

State of the art

Genetic impacts of escapees and restocking and introduction of non-native strains

The continuing global decline of wild fin-fish and shell-fish stocks has been accompanied by a parallel increase in aquaculture. Over the past 15 years, worldwide production of farmed fish and shellfish has more than doubled, with farming activities now producing more than a quarter of all fish/shellfish directly consumed by humans (FAO, 2004). Although it is generally assumed that farming relieves pressure on wild fisheries, its effects (direct or indirect) on aquatic resources are not yet fully understood and indeed have been the subject of intense debate. Particular focus has been placed on the potential negative genetic impacts of escaped farm-fish/shellfish and/or deliberate introductions of farmed and non-native organisms into wild stocks.

The physical structures of aquatic culture are such that caged fish can escape and therefore interbreed with wild conspecifics in the natural environment. For example, whilst the worldwide catch of wild Atlantic salmon is around 4,000 tons (ICES, 2003/ACFM:19), it is estimated that some two million individuals escape every year from fish farms in the North Atlantic. In addition to accidental escapes, deliberate restocking of farm-reared or non-native individuals is undertaken in an attempt to supplement recruitment in wild populations and increase subsequent harvests (e.g. Svåsand and Moksness, 2004). Although the survival of introduced organisms is often low, successful hybridisation with wild conspecifics has been documented (e.g., Crozier, 1993; Clifford *et al.*, 1998).

Escapees and restocked individuals are genetically different from wild individuals for various reasons. First they are often of non-native origin and are therefore genetically distinct as a result of natural inter-population genetic heterogeneity. More importantly, when any organism is retained in culture, changes occur due to the process of domestication. This involves both deliberate and inadvertent selection, and may also bring about random genetic changes known as genetic drift, i.e. loss of genetic variation due to stochastic changes in allelic frequencies over several generations (Crow and Kimura, 1970). As a consequence, cultured organisms often display poor survival in the wild (e.g. McGinnity *et al.*, 1997, 2003; Fleming *et al.*, 2000). However, some individuals do survive and interbreed with wild conspecifics. The resulting hybrids may also display reduced survival and reproduction success, thereby lowering the overall fitness of the wild population. With continued releases, weakened populations may enter an extinction vortex.

Much of the research on the genetic impacts of farm escapes and restocking has involved salmonid fish, especially Atlantic salmon and brown trout (Flemming *et al.*, 1996; Johnson *et al.*, 1996; Fleming and Einum, 1997; Fleming *et al.*, 2000; McGinnity *et al.*, 2003). As such, several studies have demonstrated changes in gene pools of wild stocks due to accidental releases or deliberate stocking (Crozier, 1993; Clifford *et al.*, 1998; Berrebi *et al.*, 2000; Hansen, 2002) as well as changes in behaviour and morphology associated with domestication (Johnson *et al.*, 1996; Fleming and Einum, 1997; Alvarez and Nieceza, 2003). Cultured Atlantic salmon in Europe currently amount to around 700,000 tons per year, while wild catches are below 5,000 tons (Figure 1). Atlantic salmon are thus an important model organism for the assessment of the genetic impact of escapes and restocking of novel aquaculture organisms.

