Review of the genetic and genomic information regarding the potential genetic interaction and impacts of aquaculture on native fish stocks

Preparatory work for the risk assessment in the frame of the FP7 project AquaTrace

Johann Hofherr
Jann Th. Martinsohn

The project is funded by the 7th Framework Programme for research (FP7) under "Knowledge-Based Bio-Economy - KBBE", Theme 2: "Food, Agriculture and fisheries, and Biotechnologies"
Project identifier: FP7-KBBE-2012-6-singlestage
Grant agreement no.: 311920
Funding scheme: collaborative project
Abstract
The present report aims to review knowledge and resources from previous research relevant for the assessment of risks emerging from aquaculture, in particular with a view on the three commercially important target species gilthead sea bream (Sparus aurata), European sea bass (Dicentrarchus labrax) and turbot (Scophthalmus maximus) in the context of the FP7 project AquaTrace.

The limited information on escapees and of genetic and genomic knowledge in the target species result in specific challenges for a risk assessment which are addressed here. In particular the development of management goals and assessment endpoints, indispensable to any robust risk assessment are discussed.
Table of Contents
Abbreviations ................................................................................................................................. 4
Executive Summary ............................................................................................................................ 5
Introduction ........................................................................................................................................ 6
Objective of the present report ........................................................................................................... 8
Knowledge review ............................................................................................................................. 8
  Escapes from aquaculture structures ...................................................................................... 8
  Cause of escapees and costs .................................................................................................. 10
  Behaviour of escapees ........................................................................................................... 12
  Factors for introgression ....................................................................................................... 14
  Identification of escapees ...................................................................................................... 15
  Management practice ............................................................................................................. 16
The species subject to the risk analysis .......................................................................................... 18
  Gilthead sea bream (Sparus aurata) .................................................................................... 18
    Population structure .......................................................................................................... 18
    Breeding practice ............................................................................................................... 19
    Genetic resources and selective breeding ...................................................................... 20
    Escapees and restocking - interaction with wild stocks ................................................ 21
  European sea bass (Dicentrarchus labrax) ........................................................................... 22
    Population structure .......................................................................................................... 22
    Breeding practice ............................................................................................................... 22
    Genomic resources and selective breeding .................................................................... 24
    Escapees and restocking - interaction with wild stocks ................................................ 24
  Turbot (Scophthalmus maximus) ........................................................................................ 25
    Population structure .......................................................................................................... 25
    Breeding practice ............................................................................................................... 25
    Genomic resources and selective breeding .................................................................... 26
    Escapees and restocking - interaction with wild stocks ................................................ 27
Next steps .......................................................................................................................................... 27
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFP</td>
<td>Common Fisheries Policy</td>
</tr>
<tr>
<td>EBM</td>
<td>Ecosystem-Based Management</td>
</tr>
<tr>
<td>EST</td>
<td>Expressed Sequence Tag</td>
</tr>
<tr>
<td>FA</td>
<td>Fatty Acid</td>
</tr>
<tr>
<td>GES</td>
<td>Good Environment Status</td>
</tr>
<tr>
<td>JRC</td>
<td>Joint Research Centre</td>
</tr>
<tr>
<td>IMR</td>
<td>Institute of Marine Research</td>
</tr>
<tr>
<td>MAS</td>
<td>Marker Assisted Selection</td>
</tr>
<tr>
<td>PCA</td>
<td>Principal Component Analysis</td>
</tr>
<tr>
<td>QTL</td>
<td>Quantitative Trait Loci</td>
</tr>
<tr>
<td>RAPDs</td>
<td>Random Amplified Polymorphic DNA</td>
</tr>
<tr>
<td>SNP</td>
<td>Single Nucleotide Polymorphism</td>
</tr>
</tbody>
</table>
Executive Summary

Aquaculture in the EU provides potentially major opportunities for Blue Growth and can contribute to food security. However, compared to many other regions in the world, the growth of EU aquaculture activity is dim.

There exist a number of reasons for this lack of aquaculture expansion, one of them being concerns about the environmental impact generated by fish farms. A major issue in this context is the escape of farmed marine fish into the wild, where they could interbreed with their wild counterparts, thereby lowering the fitness of wild populations.

