the sperm of male fish. There are a number of methods to do this, including:

- \* Attaching the new genes to viruses which then 'smuggle' these genes into the sperm,
- Using an electric shock to create pores in the sperms' cell membrane, and then introducing the genes into the sperm.<sup>6</sup>

However, by far the most commonly employed method used to engineer fish is by microinjection,  $^{\rm 7}$  where:

- \* Scientists make billions of copies of the DNA that they want to end up in the GE fish by inserting the genes into a piece of DNA called a plasmid that can be reproduced inside bacteria in the laboratory.
- \* The genes are then isolated and millions of copies are injected with a very fine needle into fertilised fish eggs as soon as possible after fertilisation (usually at the one or two cell stage). A piece of DNA called a 'promoter' is also inserted along with the 'new' gene in order to 'switch it on' in its new host. Promoters, that often force genes to express their traits at very high levels, also have the potential to influence other genes in the organism.<sup>8</sup>
- \* Some of these eggs will have successfully incorporated the new genes, and some of the fish that hatch will therefore be GE. As it is not possible to insert a new gene with any accuracy, the gene transfer may also disrupt the tightly controlled network of DNA in the fish. Current understanding of the way in which genes are regulated is extremely limited, and any change to the DNA of an organism at any point may well have effects that are impossible to predict or control. The new gene could, for example, alter chemical reactions within the cell or disturb cell functions. This could lead to changes in behaviour, instability, and the creation of novel substances in the fish such as new toxins or allergens or changes in nutritional value.<sup>9</sup>

An example of unexpected effects in GE fish:

When GE coho salmon were compared to a control group of non-GE coho salmon, it was found that the genetic engineering process had affected the activity of a number of non-target genes in the GE fish. These changes included an increased amount of the protein parvalbumin-b in the GE salmon, a protein that has been identified as a major food allergen in fish.<sup>11</sup>

For further examples of unexpected effects in GE fish, see Section iv ii "Unexpected effects and Trojan genes".

### II FAST GROWING, UNHEALTHY GE FISH

Most aquaculture-related research on GE fish has focused on increasing the growth rates of farmed fish so that they reach marketable size more quickly.<sup>12</sup> Growth hormone genes taken from humans, other mammals or fish have been engineered into at least 15 species of fish.<sup>13</sup>

- \* In one experiment, Atlantic salmon were engineered with growth hormones to enable them to reach adult size more quickly. After one year, most had a two- to six-fold increase in growth, whilst the largest was 13 times normal size for its age.<sup>14</sup>
- \* A company called Aqua Bounty Farms (a division of A/F Protein) is currently seeking approval in the United States to commercialise a GE Atlantic salmon. This salmon has been engineered with an extra gene for growth hormone production taken from a chinook salmon and a 'promoter' gene which forces the salmon to produce high levels of the growth hormone all year round. This means that instead of only growing during the spring and summer months, the GE salmon grows constantly, reaching adult size in about 18 months. This compares to 24-30 months for a non-GE salmon.<sup>15</sup>
- \* One study noted that when growth hormone is engineered into salmon, "the endocrine stimulation can be elevated to pathological levels in some cases", such as abnormally enlarged skulls in the GE fish, leading to difficulty in respiration and feeding.<sup>16</sup> Other studies have reported that GE carp have suffered from similar abnormalities.<sup>17</sup>

In summary, forced expression of the growth hormone in GE fish raises a number of animal welfare concerns.



### **III CURRENT PROBLEMS WITH AQUACULTURE**

GE fish are to be introduced within the context of intensive fish farming. In order to begin to understand the hazards and risks posed by GE fish, it is first necessary to examine the many problems that already exist within current aquaculture systems.

According to the Food and Agriculture Organization of the United Nations, global demand for seafood may double by 2040.<sup>10</sup> With wild fisheries suffering major declines due to over-fishing and habitat destruction, it is widely suggested that the projected shortfalls in fish supply will be met by expansion within the aquaculture sector.<sup>19</sup>

Since 1987, global aquaculture production has more than doubled. By 1995, it accounted for 18.5% of the total world seafood supply.<sup>20</sup> More than 160 species of finfish and shellfish are currently farmed, including catfish, trout, salmon, tilapia, oysters, crayfish, clams and shrimp.<sup>21</sup>

Aquaculture has a long history – fish farming has been integrated into diverse sustainable farming practices in Asia for at least 2000 years. Modern intensive aquaculture operations, however, are often grossly inefficient, damaging to the environment and raise many welfare concerns.

One of the first kinds of GE fish expected to reach commercial approval is salmon.