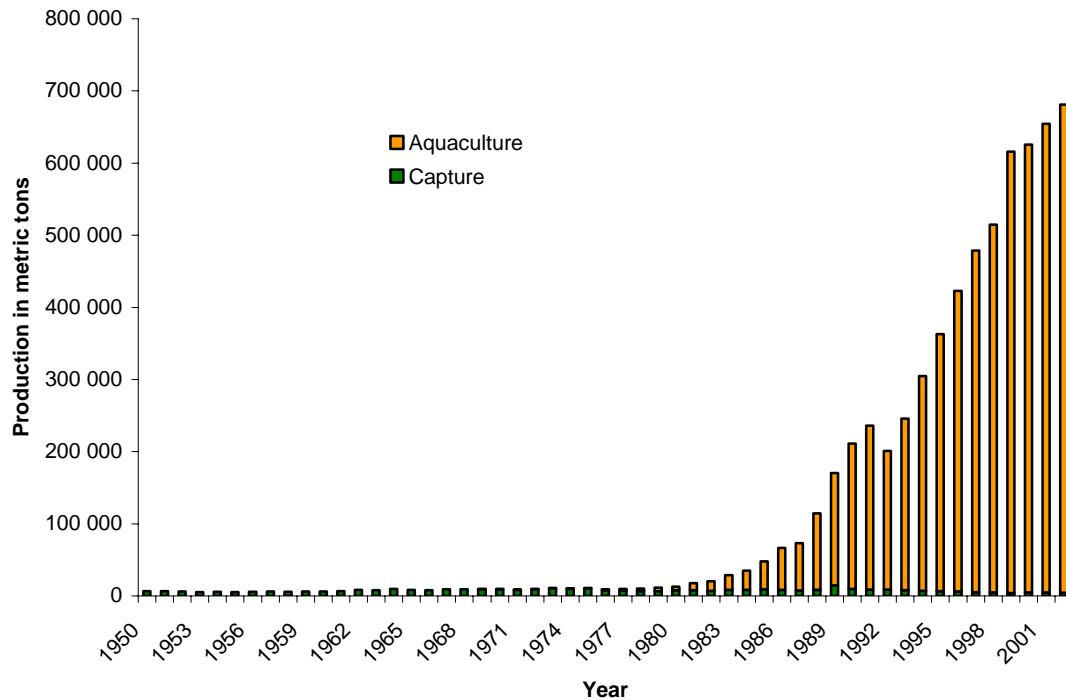


Figure 1. Production and capture of Atlantic salmon in Europe in the period 1950 to 2002 (based on data from www.FAO.org)

A recent study by McGinnity *et al.* (2003) examined, for the first time, the lifetime fitness of multiple families of both first- and second-generation hybrids between wild and farm-salmon during the freshwater and marine life history phases. Offspring of farm and ‘hybrids’ (i.e. all F_1 and F_2 hybrids and backcrosses to wild and farm groups) displayed reduced survival compared to wild salmon but grew faster as juveniles and displaced wild parr, which as a group were significantly smaller. When suitable habitats for these emigrant parr are absent, such competition tends to reduce wild smolt production. In the experimental conditions, where emigrants survived downstream, relative estimated lifetime success ranged from 2% (farm) to 89% (BC_1 wild) of wild, indicating additive genetic variation for survival.

There is a need to assess the extent to which disease transmission has, or has had, an impact on genetic impact on natural fish populations. Genetic signatures of impact of aquaculture have been identified by the study of patterns of genetic variability at loci involved in the immune response (data not yet published). Evidence of spatial and temporal differences in genetic variation has been observed in areas affected and unaffected by aquacultural activity. There is also evidence from natural and semi-natural field experiments that there are significant fitness consequences of the genetic changes that can occur because of indirect genetic changes arising from disease impacts of introductions of cultured fish into the wild (Stet *et al.*, 2005).

Besides Atlantic salmon, Genimpact will cover the species that already are, or are on their way to become, important aquaculture species in Europe: Atlantic cod, European sea bass, gilthead sea bream, turbot, carp, halibut, carp, scallops, mussels, oysters and European lobster (Figure 2). In the case of the sea bass, recent indications show that some wild stocks may have already been affected by escapees (Bahri-Sfar *et al.* 2005).