The FP7 project AquaTrace applies state-of-the-art genomic analyses to develop tools enabling traceability of the major commercial farmed marine species sea bass, sea bream and turbot, to assess the current genetic diversity of their natural populations and to identify traits that change due to selective breeding (domestication).

This research also provides the possibility to carry out a risk analysis, assessing the potential impact of farmed escapees on wild populations, thus helping to adjust aquaculture management such that risks are contained as much as possible.

This review concisely summarises current knowledge on the impact caused by escaped farmed fish, and illustrates the structure and aims of a risk assessment through the example of farmed Atlantic salmon in Norway.

While Atlantic salmon is a very well-studied species, for which a considerable amount of information on escapees and of genetic and genomic knowledge is available, this is not the case for the AquaTrace target species. This results in specific challenges for a risk assessment which are addressed here. In particular, the development of management goals and assessment endpoints, indispensable to any robust risk assessment are discussed.

Additionally in contrary to Norway the legal framework underpinning aquaculture activity in the EU is not extensive. This review identifies EU-legislation with relevance for aquaculture, which can also be used as a reference for the risk assessment, namely the Common Fisheries Policy basic regulation and the Marine Strategy Framework Directive.
Introduction

Aquaculture production is on a steep rise worldwide. It will be key to meet the escalating demand for fish worldwide, ensure food security, will contribute to employment. This is why, also on EU level much attention is paid to how a thriving EU aquaculture industry can be developed. However, to be sustainable, EU aquaculture has not only to be economically viable, but also compatible with the surrounding ecosystem and socially acceptable.1,2

Fostering sustainable aquaculture is one of the pillars of the Common Fisheries Policy (CFP)3. Concerning environmental aspects, Ecosystem-Based Management (EBM) has, since 20034,5, been an integral part of the CFP, and considerations emerging from the EBM include as well wild capture fisheries as aquaculture. An important corollary of the EBM is the fundamental requirement to conserve the integrity of exploited fish stocks and their natural environment. Any fishing or aquaculture activity that threatens to disrupt such integrity needs to be identified, and measures for mitigation implemented. The basic principles of EBM have further become entrenched within the Marine Strategy Framework Directive (MSFD)6 descriptors for monitoring of progress towards Good Environment Status (GES).

In addition to overexploitation of capture fisheries, the integrity of wild stocks can be impacted by aquaculture practices. Foremost among the concerns in relation to "environmentally friendly" is the issue of fish escaping from their aquaculture production enclosures (‘escapees’), which pose a hazard to the integrity and levels of biodiversity both through direct competition for resources and through genetic ‘pollution’ of local populations of conspecifics.7 These escapees are a feature of aquaculture that can occur

1 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 final
4 Council Regulation (EC) No 2371/2002 of 20 December 2002 on the conservation and sustainable exploitation of fisheries resources under the Common Fisheries Policy
both acutely and chronically (e.g. through leakage\(^8,^9\)). Restocking with farmed fish can constitute similar risks.\(^10\)

To respond to the rising concerns about aquaculture impact on the environment, but also due to the costs arising for producers from farm escapees (see below), there is an urgent need to identify methods that allow identifying farmed fish, but also to enable assessment and monitoring genetic effects of aquaculture escapees on wild populations. To that end it is necessary to establish tools which allow discriminating between marine fish populations on one hand and between farmed and wild animals on the other hand. Ideally such tools would also allow tracing back farm escapees to their farm of origin. However, despite recent progress (e.g. Glover et al. 2008\(^11\), Glover 2010\(^12\)) the identification of marine fish populations remains, primarily owing to standard genetic markers, exhibiting relatively low levels of population differentiation. Accordingly, there are yet few coordinated databases that allow collation of information on population boundaries and dynamics of populations beyond local scales, although exceptions exists for a database on European-wide population genetic signatures of four marine species, Atlantic cod, herring, sole and hake that has been established by the international consortium FishPopTrace with JRC involvement\(^13\). Discrimination between wild and farmed strains of marine fishes is further complicated by the fact that each farmed strain may have its own history of selection and domestication, sometimes including recurrent backcrosses to wild-origin brood-stock, thereby blurring the genetic distinction between wild and farmed animals. These breeding processes have typically not been documented, and may mask the frequency and direction of interactions.