### In salmon farming:

- \* The fish are kept in net pens, which are tethered in coastal waters. The fish are so densely stocked that ideal conditions are created for disease. These diseases race rapidly through the crowded fish pens, and in order for the salmon to survive they are fed antibiotics, and dosed with pesticides. The pesticides, uneaten feed and wastes from the fish smother and pollute the sea floor beneath the fish farms, affecting bottom dwelling organisms.<sup>22</sup>
- \* The salmon often incubate sea lice and other parasites, which then spread to wild salmon populations. In Norway, parasites from fish farms were so devastating to wild fish that the government made the decision to poison all aquatic life in dozens of rivers and streams in the hope that disease-free populations could then be restored.<sup>23</sup>

- \* Farmed salmon are fed synthetic pigments in order to give their flesh the pink colour normally associated with salmon. Without this pigment, the flesh of these salmon would be an unhealthy looking pale grey.<sup>24</sup>
- \* Farmed salmon accumulate cancer-causing chemicals in their bodies called polychlorinated biphenols (PCBs) from the fishmeal they are fed. On average, farmed salmon have 16 times the PCBs found in wild salmon. PCBs not only cause cancer but also present other health risks as well. These include neurodevelopmental risks to unborn children from maternal consumption of PCB-contaminated fish.<sup>25</sup>

It is often suggested that fish farming will compensate for the collapse of wild fisheries and is a means to provide more animal protein to hungry people. Most intensive fish farming operations are, however, extremely inefficient and require more protein in the form of fishmeal to feed the farmed fish than is supplied by the end harvest. The fishmeal is commonly made with wild-caught fish such as anchovy, jack mackerel, pilchard, capelin, menhaden, herring, and sardines.

 $\ast$  It can take three to five pounds of fishmeal to produce a single pound of farmed salmon.  $^{\rm 26}$ 

In summary, modern intensive aquaculture operations are often grossly inefficient, damaging to the environment, and raise animal welfare concerns.

#### i fish escape from fish farms

In commercial aquaculture, fish are farmed in pens in contact with aquatic systems for practical and economic reasons. However, fish frequently escape in large numbers from fish farms. These escapes can occur due to human accidents, harsh weather conditions or because of predators such as seals tearing holes in the net pens and cages the fish are kept in.

\* In 1988, a storm tore apart the moorings and nets of hundreds of sea pens along the Norwegian coast, allowing a million farmed salmon to escape.<sup>27</sup> Between 1992-1996, losses of salmon from fish farms in Norway averaged 1.3 million a year.<sup>28</sup> As a result of incidents such as these, in some parts of the country escaped fish that have bred and formed their own populations outnumber wild ones by five to one.<sup>29</sup>



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- \* Between 1994 and 1996, at least 120,000 salmon escaped from fish farms in British Columbia. About 20,000 of them escaped from a tanker truck spill on Vancouver Island.<sup>30</sup>
- \* From 1996 to 2000, almost 600,000 Atlantic salmon escaped from the net pens in Washington State waters. There is evidence that Atlantic salmon that have escaped from Washington or British Columbia fish farms are beginning to establish a breeding population off Vancouver Island.<sup>31</sup>
- \* During 1999, 255,000 salmon escaped from Scottish fish farms.<sup>32</sup>
- \* A storm in December 2000 caused steel cages containing salmon to buckle in Machias Bay in the US state of Maine, leading to the escape of 100,000 fish.<sup>33</sup>
- \* In the Magaguadavic River in New Brunswick, Canada, the number of farmed fish entering the river between 1992 and 1999 was two to eight times that of the wild salmon returning to the same river to spawn.<sup>34</sup>

Farmed salmon are genetically very uniform, bred to the needs of the industry with traits such as rapid growth or high tolerance to the crowded conditions in the fish farms. Wild salmon populations, on the other hand, are genetically diverse and "highly adapted to the specific conditions of the local river systems to which they return to spawn".<sup>35</sup>

Interbreeding between wild salmon and those that escape from farms alters the genetic makeup of the wild salmon populations.

- \* There is evidence that escaped Atlantic salmon are beginning to establish a breeding population in the Pacific, off Vancouver Island in Canada. There is much concern that these Atlantic salmon may compete for food and habitat with the fragile populations of Pacific salmon, many of which have already been listed under the Endangered Species Act.<sup>36</sup> The Washington State Hearings Board in the northwestern US has ruled that Atlantic salmon that escape from fish farms should be classified as a "pollutant".<sup>37</sup>
- \* In Maine, on the Atlantic coast of the US, Atlantic salmon have been listed under the Endangered Species Act. It is estimated that only 500 Atlantic salmon with a truly native genetic makeup remain in the wild. Escapes of farmed salmon pose a major threat to the recovery of these genetically distinct wild salmon.<sup>38</sup>

In summary, fish regularly escape from commercial aquaculture facilities and can threaten native fish populations.