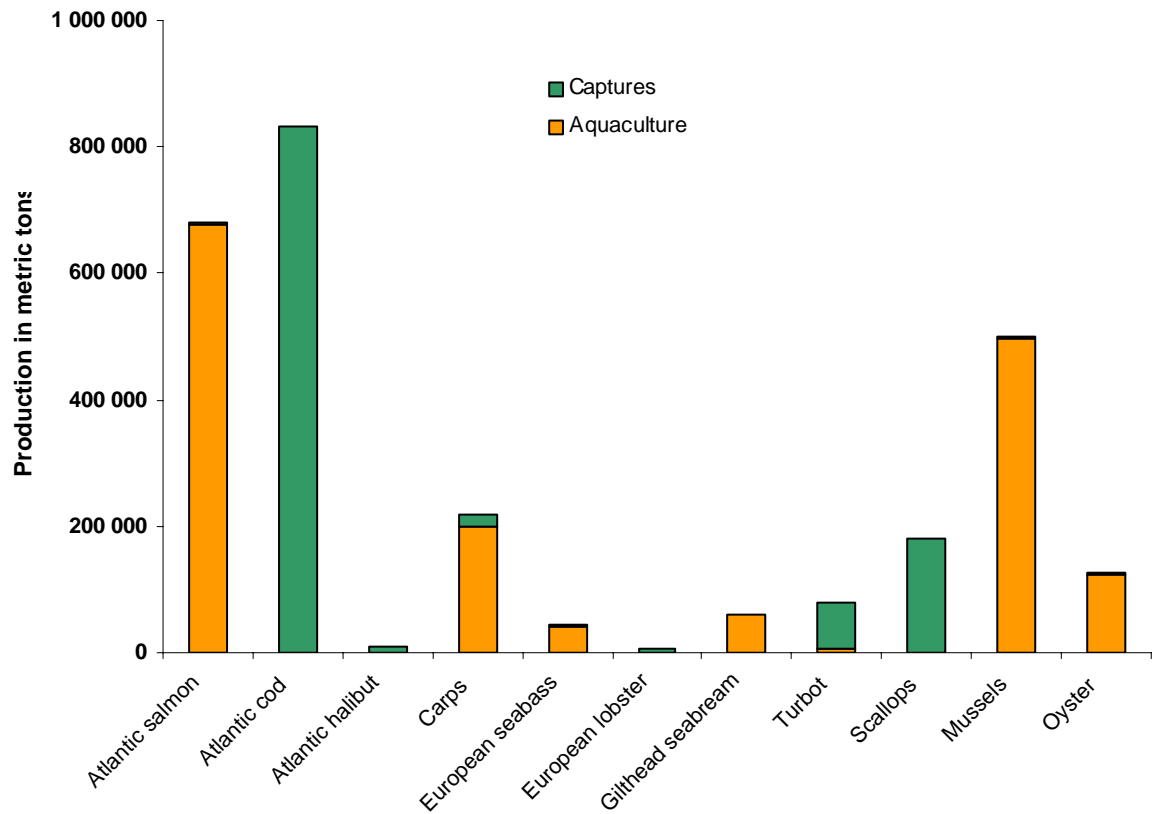


Figure 2. Production and capture and aquaculture production of selected important aquaculture species in Europe in 2002 (data from www.FAO.org)

Objectives of “Genimpact”: To review the genetic structure and biology of wild and cultured stocks of the selected species, in order to identify the genetic threats that escapes and released cultured organisms may have on native stocks.

Genetic impacts of culture practices

There is an abundant literature on the breeding programmes of some species (especially salmonids), but little has been written on cultivation practices and procedures, which could have adverse genetic consequences on cultured stocks and eventually on native populations. For many aquaculture species with a recent history of domestication, and for which breeding programmes are lacking or in their infancy, aquaculturists often rely on practices that may severely affect the genetic structure of the cultured stock and which may have negative effects on wild populations in the case of escapes or restocking. There is evidence of such unwanted effects even by hatchery-reared (sea-ranched) fish on wild populations, although the significance of such negative effects is still a matter of debate. In general, there is a need to distinguish between environmental and genetic effects in order to develop better management strategies.

Objectives of “Genimpact”: To review the cultivation practices and procedures that could have adverse genetic consequences on cultured stocks and eventually on wild populations,

and in particular to review methods of selecting and managing broodstocks and offspring in order to evaluate the genetic impacts of escapes, restocking and breeding in the wild. To review management strategies of current interest, such as rules for giving priority to stocks for conservation purposes, depending on population size or local adaptations.

Triploids (both fish and shellfish), tetraploid mother oyster strains

The induction of triploidy has been reported in most aquaculture species. In fish, triploidy is generally induced by pressure or thermal treatment, with the aim of blocking the resumption of the second meiotic division in fertilised eggs. In shellfish, however, it is induced by suppressing the formation of either the first or the second polar body in fertilised eggs, giving rise to triploids with increased or decreased homozygosity respectively, in comparison with diploid parentals (Beaumont and Fairbrother, 1991; Desrosiers *et al.*, 1993; Gérard *et al.*, 1999). An alternative method is based on the mating of tetraploids and diploids to obtain all-triploid stocks (Chourrout *et al.*, 1986; Guo *et al.*, 1996). In species in which all this could be achieved, the management of tetraploids needs to be carefully considered.

