Genetic Impacts of Marine Aquaculture Escapees

Risk Assessment and Management Recommendations

AquaTrace White Paper

Highlights and Summary

Why a risk assessment?

Aquaculture is key to meeting the escalating demand for fish worldwide, and is also becoming a major protein source and source of employment and income for the European Union (EU). Fostering sustainable aquaculture is one of the pillars of the Common Fisheries Policy and an important component of the Blue Economy and Blue Growth.

Europe represents the largest market for fish in the world and seafood consumption has steadily increased over past decades. Per capita consumption was estimated as 25.5 kg in 2014. On the other hand, the EU's self-sufficiency for seafood is currently estimated at only 47.5%, thereby necessitating high dependency on imported seafood for the EU market.

Thus, while EU aquaculture provides a major opportunity to enhance Blue Growth and to decrease the EU's dependency on seafood imports, the Scientific, Technical and Economic Committee for Fisheries (STECF), which advises the European Commission[1], confirms in its latest report[2] that aquaculture production is dominated by Asian countries, which are responsible for almost 90% of global production volume. In contrast, the EU-28 contribution to world aquaculture production has been decreasing significantly in terms of both volume and value, representing only 1.7% (by volume) and 3.2% (by value) of global production in 2014.

One impediment to enhanced EU aquaculture growth are concerns regarding the environmental sustainability of aquaculture activity. There is a clear need to reconcile aquaculture with existing environmental legislation, both national and EU. According to STECF, environmental regulations and inherent difficulties in the licensing process, due to multilevel governance and competition for space both on land and in coastal zones, continue to be the most important issues that require ongoing attention to increase growth in the EU aquaculture sector.

Foremost among these environmental concerns are fish escaping from their aquaculture production facilities ('escapees'), which typically pose a hazard to fish stock integrity and levels of biodiversity through genetic impact on wild populations of conspecifics. Escapees are a feature of aquaculture which can occur both acutely and chronically, e.g. through leakage. Restocking of wild populations with farmed fish can create similar risks.

[1] Commission Decision of 25 February 2016 setting up a Scientific, Technical and Economic Committee for Fisheries, C(2016) 1084, OJ C 74, 26.2.2016, p. 4–10.

[2] Economic Report of EU aquaculture sector (STECF-16-17) Edited by R. Nielsen, J. Guillen and N. Carvalho . JRC Scientific and Policy Reports.

In line with recent EU initiatives[3], AQUATRACE aims to support the growth of aquaculture activity by providing genetic and genomic tools that facilitate identification, risk assessment and monitoring of farm escapees, and of escape events. The latter, supported by the AQUATRACE risk assessment, is delineated below. The AQUATRACE risk assessment should become an integral part of EU aquaculture management and governance, facilitating compliance with environmental protection and conservation targets laid down in EU legislation.

The risk assessment described here is targeted at risk assessment managers and stakeholders, including the aquaculture industry, policy makers and authorities.

That the risk of genetic introgression from farmed animals into wild populations is real has been demonstrated repeatedly, as has the inherent risk that the fitness of wild populations 'infiltrated' by escaped conspecifics can decline. Experiments have shown that a single generation of domestication can alter the expression of hundreds of genes, and that even at early stages of domestication, adaptation to specific conditions in aquaculture may occur. In general, the fitness of farmed fish in the wild decreases with the number of generations in captivity.

In Norway, which is home to the most extensive aquaculture fish production in Europe, the genetic impact of farmed Atlantic salmon (*Salmo salar*) on their wild conspecifics are regularly assessed by a dedicated monitoring programme, the largest of its kind on escaped farmed fish in the world. These assessments refer to specified requirements that fish farming activities must not cause lasting genetic changes to wild populations. The assessments look into the likelihood of exceeding certain thresholds of environmental impacts, based on selected proxies such as the number and proportion of escaped salmon observed in the spawning grounds of wild salmon stocks, and on the level of actual introgression into wild stocks.