### IV POTENTIAL ECOLOGICAL IMPACTS OF GE FISH

"For more than half a century theoretical biologists have been developing ideas about biological networks and hierarchies and applying them to our knowledge of molecular genetics. The task of assessing the environmental impact of a genetically engineered organism is to understand what effect, no matter how small, the genetic modification has had on every one of the characteristics relevant to the organism's interactions with members of other species (as well as the physical environment); and then to predict all of the resultant effects that may occur in the organism's ecological network. With our current, very sketchy knowledge of how complex networks behave, it is impossible to carry out this task in a satisfactory way. Our understanding of ecological networks and their evolution is insufficient for any meaningful assessment of the impacts of artificial genetic change accomplished by genetic engineering." Dr. Peter Wills, theoretical biologist."

When examining the potential ecological consequences of the release of a GE fish, it is important to keep in mind that the complexity and dynamic behaviour of biological systems means that there are fundamental limitations inherent in any risk assessment.<sup>40</sup> It is possible to make an evaluation of the behaviour of a GE fish based on any new traits it may have (e.g. engineered cold tolerance increasing the geographic range of GE fish, leading to possible invasion of any escaped fish into new ecosystems). However, experience shows us that the most devastating ecological consequences can often be completely unexpected.

#### For example:

\* The intentional introduction of the myxoma virus (causing myxomatosis) to control rabbit populations in England is one such example. As desired by those introducing it, the myxoma virus caused a dramatic decline in rabbit populations. However, it also led to a surge in the abundance of a particular species of grass on which the rabbits previously grazed. Another previously dominant species of grass was unable to compete and declined substantially, giving rise to a shortage of nesting material for a certain variety of ant. This, in turn, triggered the extinction of the already endangered Large Blue butterfly (*Maculina arion*) due to a symbiotic relationship between the ant and the butterfly.<sup>41</sup>

In summary, ecological networks are complex and poorly understood. Therefore, the ecological impact of any escapes of GE fish cannot be fully taken into account by risk assessment; the precautionary principle must be applied.<sup>42</sup>

#### i impacts on native fish populations

Escaped GE fish will probably be able to travel over considerable distances and may have novel traits that have never previously existed within a particular ecosystem. Characteristics such as increased rates of growth, cold tolerance or faster breeding cycles could give GE fish a competitive advantage over native fish and potentially lead to the displacement of native fish populations. In many cases, they may disrupt ecological systems in some or all of the same ways as non-native species that have been introduced into new habitats.<sup>43</sup>

- \* In 1954, the British colonial administration in Uganda first introduced Nile perch into Lake Victoria, with the intention to increase fish production. Within 30 years the carnivorous perch, which can grow up to 6 feet in length, had decimated the indigenous fish populations, leading to the extinction of 50% of the over 400 different species of small fish in the lake. With the loss of so many species of algae-eating fish, the waters of Lake Victoria are now frequently choked with algal blooms and oxygen levels are seriously depleted. Several species of insect, which were once kept under control by fish now gone from the lake, breed in unprecedented numbers. Also, unlike the native fish, the Nile perch do not dry well in the sun, and the wood fires needed to dry the Nile perch have increased the rate of deforestation and consequent erosion of the lake's shoreline.<sup>44</sup>
- \* Forty-four native species of fish in the United States are currently threatened or endangered by non-indigenous fish. These introduced fish have had far reaching impacts on the ecology of aquatic ecosystems and have competed with native fish for food, shelter, mates, habitat and breeding sites.<sup>45</sup> In Florida, for example, blue tilapia escaped from two aquaculture facilities and have since become established in Everglades National Park and other waterways. In a number of streams where these fish have become abundant, almost all vegetation and native fish species have disappeared.<sup>46</sup>
- \* The European zebra mussel has spread into most freshwater habitats in the eastern United States, having arrived in the Great Lakes via ballast water released from ships that travelled from Europe. Zebra mussels compete directly with native mussels, clams, and snails, and reduce food and oxygen for native fauna.<sup>47</sup> The invasive mussels also clog water intake pipes and water filtration and electric generating plants, with an estimated US\$3 billion a year spent on control and cleaning measures.<sup>46</sup>

#### COLD TOLERANCE - AN INVASIVE TRAIT?

In 1982, a scientist at Memorial University of Newfoundland in Canada accidentally froze a tank filled with a species of arctic fish called a flounder. When the tank was thawed out it was found that the fish were still alive. This led to the discovery of a gene in the flounder that produces an 'antifreeze' protein, enabling it to tolerate freezing waters.<sup>49</sup> Since then, biotech researchers have been engineering salmon with this flounder gene in an attempt to incorporate the antifreeze protein into the GE fish, in the hope that salmon farms could operate in waters that would normally be excluded because of subzero water conditions. Although researchers have managed to engineer the salmon with the antifreeze gene with some success, the antifreeze protein in the GE salmon is currently being expressed at levels that are too low to make it commercially useful for this purpose. However it is expected that the use of more powerful 'promoter' genes, taken from insects, may increase the amounts of antifreeze protein in the salmon. Increased tolerance to cold water could increase the geographic range of GE fish, leading to possible invasion of any escaped fish into new ecosystems.