While genetic changes in wild populations over time have been demonstrated, it is more difficult to prove that such changes have been the consequences of interbreeding with escaped farmed fish. The recent technological advances in development and application of genome-wide Single Nucleotide Polymorphism (SNP) markers in fishes offer a highly promising approach that can be specifically employed at tracing the genetic origin and potential hybrid status of individual fish in a wild/farm context in terms of genetic impacts and magnitude of such impacts.

Based on these recent SNP marker techniques, the genetic impacts of farmed Atlantic salmon are regularly assessed in Norway. As a result of the biggest monitoring program for escaped farm salmon worldwide, estimates of proportions of escaped farmed salmon in wild populations exist for ca. 100 Norwegian salmon rivers from 1989 to the present. There are large differences between rivers with respect to magnitude and trend in proportion of escapees, and the effect the escaped farm salmon may have had on the wild populations. The Institute of Marine Research (IMR), Norway, conducted risk

---


\(^13\) https://fishpoptrace.jrc.ec.europa.eu/
assessments on the environmental impact for different rivers in Norway since 2010. These assessments build on goals specified in the Ministry’s “Strategy for an Environmentally Sustainable Norwegian Aquaculture Industry” from 2009. The strategy for sustainable aquaculture expressly states the goal 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 on the spawning grounds of wild salmon stocks and on the level of actual introgression into the wild stocks as quantified by SNP markers.

These works are a valuable paradigm for the envisaged risk assessment under the AquaTrace project which will align to general principles of environmental/ecological risk assessment procedures and methods proposed for coastal aquaculture.

Objective of the present report
The present report aims to review knowledge and resources from previous research relevant for the assessment of risks emerging from aquaculture, in particular with a view on the three commercially important target species gilthead sea bream (Sparus aurata), European sea bass (Dicentrarchus labrax) and turbot (Scophthalmus maximus) in the context of the FP7 project AquaTrace.

Knowledge review
This chapter assesses available data on escapees, especially the impact of introgression into wild populations, degree of introgression as monitored with new molecular tools, the potential impact on the genetic structure of the wild populations, and ecological consequences with focus on potential changes in life-history traits and fitness.

Escapes from aquaculture structures
Several countries, such as Norway, Scotland and Ireland have mandatory reporting requirements whereby farmers are obligated to report escape incidents, their size and number.

15 K.A. Glover, C. Pertoldi, F. Besnier, V. Wennevik, M. Kent, Ø. Skaala; Atlantic salmon populations invaded by farmed escapees: quantifying genetic introgression with a Bayesian approach and SNPs; BMC Genetics 2013, 14:74
cause when they occur. In contrast, Mediterranean countries have no such requirements, thus no statistics are available on the number of escapes or the underlying causes of escapes.\textsuperscript{21, 22, 23} Also on EU level no such provision exists.

Most comprehensive information on fish escapes from aquaculture installations derive from Atlantic salmon production in Norway where the mandatory reporting of escape incidents was introduced in the 1980s and a national statistic of escape incidents was established in 2001. Annually, several hundred thousand individuals of Atlantic salmon escape from fish farms in Norway. A technical standard for sea cages followed in 2004 (NS9415, Standards Norway 2009) in order to prevent or minimize escape incidents. To assess the risks of farm escapes on the wild populations of Atlantic salmon, the MR Norway conducted initial risk assessments of the environmental effects of Norwegian Aquaculture\textsuperscript{24, 25}. The analysis of recorded escape events in Norway since 1989 suggests that during the 1990s on average more than 20\% escaped farmed salmon constituted of wild spawning populations, and between 10 and 20\% during the last decade.

The first step to mitigate potential impacts of farmed fish on wild populations is to lower the probability of escape events. The FP 7 project \textit{Prevent Escape (2009-2012, www.preventescape.eu)} involved on a pan-European scale a combination of approaches: fish ecology, fish behaviour, genetics, cage technology etc. to improve the understanding of the escaping process at the stages before and after and to improve the design of the farms and the management in order to minimize the number of escapees.