Using the Norwegian assessments as a paradigm, the AQUATRACE project aimed at quantifying the risks aquaculture pose to wild stocks of three European marine fish species which are intensively farmed in Europe. These are European sea bass (*Dicentrarchus labrax*), gilthead sea bream (*Sparus aurata*) and turbot (*Scophthalmus maximus*). The project was able to take advantage of the most up-to-date genetic and genomic analytical approaches.

This white paper starts out by looking at the populations at risk among the three target species. It includes a concise summary of the biology and exploitation patterns of teach species. Such collective knowledge is needed to enhance understanding of the vulnerability and tolerance towards natural and anthropogenic impacts.

The white paper continues by describing stock structures followed by a depiction of possible hazards emerging from aquaculture activities. Moreover, based on published evidence, new empirical data, simulations and modelling, it provides a preliminary assessment of existing risks emerging from exposure of wild animals to farmed fish due to release and escape events. Finally, based on compiled and reviewed existing knowledge and AQUATRACE research results, a set of recommendations is delineated.

The target species

The capture fisheries of all three target species are small in comparison with aquaculture production.

For European sea bass, capture fishing was until recently – apart from a few national rules – not regulated. In 2015 scientific analyses reinforced previous concerns about the stock decline as also observed by the International Council for the Exploration of the Sea. For commercial and recreational fishing of wild gilthead sea bream some restrictions are in place, but in general no management measures are implemented for wild stocks, except for fish, mostly juveniles, entering estuaries and coastal lagoons in the Mediterranean Sea. Turbot is an important by-catch species in demersal fisheries and is not considered endangered. Declines in wild catches and some genetic evidence suggest a historical reduction in population size, however.

In contrast to the sea cage farming of sea bass and sea bream aquaculture, turbot farming is almost exclusively land-based, in closed-recirculation systems during the early stages of cultivation and later in open-flow systems for-growing out. Genetic introgression in wild populations can occur though as farmed bloodstock is being used for restocking activities (see below).

[3] Communication from the Commission to the European Parliament and the Council, Building a sustainable future for aquaculture, A new impetus for the Strategy for the Sustainable Development of European Aquaculture (COM/2009/0162 final); Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions, Strategic guidelines for the sustainable development of EU aquaculture (COM/2013/229).

The AquaTrace 'Survey on selective breeding programmes in European Aquaculture' highlighted significant differences in selection efforts in the aquaculture industry. Some major seed-producing countries are still producing almost exclusively unselected material, or they use F2 animals, that is, animals of the second generation of a breeding program, derived from other commercial breeding programs. This is particularly the case for sea bass (Italy and Spain) and sea bream (Italy and Turkey). In contrast, sea bass, sea bream and turbot production in France, and turbot in Spain are produced mainly or exclusively from genetically improved seed.

Despite its rapid development, information on the impact of breeding programmes on cultured stocks remains limited. In 2004, a survey on 13 Italian hatchery broodstocks revealed no significant reduction in genetic variability compared with wild populations, indicating a reduced risk of genetic impacts on wild populations by farm escapees. However, different results obtained from Greece showed a significant reduction in the genetic diversity in farmed stocks. A similar loss of diversity was observed in Spain for farmed F1 progeny.

Selective breeding is expected to become more common, as it creates significant effective opportunities for aquaculture through enhanced productivity. This implies an increased drive for genetic differentiation between farmed and wild fish.

Genetic baseline results from AquaTrace

From extensive tissue samples, which covered of fish collected across a large part of the native distribution range of all three target species, DNA based genomic markers were developed and used to genotype the sampled individuals, to characterise the wild and farmed populations and signs of introgression.

European sea bass is composed of three main wild population groups, an apparently almost homogenous population in the north-eastern Atlantic, and two Mediterranean populations which are population groups separated at the Siculo-Tunisian Strait. The eastern Mediterranean sea bass is genetically split into several distinct subpopulations while its western counterpart is relatively homogenous and shows closer affinity to the eastern Mediterranean than to the Atlantic group. In the western Mediterranean Sea there is some evidence for introgression of the Atlantic lineage. Off the Strait of Gibraltar there is also evidence for natural introgression of the Mediterranean lineage into the Atlantic population.