In summary, escaped GE fish could disrupt natural fish populations in a similar fashion to other escaped non-native fish. However, because of their novel characteristics, they could pose additional problems as described by the "Trojan gene effect" below.

#### ii unexpected effects and trojan genes

"Unfortunately, for an exotic organism it is almost impossible to determine either the risk of species spread or hazard to the environment before introduction because of the nearly infinite number of direct and indirect biotic interactions that occur in nature." William Muir & Richard Howard, authors of the Trojan Gene Hypothesis<sup>50</sup>

Genes are engineered into fish because the genetic engineers want to confer the GE fish with a particular trait, such as rapid growth. However, due to inherent problems in the genetic engineering process, GE fish often also exhibit a range of unanticipated characteristics.<sup>51</sup>

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With salmon GE with growth hormones, for example, growth hormone genes in the GE fish have also influenced swimming ability,<sup>52</sup> feeding rates,<sup>53</sup> muscle structure and enzyme production,<sup>54</sup> disease resistance,<sup>55</sup> body morphometry,<sup>56</sup> pituitary gland structure,<sup>57</sup> life span,<sup>58</sup> larval developmental rate,<sup>59</sup> smoltification,<sup>60</sup> risk-avoidance behaviour,<sup>61</sup> cranial morphology and gill irrigation.<sup>62</sup>

These unanticipated consequences of the genetic engineering process, which are also in many cases undesired effects, add to the difficulties of assessing the ecological hazards associated with a GE fish. For example, although fish genetically engineered with a growth hormone may have characteristics such as increased size which increase their ability to compete with their wild counterparts, the genetic engineering process often leaves them with some kind of disadvantage, such as increased mortality in their offspring.<sup>63</sup>

Some scientists have argued that disadvantages, such as increased mortality in the offspring of GE fish, mean that there is little risk that GE fish will prove to be invasive or in other ways hazardous to the environment.<sup>64</sup> Researchers at Purdue University in the US, however, found that interactions between traits that would appear to offer a disadvantage and other traits in GE fish that appear to offer some kind of competitive advantage, could actually increase the hazard of GE fish to aquatic ecosystems.

The Purdue laboratory studies used GE and non-GE Japanese medaka fish as experimental models to study the potential effects of the release of a small number of GE fish into the wild. The GE fish were engineered with a human growth hormone gene and had a competitive advantage — the males were larger, making them more attractive to female mates. But the genetic engineering process had also given the GE fish a disadvantage their offspring were less likely to survive.<sup>65</sup> These data were then fed into computer models, which came up with startling results. The first study found that **the release of just sixty of these GE fish into a wild population of sixty thousand led to the extinction of both fish populations in just 40 generations.**<sup>66</sup>

"You have the very strange situation where the least fit individual in the population is getting all the matings—this is the reverse of Darwin's model," says William Muir, one of the researchers. "The sexual selection drives the gene into the population and the reduced viability drives the population to extinction."<sup>57</sup> "What surprised us was how fast a GMO mating advantage could cause a transgene to spread in a population," said Richard Howard, another of the Purdue researchers. "A population invaded by a few genetically modified individuals would become more and more transgenic, and as it did the population would get smaller and smaller. We call this the 'Trojan gene effect'."

"Imagine a pie, and you eat 30 percent of it every day," said Howard. "Half of it is gone in two days, and within a week less than one-tenth of it remains. It is conceivable that a similar effect could occur among fish populations if GMOs with 'Trojan genes' escape into the wild."<sup>56</sup>

Further research on the "Trojan gene" effect has now been published in scientific journals.<sup>69</sup> Together, this research shows that there are numerous ways in which genes engineered into GE fish could invade wild fish populations, reduce their fitness, and potentially cause their extinction.

One of the ways in which an escaped GE fish could impact on other fish within an ecosystem, and end up being invasive, is through competition for food. One study found that genetically engineered salmon with a growth hormone gene consumed 250 % more food than non-GE salmon of the same size, suggesting that they could be highly competitive. GE predatory fish such as salmon, trout, or tilapia may also prey on other fish at unusual times during the season, as many GE fish mature at an earlier age than their non-GE counterparts. Altered feeding patterns such as these could result in major disruptions to aquatic ecosystems.<sup>70</sup>

When researchers placed non-GE coho salmon and coho salmon GE with a growth hormone gene together, they found that when food supplies were low, the genetically engineered salmon dominated the acquisition of food resources, consistently outgrew the non-GE salmon and affected their growth. Dominant GE individuals emerged which then became cannibalistic towards the other fish, including other GE salmon that were weaker than them. When there was low availability of food, all the groups containing genetically engineered salmon also then experienced population crashes or complete extinctions, whereas groups containing only non-GE salmon had good survival and growth rates.<sup>71</sup>