Triploidy is often mentioned as a possible tool for the protection of genetic resources from aquaculture escapees or to ensure the genetic confinement of transgenic organisms. However, much of the interest in triploidy in commercially important aquaculture species is due to the assumption that growth rates of triploid organisms will be higher due to retarded or non-existent gonad development. In fact triploids are assumed to be at least gametically sterile, as they do not show proper reductional division during gametogenesis (Thorgaard and Allen, 1987). The avoidance of reproduction would be of advantage in aquatic cultured species, which become sexually mature before they reach marketable size.

In bivalves, some species are unmarketable at certain times of the year due to seasonal maturation, and in this respect the use of triploidy may circumvent this problem. There are studies that have failed to demonstrate any differences in growth or other physiological parameters between triploids and diploids (e.g. Downing and Allen, 1987; Beaumont *et al.*, 1992; Thorgaard, 1992). Despite this, there is great interest in triploidy for some species, especially in oysters, where triploids clearly display better growth than diploids, a finding that has resulted in increased commercial application of this technology (Nell, 2002). In oysters, the genetic impact of such a production needs to be studied in terms of: (1) sterility of triploid oysters (Guo and Allen, 1994), and (2) risks of releases of gametes or escape of adult tetraploid oysters into the wild where the oysters are growing (both wild and farmed).

Potential extension of the use of triploids in the future will depend both on the economic advantages of this genotype and on consumer perceptions.

Objectives of “Genimpact”: Evaluate triploidy as a possible tool for the protection of genetic resources from aquaculture escapees or as a means of ensuring the genetic confinement of transgenic organisms.

Growth enhancement in fish by gene transfer

Biosafety has become a sensitive issue in fish culture owing to the possibility of the mass-scale application of biotechnology to increase productions, a step which implies the use of transgenic, genomic and cellular technologies. It is feared that this innovative approach might entail unforeseen risks for human health and hazards for the integrity of aquatic ecosystems.

Given that the United States Patent Office has recently granted a patent to a national firm on growth-enhanced transgenic salmon bearing an “all-fish” construct encoding salmon growth hormone (GH) (Diouf, 2000), and that, if a transgenic animal raised for food is ever going to reach the market and consumers, it will likely be a fish, the question of the safety of transgenic fish has become a visible part of the agenda in discussion of genetically modified organisms (GMOs).

Genetic modification by gene transfer into the germ line is a highly controversial topic because it is a truly “subversive” approach. Classical genetic amelioration by selective breeding adopts a top-down strategy by which the parental phenotypes are selected in such a way as to conveniently alter the allelic assortments in the filial genotypes. By contrast, in transgenesis, the strategy is bottom-up, as a selected gene is inserted into the genome of a gamete or embryo to alter the filial phenotype.

Man-directed selection progresses through successive waves of small genotypic changes across generations, until they become stabilized by allelic fixation. Genotypes are modified while maintaining their overall genetic integration. Hence, the associated phenotypic changes, especially of polygenic traits, express innumerable pleiotropic, dominance and epistatic effects and accommodate countless molecular trade-offs to ensure a production gain, while shifting adaptation from the wild to the culture environment.

Conversely, gene transfer relies on a strictly deterministic design, in which a single genetic change is expected to suddenly affect the phenotype conferring a novel trait favourable in culture. Any subsequent change occurring in the transgene during transmission to offspring would be counterproductive.

It is frequently argued that the commercial prospects for transgenic fish are meagre, at least because of the negative public perception and general opposition to plant GMOs, and that moratoria against the mass culture, trading and consumption of transgenic fish must be imposed until international legislation and guidelines have been established, in the first place, for transgenic agricultural crops (Bartley, 1999).

This cautionary stance, however, fails to consider the profound differences between transgenic fish and transgenic crops. In GM crops, transgenes were derived almost exclusively from non-plant sources, including bacteria, viruses and animals, an approach called allotransgenesis (Beardmore, 1997). All allotransgenes introduce a qualitative difference in both the genotype and the phenotype of the recipient. In GM fish, on the other hand, “all-fish” constructs, often from the same or related species, have normally been preferred, an approach called autotransgenesis. Autotransgenes equivalent to genes already present in the genome of the recipient, as GH-encoding transgenes for growth enhancement, introduce essentially a quantitative difference in both the genotype and the phenotype.