Farmed populations show a range of genetic diversities, reflecting the broad origins of the source material, breeding practice and selection pressure, and resulting in some cases in pronounced genetic differentiation between farms. There is evidence of genetic material from the Atlantic Ocean in Mediterranean farms and of Mediterranean material in one Atlantic farm. It is not feasible to identify a unique individual genetic profile for each farmed sea bass. However, some genetic characteristics point to unique sources of domesticated genetic material.

Some wild samples show evidence of escapees, with high proportions of individuals closely related genetically to each another. Mediterranean samples show higher numbers of related individuals (likely escapees) per sample than Atlantic samples.

In gilthead sea bream the results suggest the existence of genetic differentiation between Atlantic and Mediterranean population groups. and, within the Mediterranean, between Western Mediterranean, Ionian and Aegean Sea populations. The latter appear to be the most differentiated population. The population in the north Adriatic Sea were found to be more similar to those of the Greek Ionian Sea, and the population from the Italian Ionian coast was more similar to the West Mediterranean population.

The possibility that both genetically close or very different fish may escape or be released, and that both leakage and mass escape events are likely to occur, results in the requirement for a localised case-by-case assessment in order to elucidate the specific rates of introgression and fitness risks.

In turbot, four main genetically differentiated regional groups of fish across the distribution range have been identified: fish from the Atlantic area, mostly compatible with a large homogenous population; the Baltic Sea, showing moderate differentiation from the Atlantic area; and the Mediterranean and Black Sea, highly differentiated from the Atlantic region.

Farmed turbot is capable of surviving and reproducing in the wild, leading to introgression in wild populations. This introgression is likely to have occurred over several generations. A set of genetic markers was developed to monitor escapes or releases from farms and to evaluate introgression in wild populations of turbot. Pure farm and introgressed individuals may represent close to 15% of individuals collected in some wild populations. The impact is related mainly to intentional releases, i.e. restocking, intended to supplement depleted fisheries in threatened coastal habitats, although a minor risk could be associated with farmed escapees.

Modeling results transferred from salmonids

Salmonids are the most affected fish when it comes to introgressive hybridisation. This is due to widespread exposure to genetically divergent farmed fish, mainly from escapes; the Atlantic salmon is emblematic of this problem. As a group, salmonids have therefore been intensively studied in attempts to clarify how hybridisation and introgression with farmed fish affect wild populations, serving as a model system for less well-studied marine fish species.

As experiments with salmonids as the model species indicate, non-native strains generally show maladaptation compared with native populations, and hence introgression, will usually reduce fitness in wild populations. For the marine target species, farmed fish may genetically be very similar to or very different from their wild conspecifics, thus the prediction of fitness effects caused by escapees in wild populations will be population and scenario specific.

The majority of results obtained in model studies with salmonids are also valid for the marine target species. Firstly, hybridisation between escaped farmed fish and wild fish will lead to genetic changes that can be assessed using genetic markers. Secondly, introgression can be monitored using genetic markers. That is, the degree of genetic change caused by introgression can be determined, and likewise, whether introgression leads to a general reduction in genetic variation of wild populations.

Evidence from salmonids also shows that vulnerability and tolerance can be determined on a case-by-case basis (whether escapes from specific farms at specific magnitudes are likely to incur fitness costs to wild populations). Predictions will be most accurate if information can be obtained on (1) the genetic identity and variability of farmed strains and wild populations, (2) the structure and size of wild populations, and (3) the frequency, volume and age structure of escapes.

Factors likely to affect impact of introgression in salmonids can be directly transferred to marine fish, and mitigation actions can be planned to address them. They include the genetic differences and heritable trait differences between wild and farmed fish, the frequency of intrusion and the relative frequency of escaped to wild fish in spawning grounds. Efforts to avoid introgression can also be prioritised, incorporating aspects of conservation and economic goals. It would be possible, for example, to conduct a spatially explicit assessment of whether the use and escapes of specific farm strains is more likely to inflict genetic damage in some areas than others, e.g. in areas inhabited by vulnerable, genetically unique populations. The usefulness of 'indicator-based management systems', as suggested for Norwegian Atlantic salmon, could be examined.

