Genetic considerations in the introduction of aquacultured fish to natural ecosystems

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ABSTRACT: Introduction in the natural aquatic ecosystems of fish grown in captivity is a common phenomenon, which usually results either from planned stocking activities or from accidental escapes from farms. Although the introduction of genetic material in wild populations may be in some instances advisable, in most of the cases this practice is loaded with several threats for the genetic integrity of receiving populations. The inundation of a broad area by introduced genetic material may result in the loss of the genetic structure and variability and thus in the genetic homogenization of the populations in this area. Valuable gene pools, such as genes or co-adapted gene complexes could be lost through their replacement by exogenous genes, a phenomenon known as *genetic introgression*. This happens when the released fish interbreed with the wild ones and the genetic characteristics of the former differ from those of the latter.

Since the existence of natural population subdivisions may imply adaptation to local conditions, genetic assessments of the degree of population structuring and gene flow are necessary not only to preserve the existing biodiversity, but also to keep valuable adaptive resources. The assessment of the degree of genetic differentiation between cultivated and wild populations, as well as monitoring of the changes in genetic composition of the receiving populations after release, should constitute an integral part of any translocation or restocking program. Several types of genetic markers can be used for the analysis of genetic variation in populations, most of which are considered as evolutionary neutral. Between them, microsatellites are currently the most extensively used, since their high mutational rate and polymorphism offer high-resolution power. The inclusion in the studies of markers under selection could help in the understanding of the relationship between genetic divergence and fitness differences implying local adaptation in the populations.

A common practice of stocking is supportive breeding, whereby a certain number of wild fish are caught and reproduced in captivity and the progeny is released into the environment. This practice may be harmful for the native populations, even if no exogenous genetic material is introduced and the released fish are not adapted to the artificial rearing conditions, because it may lead in a reduction of the effective population size and hence of the genetic diversity of the receiving stocks.

To avoid problems caused by the accidental escapes of reared fish, specific measures such as farming of local stocks, manipulation of sex and ploidy (e.g. production of mono-female or sterile triploid fish) etc, should be employed.

1. INTRODUCTION

The term translocation is used to describe the movement of species or strains outside their native origin. Translocations are usually performed for the purposes of *stocking* or *farming*, and in both cases the majority of donor strains are of hatchery origin. The release into the natural aquatic ecosystems of fish grown in captivity is a common phenomenon nowadays, especially after the abrupt expansion of aquaculture activities in the last decades. This release can be either intentional, in the case of stocking (introduction, reintroduction, enhancement), or accidental, in the case of escapes from aquaculture farms. Although the introduction of genetic material in wild populations may be in some instances advisable, for example when a natural stock has been depleted for a long time, in most cases this practice is loaded with risks concerning the genetic integrity of receiving populations. Several studies have explained the possible genetic impacts of the release of stocked fish into the aquatic environment (Hindar *et al*., 1991; Ryman *et al*, 1995; Rhymer and Simberloff, 1996; Cross, 2000; Johnson, 2000; Youngson *et al*., 2001).

In broad terms, there are two kinds of genetic impacts on wild populations from the release of captive-bred fish: a) *introgression* of exogenous genetic material, and b) reduction of effective population size, a specific situation occurring in the case of stocking with natives (or *supportive breeding*).

2. INTROGRESSION OF EXOGENOUS GENETIC MATERIAL

2.1. Risks involved

Introgression of exogenous genetic material in the receiving populations happens when the genetic characteristics of the cultivated populations are different from those of the wild populations, and there is interbreeding between introduced and wild individuals. There are several threats associated with this phenomenon. The first is that the natural genetic architecture can be altered by the loss of valuable genetic material, like locally adapted genes or gene complexes, or the homogenization of a previously structured population through continual flooding with exogenous genes (Ryman *et al*, 1995). Genes or gene complexes promoted by artificial or domestication selection, which most likely will not be fit in the natural environment, may erode the native genetic pool. Moreover, re-adaptation of local populations, after the first introduction of exogenous genetic material, may be prevented. Finally, weakening or even loss of natural populations may occur, through the exclusion of native fish by introduced fish, which finally demonstrate low reproductive success.