If the overall protein profile of GH-autotransgenic fish is qualitatively equivalent to that of non-GM conspecifics, the former satisfy the requirement of substantial nutritional equivalence and safety for human consumption. While the satisfaction of the first requirement is straightforward, compliance with the second requirement, that of the substantial environmental equivalence of GM and non-GM fish remains problematic.

The main difference lies in the degree of reversibility of the genetic impacts caused by interbreeding with wild conspecifics and the spread of selected alleles or transgenes by

introgression and intergradation. Theoretically, alterations in allelic frequencies of structural genes in a wild fish population caused by mixing with allelic assortments from domestic fish is likely to be eventually normalized with time by natural selection, if the impact is not catastrophic (extinction of the population). In contrast, the natural eradication of a non-self-eliminating transgene in a wild population is a less certain outcome.

For this reason, there is a need to better evaluate the applicability of field tests in risk assessment studies with GM fish. Performing the test in the context of real ecosystems is troublesome because it might well turn out to be impossible to remove all GM fish or their transgenic progeny. If carried out in a secluded facility, the test would be of limited significance, given the arbitrary environmental constraints that were enforced. Analogously, mathematical modeling may predict some general consequences under certain assumptions that are just as arbitrary.

This part of the present project aims to examine all possible strategies to overcome this deadlock caused by the implicit premise that GM fish can be considered safe for culture only if they inflict genetic damage on wild conspecifics equivalent to that commonly inflicted by selected strains. An effort must be made to leave aside any such comparison and go straight for the best solution, i.e., not to cause genetic damage at all. All possible options will be explored, including how to eliminate any natural fitness in GM fish, where to raise GM fish without the possibility of interbreeding with wild fish populations, and how to exploit natural gene transfer for safe transgenesis. As the massive culture of GM fish may in any case cause environmental damage due to predation or competition by escapees, guidelines will be proposed regarding which species might be subjected to this technology to achieve benefits that not only outweigh the costs, but are truly significant in coping with the pressing demands of a crowded world.

Objectives of “Genimpact”: To evaluate the risks associated with the farming of transgenic fish, such as their probability of escape and capacity for naturalization and self-perpetuation, predation upon and competition with wild fish for resources (food, space and mates), consequences of crossbreeding with wild fish and spreading of different types of transgenes, and possibility of genetic containment of GM fish

Recombinant DNA vaccines

It has been estimated that about 10% of cultured fish are lost annually to infectious diseases (Leong and Fryer, 1993). Moreover, infected stocks may diffuse pathogens among wild populations by direct contact or through discharges into open environments. Prophylaxis rather than chemotherapy has been advocated as the best countermeasure (Heppel *et al.*, 1998). The prophylactic potential of DNA vaccination in fish culture rests on several advantages, including identical and inexpensive production processes, the possibility of co-administration of multiple vaccines (multivalency), simplicity of storage due to the high chemical stability of plasmid DNA, rapid modification of vaccine DNA sequences to confront new pathogen mutants, no risk of disease transmission (as occurs with live attenuated vaccines), proper conformational folding of the antigen protein of the pathogen (not always achieved with recombinant protein vaccines produced in bacteria), no need for adjuvant use and boosting to elicit immune responses, and effectiveness in stimulating both humoral and cell-mediated immunity (Heppell and Davis, 2000).

Despite unsolved technical and biological problems regarding a suitable administration

method and unwanted integration of the plasmid DNA into the host genome at the germinal line, it is expected that these difficulties will be overcome, permitting the widespread use of DNA vaccines as a viable vaccination method in the future. The genetic impact that integrant escapees may exert by out-crossing with wild conspecifics is serious, because the constitutive expression of the antigenic protein at the time of immunocompetence development and self-recognition would deprive the fish of both innate and acquired immune protection against the pathogen, thus exposing natural fish populations to devastating epizootics. Hence, it seems appropriate to demand specific requirements for DNA vaccination in fish in order to deal with this problem in practice rather than merely theoretically.