Knowledge gaps

Knowledge of the factors impacting introgression has until recently been very limited for all three marine target species, and AquaTrace has contributed to addressing relevant knowledge gaps.

In contrast to most countries with extensive salmon aquaculture, notification of escape episodes is not officially required in EU countries with substantial aquaculture production of sea bass, sea bream and turbot. Hence, escapes episodes from the during various stages of production, from hatcheries to grow-out cages, remain mostly unreported in terms of geographical and temporal distribution, estimated number of cases and individuals, production stage, frequency or closeness to spawning grounds.

Intentional stocking can be considered to be the main source of release of turbot into the wild. Experimental wild stock enhancement with farmed turbot has been conducted in Belgium, Denmark, Norway and Spain. With the exception of Spain, and perhaps Denmark, no specific information exists on the use of marine fish broodstocks for population enhancement.

The general lack of data on escape events does not allow for a comprehensive evaluation of long-term survival of escapees and associated degree of exposure to wild conspecifics. On the one hand, it is possible that the cumulative impact of escapees may be substantially reduced by the high mortality rate observed, typically occurring after escape. On the other hand, and as is the case with Atlantic salmon, even a modest survival rate after escape might entail negative fitness consequences for wild populations.

An additional source of concern lies in the spawning in sea cages during the grow-out phase of farmed fish. Recent studies in Cyprus indicate that substantial numbers of female sea bass do mature in sea cages under normal farming conditions. Spawning during the grow-out phase has also been observed in gilthead sea bream in floating cages at sea, which, may lead to continuous release of eggs and juveniles into the environment. This presents a potential source of escapees through spawning. The impact on natural populations is therefore difficult to assess.

General conclusions

The current knowledge on genetic structure and dynamics of the AquaTrace target species does not allow a single quantitative risk assessment covering the entire distribution area across European waters.

In all three species, broodstocks from distant populations are used for breeding, and various levels of selective breeding is applied among the hatcheries. Release and/or escape events of farmed fish occur, though the frequencies and magnitudes of release and/or escapes cannot be quantified precisely for any of the examined target marine species. For turbot, rough estimates of escapees and intentional releases are available. As both genetically close or very divergent fish may escape/be released for all three species, and that both leakage and mass escape events are both likely to occur, (sea-bass and sea bream), a more profound evaluation would require a case-by-case assessment of introgression and fitness risks at the level of local aquaculture regional clusters.

Continuous genetic monitoring would constitute a highly valuable asset allowing a temporal assessment of the degree of genetic change and genetic variation in wild populations and farmed fish. This would be a prerequisite for the assessment of introgression and the implementation of quantitative risk assessments.

The AquaTrace consortium has taken on the challenge of developing a traceability toolbox for European sea bass, gilthead sea bream and turbot. The tools will be very useful for future monitoring of releases and hatchery practices, and for appropriate management with the aim of preserving wild resources in a sustainable aquaculture framework.

For the time being, on the basis of the available knowledge, AquaTrace proposes the use of a semi-quantitative evaluation grid for risk assessment purposes in order to support risk managers responsible for aquaculture activities at a local level.

Recommendations for risk management and research

Based on its research results and the knowledge gaps identified, the following recommendations are provided in order to enable a quantitative assessment of genetic risks emerging from marine farmed fish that can be integrated into management frameworks underpinning sustainable and profitable aquaculture:

R1. Management goals: There is a need to formulate management goals linked to potential genetic risks emerging from EU aquaculture, goals which set thresholds against which such risks could be evaluated and assessed. The management goals established for Norwegian aquaculture could serve as an orientation paradigm. An example is the development of species-specific indicators and threshold values for acceptable levels of farmed fish in wild populations. Until further progress in this field, AquaTrace strongly recommends applying the **risk assessment management support actions** depicted below under R8.