### iii Sterilisation will not be 100 % effective

"One of the most significant issues raised by environmentalists is the potential for negative effects on wild stock posed by "gene flow" [from GE fish]. Use of sterile fish will eliminate this issue." Elliot Entis, President of Aqua Bounty Farms<sup>72</sup>

Companies currently seeking commercial approval to market GE fish are claiming that sterilisation of these fish can protect the environment from the hazards associated with the escape of GE fish from fish farms. They claim that the methods used can be 100 % effective.<sup>73</sup> However, other evidence indicates that this cannot be achieved under commercial conditions.

A number of different methods can be used to sterilise fish, but there is one principal technique being proposed for GE fish, called 'triploidy.' With this method, temperature or pressure shock is applied to fish eggs shortly after fertilisation. This produces eggs that contain three chromosomes (triploids) instead of the normal two and fish grown from these eggs usually do not develop normal sexual characteristics.<sup>74</sup>

Although most of the fish produced by triploidy will be sterile, it is widely acknowledged that a small percentage of triploid fish will develop sexual characteristics and be fertile.<sup>75</sup>

\* A study of GE tilapia fish that were triploids, for example, found that some of the male fish had sperm in their testes, 'indicative of reproductive functionality'.<sup>76</sup> One of the reasons that low fertility in male fish is of concern was highlighted in publications by Muir and Howard, authors of the Trojan Gene Hypothesis. Among their scenarios where escaped GE fish could lead to extinction of both GE and native fish populations,<sup>77</sup> was one where GE male fish have lowered fertility,<sup>76</sup> while at the same time being more viable (through a trait such as increased disease resistance).<sup>79</sup> The researchers concluded that *"attempts to reduce GE male fertility that do not result in complete male sterility may increase the hazard rather than reduce it".<sup>80</sup>* 

Aqua Bounty Farms, the company currently seeking commercial approval for its GE salmon, say that the company will ensure that all the triploid eggs will be female and that every batch of eggs will be thoroughly screened.<sup>61</sup> While it is possible to achieve close to 100% sterilisation of eggs through careful screening, many people in the industry (including from companies developing the GE fish) are doubtful that this kind of screening would be economical under conditions of commercial production.<sup>82</sup>

When GE crops were commercialised, the biotech industry demanded 'tolerance' levels to legalise genetic contamination of other crops with pollen from GE plants, having denied for years that such contamination would ever occur. Since the widespread commercialisation of these crops in the 1990's, there have been numerous instances of contamination from GE crops.<sup>83</sup> It is extremely likely, therefore, that faced with economic considerations under commercial production, the companies developing GE fish will demand acceptance of less than 100 % success rates in the sterilisation of GE fish.<sup>84</sup>

Even without GE fish reproducing, escapes of sterile GE fish could still have a major impact on wild fish populations. This could occur through the spreading of diseases or through competition with wild fish for resources, such as food or habitat.<sup>85</sup> Damage to wild fish populations could also occur if sterile GE fish compete with wild fish for mates.<sup>86</sup> This fruitless mating could depress populations of already endangered species of fish.<sup>87</sup>

There are other major problems associated with triploid fish, which raise questions as to whether the technique will be suitable for commercial aquaculture operations, as well as major animal welfare concerns.

\* Triploid Atlantic salmon exhibit greater variability in growth,<sup>86</sup> diminished tolerance of chronic stress,<sup>89</sup> reduced survival during egg incubation and, during marine grow-out,<sup>90</sup> a number of abnormalities including spinal abnormalities,<sup>91</sup> abnormalities of the lower jaw,<sup>92</sup> and greater susceptibility to cataract formation.<sup>93</sup> Triploid fish have also been found to suffer from breathing difficulties, low blood haemoglobin levels and higher rates of mortality.<sup>94</sup>

In summary, sterilisation is unlikely to be 100 % effective in a commercial situation. In addition, there are welfare concerns regarding triploid fish.

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### V NO! TO THE COMMERCIALISATION OF GE FISH

GE fish could be the first GE animals to receive any government approval for sale as food.<sup>95</sup> No official approval has yet been granted for the sale of GE fish for human consumption, but there are at least three countries where approval could be in the pipeline.