Objectives of “Genimpact”: To review the benefits of DNA vaccines in terms of their applicability and cost-effectiveness, as compared with traditional vaccines. In addition, to review the potential beneficial effects of DNA vaccines (for example reduced use of chemotherapeutics and associated benefits). Importantly, the probability of integration of the vaccine DNA into the host genome and its possible transfer by vaccinated escapees into wild fish will be evaluated.

Genetic impact evaluation, monitoring tools and modeling

The genetic impacts of ecological and reproductive interactions between introduced cultured conspecifics and wild populations will be highly dependent on the specific circumstances prevailing in a given situation. The results of research studies such as those on the impacts of escaped farmed Atlantic salmon are largely species-specific (e.g. McGinnity *et al.*, 2003) and, while they do offer information on the potential for impacts, they fail to do so with regard to impacts over the range of scenarios which confront managers and policy makers. As is the case with many management issues, time and cost constraints preclude research into a sufficiently wide range of conditions to enable the impact to be generalised across a realistic range of management scenarios.

An abundant literature has been dedicated to the effects of hybridisation and introgression following secondary contact and to methods of evaluating such effects. However, this literature is based on case studies, mostly natural situations, or single-species monographs. Few reports have been devoted to aquaculture species, and those mainly deal with Atlantic salmon (Gross, 1998; Vandeputte and Prunet, 2002). To the best of our knowledge, Workpackages 2-3 will provide the first overall evaluation of existing methods and perspectives for monitoring genetic impacts of fish-farming activities on native populations.

In order to improve predictive capacity to guide management, predictive impact models can be developed to evaluate the risk of significant impacts. The potential for developing such models is considerable, but model development to date has been limited, and confined to deterministic models (e.g. Hutchings, 1991; Kanaiwa and Harada, 2000; Muir and Howard, 2001), while the use of probabilistic, individual-based simulation models incorporating realistic genetic and population dynamics remains unexploited.

Some models have been developed with the aim of describing and predicting genetic variations introduced into wild populations by released hatchery fish and farmed escapees. However, there is a great need for models capable of integrating available knowledge and reconciling conflicting results and viewpoints. Such models should improve our understanding of problems, provide clues to possible solutions, identify areas in which further work is required, and suggest appropriate strategies whenever residual uncertainty must be

dealt with.

Simulations are the base and reference on which to formulate complex, realistic and powerful models. Because the random sampling process can be taken into account, the effects of both linkage disequilibrium, due to migration, and inbreeding in terms of genetic drift can be investigated in depth. In particular, simulations of stochastic and deterministic models may be used to focus on the consequences of genetic drift in quantitative traits subjected to other evolutionary forces such as migration and selection. Replicate simulations to evaluate the extent of induced genetic variations will help in assigning the risk levels associated with different management strategies.

Objectives of “Genimpact”: To evaluate current methods of monitoring, in addition to future research to improve existing, or to develop new, monitoring tools and strategies.

Predictive Tools: modeling and assessment of risk

Informative studies of the actual nature and extent of genetic impacts of ecological and reproductive interactions between introduced cultured conspecifics and wild populations are difficult to carry out. With few exceptions, they can be expected to be logistically difficult to perform, given the need to monitor individual performance over time and across generations. This makes such work costly and has limited the number of studies to date. This constraint will continue on future work. Nevertheless, a consideration of the nature of the interactions expected indicates not only that the impact is likely to vary among species, but that it will also be highly dependent on the specific circumstances prevailing in a given interaction situation. This makes generalising from individual studies difficult, particularly when we try to define whether there are acceptable levels of escapes below which impacts can be absorbed by the natural resilience of wild populations.

There exists no review of existing modeling work or of current research in this area. Furthermore, little consideration has as yet been paid to the extent to which useful predictive models can be developed to help with the assessment of risk. There is an urgent need to look at the use of models in other areas of risk assessment and population genetics and to determine to what extent the results of work in other areas can be applied to the issue of genetic impacts of aquaculture escapes. These issues will be addressed in WP3.

Objectives of “Genimpact”: To review the potential of models in evaluating the risk of genetic impacts of cultured fish on wild populations, and define research priorities for this area of work.

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