R2. Notification of escape events: Establishment of an EU-wide harmonised compulsory and immediate notification requirement of escape incidents from marine aquaculture installations, covering production from hatcheries to harvest should be prioritised This should include date of event, location, number of cages affected, age group of fish and number of fish, following established enforced requirements in Ireland, Norway and the United Kingdom.

R3. Recording of stocking events: Stocking actions should be documented and recorded on a mandatory basis. The documentation should encompass a comprehensive record including date of event, location, level of location confinement and origin, number and size/age of fish used.

R4. Research on populations at risk: Scientific studies and assessments on populations at risk should be pursued and enhanced. This is particularly relevant for stock assessments in the Mediterranean Sea, identifying the main spawning grounds and improving knowledge on the breeding behaviour of sea bass and sea bream, but also for turbot in the Atlantic region (North Sea).

R5. Trade of eggs and juveniles: A traceability scheme for the trade and exchange of fish eggs and juveniles should be established.

R6. Risks to confined stocks and aquaculture areas: The risk assessment approach should be refined to address confined wild stocks and aquaculture areas. To this end, regional monitoring programmes should be established taking advantage of the genetic traceability toolbox for European sea bass, gilthead sea bream and turbot developed by AquaTrace.

R7. Model studies: The evidence of introgression observed in farmed marine fish demands strongly a direct evaluation of the fitness impact on wild populations through common garden experiments, similar to those carried out in Atlantic salmon and brown trout by AquaTrace.

R8. Risk assessment management support: Implementation of decision thresholds that are easy to apply and will help to establish a more robust knowledge baseline about farm escapees and their impact, as well as to contain possible risks. Levels of below 5%, between 5% and 20% and above 20% could serve as general rules to define actions regarding the intensity of monitoring. These levels could be refined as more specific knowledge on genetic risks (levels of integration and fitness loss) is gained for species and geographical regions. This is further delineated in Table 1 and 2.

Risk of genetic change of wild population due to introgression	Threshold value for escaped fish [%] of identified escapees among the fish sampled in the wild]	Action
Low	<5%	Monitoring: Sampling and analysis at two-year intervals
Moderate	5-20%	Monitoring: sampling and analysis at 6 month intervals
High	>20%	Management Action: Identification of exact source and cause. Application of table 2 in a collaboration between risk managers and geneticists.

Table 1 Risk Assessment Management Support Table. The application of this table should assist the decision finding on actions needed to contain risks inherent to aquaculture escapees. The implementation and application of this table will also contribute to the enhancement of the knowledge base needed to foster increasingly robust risk assessments and management measures.

Factor (question point in decision tree)	Relevant variables (information required)
Is fish farming extensive or intensive?	•Number of generations
	•Ne (Intensity of selection)
Are escapees likely to be from a different genetic population to the local wild fish?	•Geographic source of broodstock
	•Level of geographic genetic structure in wild
	•Level of gene flow between wild and farmed stocks
Are farmed fish likely to show different adaptations to local wild fish?	•No. generations
	•Purpose and degree of selection
	•Ne (selection intensity)
	•Heritability of traits
Are farmed fish escapees likely to reduce diversity of natural populations?	•Wild population diversity (nucleotide diversity and individual heterozygosity)
	•Farmed population diversity (nucleotide diversity and individual heterozygosity)
Does the wild population have capacity to absorb escapees?	•Estimates of census and effective wild population size
	•Escape scenarios – how many fish might escape?
	•How do fish mix and disperse regionally?

Table 2 Management Action Support Table to be applied if the risk of genetic change of wild population due to introgression has been assess as being 'High'. Risk managers and geneticists can address a number of relevant variables to define the exact source and cause of escape events as well as to assess in greater detail the level of risks and expected impact.

For more detailed information consult the **AquaTrace website** (https://fishreg.jrc.ec.europa.eu/web/aquatrace) where the consortium put together the most updated information on the project.