- \* In the United States, commercial approval is currently being sought for GE salmon and catfish. The company developing the GE salmon, Aqua Bounty Farms, says that it has pre-orders for 15 million eggs and it had hoped to receive commercial approval by 2002. There has been intense controversy over GE fish in recent years, however, and the company now says that it does not expect its application to be approved until late 2004 at the earliest. The US Food and Drug Administration (FDA) decided to regulate the salmon, GE with a gene for a growth hormone, as a new 'animal drug'. The FDA has been widely criticised as lacking both the legal authority and ecological expertise to regulate GE fish.<sup>96</sup>
- \* Researchers at the Center for Genetic Engineering and Biotechnology in Cuba are seeking government approval for GE tilapia. Mario Estrada, leader of the project, says that feeding studies have been conducted, including an experiment in which the tilapia were eaten by human volunteers. The researchers expect that it will be 2005 before they hear whether or nor approval will be granted.<sup>97</sup>
- \* In China, GE carp is currently being tested for possible commercial production.<sup>96</sup> However, concerns about their impact on the environment mean that commercialisation is unlikely in the near future.<sup>99</sup>

Although regulatory bodies in the US or elsewhere may soon decide to give commercial approval for a GE fish, it is not possible to say if these GE fish will be safe to eat. The process of genetic engineering itself typically results in unexpected and unintended changes in the organism that is being genetically engineered. In the case of a GE fish, this could include the modification of an existing fish protein, or the creation of a new protein that could cause allergic reactions.<sup>100</sup> Such modifications could go unnoticed by government regulators who often depend on the very companies who stand to profit from commercial approval of a GE organism to do the safety assessment. In most cases, government regulators require companies to assess the safety of GE organisms by examining a narrow range of known characteristics, while

failing to require any thorough analysis of the hazards posed by unexpected new molecules that could be harmful.  $^{\rm tot}$ 

#### GLOFISH - A GE PET

For just US\$5, you too could be the proud owner of the world's first GE household pet. On sale since January 2004 in pet stores across the United States, the GE fish called GloFish, are being marketed by a Texas biotech firm called Yorktown Technologies. Originally developed from zebra fish by researchers at the University of Singapore, GloFish engineered with a fluorescing gene taken from a jellyfish glow green, while those engineered with genes taken from Discosoma (a relative of sea anemones and coral) have a reddish hue when exposed to black or fluorescent light.

While this GloFish might sound cute to some aquarium owners, the attempt to win acceptance for this GE pet is really the thin end of a much more ambitious wedge as far as the genetic engineering industry is concerned. The industry has been told again and again by its public relations advisors that there might have been more success in winning global acceptance of GE foods if the first ones introduced had offered something perceived as a 'consumer benefit', such as increased vitamin content. The industry hopes a cute GE pet could be a good way to prepare the public for a flood of other GE animals in the pipeline, such as fast growing fish or pigs engineered for intensive factory farming operations.

The US Food and Drug Administration declined to regulate the GE GloFish, because there are currently no regulations that pertain to ornamental fish. *"Allowing the unregulated sale of GloFish provides a gateway for GE fish to find their way on to our dinner plates and into our environment,"* commented Joseph Mendelson, legal director of the Washington based Center for Food Safety. *"By not stepping in to regulate these fish, the US regulatory agencies are establishing a dangerous precedent for all future gene-altered animals, whether created as food or pet fads".*<sup>102</sup>



#### i widespread opposition to the commercial development of GE fish

Increasing awareness of the considerable hazards associated with GE fish means that the commercial future of GE fish is far from certain. Opposition to the commercial development of GE fish has been voiced by governments, industry bodies, scientific institutions and a wide range of other organisations.

- \* Deep concern about the ecological impacts of GE fish has been expressed by highly influential scientific bodies such the Royal Society and the US National Academy of Sciences.<sup>103</sup> The Royal Society called for a moratorium on the release of GE fish in sea pens and recommended that commercial approval only be granted for GE fish kept in contained landbased facilities. Although land-based facilities are an improvement on fish farms located in open water, experience with such facilities shows that animals, floods and human accident mean that fish could still end up escaping into open waterways.<sup>104</sup> Birds, for example, pick up fish from land-based farms and then sometimes drop them into open water. Flooding, which is increasingly common as climate change alters weather patterns, can also provide routes for fish to escape.
- \* In 1992, the Oregon Department of Fish and Wildlife in the US published an administrative rule, stating that: *"The Department shall consider releases of GE fish to pose a serious risk to wild populations. The Department shall not authorize the release of GE fish into locations where such fish may gain access to wild fish populations"*.<sup>105</sup> In 2001, the state of Maryland in the US placed a five-year prohibition on the introduction of any GE fish into any state waterways that flow into any other body of water.<sup>106</sup> In 2002, the Washington Fish and Wildlife Commission banned all GE fish from Washington State fish farms in marine waters.<sup>107</sup> In 2003, California passed a law making it illegal to spawn, cultivate, or incubate any GE fish in the waters of the Pacific Ocean over which the state has jurisdiction.<sup>108</sup>
- \* During recent meetings of the National Atlantic Salmon Conservation Organization (NASCO, established by international treaty and consisting of Canada, Denmark, the European Union, Iceland, Norway, the Russian Federation, and the United States), the parties agreed to support a resolution, which states that the parties will *"take all possible actions to ensure that the use of GE salmon, in any part of the NASCO*