A critical question concerns what fraction of wild genes can be replaced by exogenous genes without compromising the genetic characteristics of wild populations, especially the ones with adaptive significance. Unfortunately, only very general *a priori* predictions can be made. Based on the population genetics theory we could make sound predictions if we knew a number of parameters, like the size and evolutionary history of both the cultivated and natural populations, the acting selective forces etc. But actually we usually have very limited knowledge of the genetic characteristics of the populations, especially the wild ones (Ryman *et al*., 1995). Our knowledge pertains mainly to a small number of neutral genetic markers and we have no information about loci related with characteristics with adaptive significance.

2.2. Assaying the genetic effects of the introduction of captive-bred fish

The most basic requirements for assaying the genetic effects of the introduction of captive-bred fish is to obtain an estimation of the degree of genetic differentiation between cultivated and wild populations, to determine the genetic population structure of the wild fish and to monitor changes in the genetic make-up of receiving populations after release.

It has been recommended (Ryman *et al*, 1995) that acceptable levels of introgression should be correlated to levels of gene flow occurring naturally. The idea is that an estimation of the degree of gene flow between natural populations should be obtained and similar levels of introduction of captive fish into the wild populations should be allowed.

Gene flow can be estimated either by observations of actual migrations of fish or from the observation of the patterns of genetic differentiation, based on the methods of population genetics. The first approach can produce both overestimations and underestimations of the actual gene flow, while the second appears more realistic, even if theoretical assumptions of the population genetics theory are not strictly met. The level of the gene flow is usually approximated by using the fixation index F_{ST}, which expresses the portion of total genetic variation of a set of populations that is attributable to differences between the populations. F_{ST} is calculated from the formula:

$$F_{ST} = \sigma^2(p) \,/\, \overline{p}\,(1-\overline{p})$$

where $\sigma^2(p)$ is variance of the allelic frequencies in the populations and $\overline{p}$ is the mean allelic frequency. F_{ST} is related to gene flow through the formula:

$$F_{ST} = 1/\,(4Nm+1)$$

where N is the effective population size, m is the migration rate and Nm is the number of migrants per generation. Therefore, the critical parameter is the number of migrants rather than the migration rate. The estimated number of migrants per generation (Nm) can be used as a guideline for the acceptable levels of introgression. For example, if F_{ST} is estimated at 0.10 (which, as explained above, means that 10% of the total genetic variability residing in the populations is due to population subdivision), only two individuals per generation can be released if the local population is to keep its genetic identity.

2.3. Examples from salmonid fish

Salmonid fish provide the most appropriate fish group to study the impact of the introduction of aquacultured fish into the environment, since they are characterized by strong population subdivisions, both at macro- and microgeographical levels, and deliberate (stocking) and unintentional (escapes) releases of farmed fish in the natural aquatic environment is a very common phenomenon. From the numerous studies contacted there are not examples of better performance of captivity-bred fish than the wild ones in the natural environment, and reduction of survival rates is the most frequent observation.

In an experiment designed to compare the fitness of native and farmed Atlantic salmon (*Salmo salar*) and their hybrids in a natural environment of Ireland (McGinnity *et al*., 1997, Cross, 2000), four categories of families (wild x wild, farmed x farmed, wild x farmed hybrids, farmed x wild hybrids) were produced. The eggs were released in a stream and some months later juveniles were recovered and assigned to families by parentage identification performed by profiling for minisatellite loci. While the expectation was for equal survival, the native fish showed significantly better survival than expected, the farmed survived significantly less well, and the hybrids were close to expectations.

A number of other recent studies have used highly variable genetic markers to assess the interactions and the degree of genetic introgression of various species of stocked salmonids to wild populations [Chilcote *et al*., 1986 (steelhead, *Oncorhynchus mykiss*); Fleming *et al*., 2000 (Atlantic salmon, *Salmo salar*); Koskinen *et al*, 2002 (grayling, *Thymallus thymallus*); Poteaux *et al*., 1998, Ruzzante *et al*., 2001, Hansen, 2002 (brown trout, *Salmo trutta*)]. In all these works lower fitness of the captive stocks in the natural environment was detected.