### Convention Area, is confined to secure, self-contained, land-based facilities".<sup>109</sup>

- \* In March 2002, European ministers agreed at the 5th North Sea Conference at Bergen, Sweden to take all possible actions to prevent the release of GE marine organisms to the marine environment, and to ensure that their culture is confined to secure, self-contained, land-based facilities.<sup>110</sup> Other bans on the environmental release of GE fish are in place at a local and national level in Europe.<sup>111</sup> In Austria, for example, the federal counties of Salzburg, Styria and Vorarlberg adopted amendments to their fisheries law, which ban the release of GE fish.<sup>112</sup>
- \* A number of industry bodies representing aquaculture and fisheries interests are strongly opposed to the use of GE fish.<sup>113</sup> The Swedish Fish Industry Association, for example, recommends its member companies *"to completely refrain from having anything to do with GE fish"*.<sup>114</sup>
- \* In June 2003, the Baltic Marine Environment Protection Commission, the Helsinki Commission and the Commission for the Protection of the Marine Environment of the North East Atlantic held a joint ministerial meeting in Bergen, Germany. In the joint declaration from this meeting, they stated that: *"Recognising that the release of genetically modified marine organisms presents an inherent threat of potentially severe, irreversible and transboundary effects, and the need to apply the precautionary principle, we agree to take all possible actions, in accordance with the requirements of the Directive 2001/18/EC and comparable national legislation, to ensure that the culture of genetically modified marine organisms is confined to secure, selfcontained, land-based facilities in order to prevent their release to the marine environment."*<sup>115</sup>

Several governments, intergovernmental organisations and conventions have already taken steps to try to prevent GE fish from being accidentally or deliberately released into aquatic systems. However, steps need to be taken globally, as any released GE fish will not respect national boundaries. A suitable multilateral instrument would be the Cartagena Protocol on Biosafety of the Convention on Biological Diversity, which seeks to protect biological diversity from the potential hazards posed by GE organisms.

GENETICALLY ENGINEERED FISH - NEW THREATS TO THE ENVIRONMENT CONCLUSIONS

### conclusions

This report has examined many of the hazards associated with GE fish. The physical containment of these fish cannot be guaranteed under commercial conditions and any escapes into the environment could have devastating effects on wild fish populations and biodiversity.

Greenpeace demands that:

- \* Genetic engineering of fish for commercial purposes should be prohibited. Once approved for commercial use, GE fish may never be contained;
- \* The Parties to the Cartagena Protocol on Biosafety of the Convention on Biological Diversity consider the special transboundary challenges posed by GE fish, including the risks they pose to the environment, with a view to prohibiting any facilities that could result in the introduction (either accidental or deliberate) of GE fish into freshwater and marine ecosystems.





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- 107 Anon 2002. Washington state issues ban on transgenic fish. Seafood News, 18 Dec 2002 <www.fass.org/fasstrack/news\_item.asp?news\_id=891>
- 108 California SB 245, Signed by Governor 10/12/03; Chpt 871, Statutes of 2003 <http://pewagbiotech.org/resources/factsheets/legislation/index.php?StatusID=1&Year=2003>
- 109 Resolution by the Parties to the Convention for the Conservation of Salmon in the North Atlantic Ocean to Resolution by the root in Additional Section 2016 and the root in Addition of Section 1 and the Work Addition 2 and the Wor

110] The Bergen Declaration (Ministerial Declaration from 5th North Sea Conference, Bergen, March 2002) The begin because that the release of genetically modified marine or galax conserved, begin in the North Sea WRecognizing that the release of genetically modified marine or galaxies is an emerging issue in the North Sea owing to the inherent, potentially severe, irreversible and transboundary effects, and the need to apply the precautionary principle, the Ministers agree to take all possible actions, in accordance with the requirements of the Directive 2001/18 EC and comparable national legislation, to ensure that the culture of genetically modifed marine organisms is confined to secure, self-contained, land-based facilities in order to prevent their release to the Full text of the declaration, and further information and background to the North Sea Conference itself, is

available at <http://odin.dep.no/md/nsc/>

111 Letter to Greenpeace from Margareta Winberg, Swedish Minister of Agriculture, Sep 2002: 'According to a decision taken by the Swedish Parliament last year "Alien species and genetically modified organisms that may be a threat to biological diversity may not be introduced". Further, the decision states "Genetically modified fish may not be released". This decision is the national position regarded as an instruction for Swedish delegations in future international deliberations concerning genetically engineered salmon. MAFF (Ministry of Agriculture, Fisheries and Food) 2001. Review of salmon and freshwater fisheries', UK

Government Response. Available at: <www.defra.gov.uk/fish/salmon/salmon.pdf> Recommendation 51: "Any GE fish kept in England and Wales should be confined to self- contained land-based and escape-proof facilities, with all necessary measures taken to ensure they cannot deliberately (by, for example, vandals) or accidentally be released into the natural environment."

112| Styria: Gesetz vom 18. Mai 1999 über das Fischereirecht in Steiermark (Steiermärkisches Fischereigesetz 2000) Styria, ocecct form zwitchen (h. 1999). http://fis.bka.gv.at/ir-steiermark/ Vorarlberg: Gesetz über die Fischerei in den Binnengewässern (Fischereigesetz) LGBI. Nr. 47/2000, <www.vorarlberg.at/vorarlberg/land\_politik/land/gesetzgebung/weitereinformationen/landesrecht/voris.htm>

113| Stoll, M. 1999 Designer fish flounder over legal hurdles. Christian Science Monitor 4th March, 1999. <www.csmonitor.com/durable/1999/03/04/p19s1.htm>

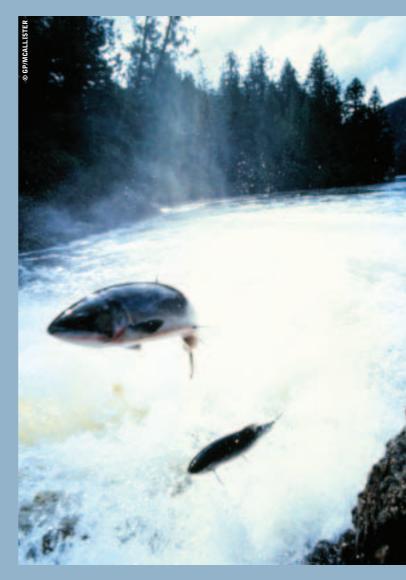
One of the biggest fish farming companies in the world, Nutreco Aquaculture, based in the Netherlands, say that

Use of the piggest rish rarming companies in the work, Nutree Aquacuture, based in the Netmerianos, say that they "will never use GE eggs." Forbes magazine, February 19, 2001 p. 100 AM (19, 2001 For the Also, "Norwegians applaud Greenpeace stance", Intrafish, 6 Apr 2001. Excerpt from Ethical rules for the aquacuture association: "The association do not accept 6M (fish which differ from natural populations and which can affect biodiversity and surrounding environment in a negative way"...,"It's marvellous that Greenpeace is also taking up the fight against genetically modified salmon," said NFF chairman, Tarald Sivertsen. On the Norwegian salmon farming organisation's website it is abundantity clear that Norwegian fish farmers and fish farmers across the globe are against plans by the American company Aqua Bounty Farms to produce genetically modified salmon on a commercial basis.

114] Comments sent in response to information requests from Greenpeace. Other replies include: FEDIS (Beigian Federation of retailers) "As agreed, we're informing you of the position adopted on 9 May by the Commission 'food safety' of FEDIS re: international commercialisation of eggs from GM salmon, ....) In the case of GM salmon, members of FEDIS cannot accept its commercialization because the risks of environmental damage have been clearly identified. It is now to competent authorities to verify them. The utmost precaution is therefore necessary. (...). Besides, if one cannot say anymore that there is global overproduction of salmon, one can still say that supply meets demand, which remains high. Consequently, an insufficient production can extainly not be invoked to justify a demand for authorisation as the one introduced by AVF Protein. We will forward this standpoint to our European organization EuroCommerce, as well as to the Ag Min Jaak Gabriels," - Letter to

stanopoint to our coopean organization corocomience, as very as to be 4g win oaak dataries. - Exter to Greepeace Way 2001. -In 1996 the ISFA (organisation for salmon-producing countries around the world), implemented the following resolution: In line with good environmental practice, the ISFA rejects unequivocally the production of genetically modified salmon. "In connection with the aim of maintaining a high animal ethics standard, the ISFA's members will not make use of growth hormones in their salmon production."...." It is not possible to make a clearer . with not make use of growth normals in their similar production. .... This not possible to make a clearer statement. Nevertheless, every year we bear the brunt of attempts to connect gene manipulation to salmon through Epublicity] from this firm. We very much appreciate Greenpeace's engagement in focusing attention on this issue. I am hoping in any case that a united salmon industry and Greenpeace's engagement in for and to these attempts. Consumers across the globe should be made aware that Norwegian fish farmers base their livelihoods on a sustainable and environmentally friendly production of a healthy and good product. The consumers deserve this much and they can rely on this."

115 Declaration of the Joint Ministerial Meeting of the Helsinki and OSPAR Commissions - (JMM 2003/3(final version)-E) <www.ospar.org/eng/html/md/ioint\_declaration\_2003.htm>



GENETICALLY ENGINEERED FISH - NEW THREATS TO THE ENVIRONMENT



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