2.4. Recent advances in the use of molecular genetic markers

The latest advances in the area of molecular genetic markers, as well as developments in the corresponding theory and methods for statistical analysis, have offered new possibilities for assaying the genetic impact of the farmed animals in the wild conspecifics. DNA can nowadays be extracted from minute amount of tissues, even from museum material (e.g. scales, otoliths), and thus the analysis of historical samples and the comparison with contemporary ones is now feasible (e.g. Nielsen *et al*., 2001; Koskinen *et al*, 2002; Hansen, 2002). Microsatellites are currently the most extensively used markers, because they offer high-resolution power due to their high mutational rate and polymorphism. Tools for the analysis of the population admixture proportions (i.e. the proportion of contemporary gene pools derived from indigenous and cultivated stocks), and the individual admixture proportions (i.e. the proportion of the genome of individual fish derived from indigenous and introduced fish), have been developed (Chikhi *et al*., 2001; Pritchard *et al*., 2000). Using these methods, Hansen (2002) examined the long-term impact of stocking domesticated trout in brown trout populations of two rivers in Jutland, Denmark. He estimated that in one river there was small genetic introgression of domesticated trout (app. 6%) and the majority of individual were non-admixed, which was interpreted as a result of low fitness and poor performance of domesticated trout in the wild. In the other river, strong genetic contribution from domesticated trout (57 - 88%) was detected. In this case, survival of domesticated trout and admixture with indigenous fish in a local broodstock was invoked to explain the high degree of introgression. Moreover, since stocking can be regarded as migration and since domesticated fish can be removed from the natural environment by selection, introgression seems to be dependent on the interplay between the opposing forces of migration and selection (migration - selection balance). In this way, it was considered that the immigration rate (*m*) might be higher in the second river, while the selection coefficient (*s*) might be lower in this river. It should be noted that from the analysis of the historical samples, the local stocks in the two rivers were found not different from each other before the beginning of the stocking activities.

2.5. Molecular markers as predictors of fitness

Another critical question is to what extent the differences between the cultivated and wild populations, as estimated by the use of the molecular markers, which are considered as neutral (not subject to natural selection), can be considered as good predictors of differences in adaptive characteristics and thus of the differences in fitness. In a pioneer experiment, Áltukhov and Salmenkova (1987) compared the rate of return of chum salmon *Oncorhynchus keta* for three different pairs of populations, which differed in the genetic distance between the source and the recipient population. They found an inverse proportionality between genetic distance and rate of return. Even if the low number of pairs examined renders this result rather preliminary, an important finding from this study was that low success of transplanted individuals can occur, even when the genetic differences are small (Johnson, 2000). It has been suggested (Cross 2000) that markers under selection should be included in the studies for better understanding of the relationship between genetic divergence and fitness differences implying local adaptation in the populations.

2.6. Escapes of farmed fish

Several methods have been proposed to minimize the escapes of fish from aquaculture farms, under the understanding that complete avoidance of escapes is not feasible. Between them the following are included (Cross, 2000):

- Matching equipment, such as sea cages and moorings, to the environmental conditions of the site.
- Introducing sanctuary zones near spawning or nursery areas, where aquaculture activities are prohibited.
- Use of sterile animals in aquaculture by manipulation of sex and ploidy.
- Farming of natives whenever possible.
- Keeping healthy wild stocks, by avoiding overfishing and habitat deterioration.

It should be noted, however, that sometimes the biological and ecological characteristics of the species help to limit adverse genetic effects of escapes. This is true for both the main aquacultured Mediterranean species, the gilthead sea bream (*Sparus aurata*) and the sea bass (*Dicentrarchus labrax*) (Youngson *et al.*, 2001). Sea bream is a protandrous hermaphrodite species and the sex reversal occurs at the age of 2-3 years. This means that most of the fish in the farms are almost entirely males, since harvesting of fish is typically performed before this age. On the other hand, wild sea bream breed in lagoonal areas, a habitat rather rare in the Mediterranean. These facts mean that escaped males have to disperse for long distances, in order to breed with wild females. Sea bass often show a highly skewed sex ratio in reared stocks, and this means again that the majority of escaped fish are males. It is not known if they are capable of migrating in breeding areas and if they have synchronous maturity cycles with local fish so that interbreeding is possible (Youngson *et al*. 2001).

3. STOCKING WITH NATIVES (SUPPORTIVE BREEDING)

3.1. Dual aim

In the case of stocking the aim is twofold: to maximize the performance of the donor strain, while minimizing the detrimental effects on receiving population(s). The ideal would be to produce a large self -sustaining population by a single or a restricted number of interventions, but what is actually done in most cases is that hatchery fish are produced and released in each generation (Cross, 2000). The genetic effects on the wild stocks can be either direct, when genetic introgression occurs through interbreeding with natives, or indirect, when the introduced species or race outcompetes native species or races.

3.2. Choice of donor strain

The focal point in translocation for stocking is the choice of the donor strain. The translocation between locally differentiated groups of populations (races) should be avoided. Also, measures to avoid genetic changes, such as *inbreeding depression*, which quite often occurs in aquaculture practices, should be applied and artificial selection (genetic improvement) not be performed. Inadvertent *hatchery* or *domestication selection* is difficult to avoid, so strains to be used in stocking should not be kept in the hatchery for more than one generation (Cross, 2000). Often, in order to enhance the local wild populations, a number of wild breeders are brought into captivity and their offspring released into the wild environment. In this case, there is no introgression of alien genetic material and adaptation in artificial conditions can be minimal.

3.3. Effects of supportive breeding

The main problem with this practice, often described as *supportive breeding*, is the impact on the genetically *effective size* of the population. The effective size is the size of the population that actually counts for the maintenance of the genetic variability and the evolutionary fortune of the population. Usually the effective size is much lower than the census size of the population. It is equivalent to the size of an "equivalent" ideal population, that is of a population with a sex ratio of 1:1, random matings, non-overlapping generations and equal number of progeny per family. Deviations from these conditions of ideal population result in reduction of the effective size. Small effective population size has detrimental effects on the fitness characteristics of the population, which are known with the term *inbreeding depression*.

Supportive breeding increases the variance in family size, since the fish bred in captivity contribute much more offspring in the next generation than the fish that reproduced in the wild. Thus, supportive breeding may drastically reduce the effective population size, even if the census size is increased. The total effective size of the population equals the sum of the effective number of wild breeders and the effective number of the breeders artificially bred, only if the contribution of the offspring are proportional to the effective sizes of the parents. For example if the captive breeders are 1% of the wild breeders, then, only if the progeny born in captivity is the 1% of the progeny of the natural population, there is no reduction in the total effective size. Since the proportion of the captive progeny is usually much higher than that of the wild progeny, the effective population size is reduced, notwithstanding the fact that the census size of the population is increased. For example, if there are 200 wild specimens, 20 of them are bred in captivity and their released progeny contribute 40% of the progeny in the wild environment, then the effective size is reduced to 100 (Ryman *et al*., 1995).

Thus, supportive breeding results in a trade-off. There is a gain in the total production of progeny, but at the same time there is a loss of genetic variability, due to reduction of the effective population size. If a population has drastically decreased and is prone to extinction, its backing by artificial breeding is of course advisable, but care should be taken to avoid the problems related to the loss of genetic variability. Increasing as much as possible the proportion of the breeders bred in captivity is a measure that can be taken towards this end. Recent studies, however, have shown that decrease in the effective population size may not be necessarily a problem over multiple generations, if supportive breeding results in a substantial increase of the census population size (Wang and Ryman, 2001; Duchesne and Bernatchez, 2002)

4. CONCLUSION

The study of genetic population structure of natural populations and the estimation of the degree of divergence between wild and cultivated ones is a basic prerequisite of any programme that includes intentional release or the risk of accidental release of captive bred animals in the environment. The use of neutral genetic markers, even if does not provide direct information about genes controlling adapting characters can provide important insights for the degree of genetic differentiation of the populations.

The estimated degree of naturally occurring gene flow could be used as a measurement of the maximum allowable introduction of cultivated animals into the natural aquatic ecosystems. Special care to avoid introductions or escapes can be taken in the case that the reared populations are considerably divergent from the local natural ones, either as a result of their origin from a genetically differentiated race or population, or because of genetic changes brought by adaptation to artificial environment. The monitoring of genetic changes after the release should be an indispensable stage to be followed, in order to be able to ascertain the degree of genetic introgression into the natural environment. This will also allow for remedial measures to be taken. Eventual use of genetically modified organism (GMO's) in aquaculture should impose even greater measures to be taken to restrict escapes and monitor their impacts to natural populations.

Modern advances in the area of molecular genetics have opened new and unprecedented capabilities. The development of the hypervariable genetic markers, like microsatellite DNA, allows for the detection of even subtle genetic differentiations of populations and modern statistical tools offer the possibility of reconstructing the demographic histories of the populations.

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