The research focused on sea-cages and their immediate surrounds, technical and operational causes of escape incidents, the extent of escapes of reproductive gametes and fish, determined the inherent behaviours that predispose certain species of fish towards a higher probability of escaping, and documented the dispersal of escapees to develop and test recapture strategies. Information from these components of the project feed into research specifically aimed at benchmarking the performance of equipment under farming conditions and thereby improving operations and equipment production, and advancing national and international standards for the design, construction and use of aquaculture equipment.

Thus far, the Prevent Escape project has assessed the extent, cause and costs of escape on a Europe-wide basis. Escapes may be more extensive and costly to producers than previously believed, highlighting the need for advances in farming technologies and operations. The project has also developed: 1) information on the inherent behaviours of fish within cages to assess which species may be more pre-disposed to escapes through holes; 2) practical, cost-effect methods to traces escapees in natural populations; 3) information on the immediate post-escape behavior of salmon, cod, sea bream, sea bass and meagre; 4) knowledge of the extent of spawning within sea-cages by cod, sea bream and meagre; 5) information on the wear and tear sea-cage equipment is subject to under farming conditions; and 6) suggestions for design modifications to make sea-cage farming

\textsuperscript{22} Scottish Statutory Instruments 2009; the Aquatic Animal Health (Scotland) Regulations 2009, Part 4A (http://www.legislation.gov.uk/)
\textsuperscript{23} Irish Statute Book - S.I. No. 253/1996 - European Communities (Aquaculture Animals and Fish) (Placing on The Market and Control of Certain Diseases) Regulations, 1996
\textsuperscript{25} K.A. Glover, C. Pertoldi, F. Besnier, V. Wennevik, M. Kent, Ø. Skaala; Atlantic salmon populations invaded by farmed escapees: quantifying genetic introgression with a Bayesian approach and SNPs; BMC Genetics 2013, 14:74
equipment more robust to escape events. These key pieces of information will allow determination of practical, implementable measures to prevent escapes and mitigate the effects of escapees, the underlying rational being that efficient prevention and mitigation lead to diminished genetic and ecological impacts and lowered costs.

The FP6 project Genetic impact of aquaculture activities on native populations – GenImpact 26 reviewed existing knowledge necessary to assess genetic effects of aquaculture on marine biodiversity and the ecosystem, identified future research needs, and disseminate this information to a wider public.

Another project - QuantEscape - started in 2012 with the objective of quantifying genetic effects of escaped farmed salmon on wild salmon populations. The project comprises four Norwegian research institutions that study interactions between escaped farmed and wild Atlantic salmon from different perspectives: population genetics, ecology, genomics, and quantitative genetics. The knowledge gained should promote sustainable management of farmed and wild Atlantic salmon, and benefit the aquaculture industry and wild salmon fisheries by providing guidelines for sustainable aquaculture.

The FP7 project FISHBOOST intends to boost selective breeding in aquaculture. Starting in 2014 it is a collaborative research project targeted to small and medium-sized enterprises (SMEs). Optimum breeding programs should be determined for each of the six species Atlantic salmon, common carp, European sea bass, gilthead sea bream, rainbow trout and turbot. One of the tasks is to quantify the genetic basis of disease resistance traits for important diseases of six target species. The genetic architecture of the traits will be evaluated with the use of genomic tools.

In summary it is generally acknowledged that farm escapees pose a serious challenge to sustainable and profitable aquaculture, which is addressed on one hand by the development of technical solutions to contain the risk of escape incidents, and on the other hand on genetic studies to understand the impact of farm escapees on wild populations and to develop traceability tools.

Importantly, as also hinted at above, the genetic/genomic research has added value for the aquaculture industry as it helps to enhance current breeding strategies and to identify genetic markers linked to favourable traits such as disease resistance.

**Cause of escapees and costs**

Escapes of farmed fish from net cages are a common phenomenon caused by severe environmental conditions and operational failures, which normally leads to mass escape events, and defects in material which leads to constant low frequency escapes (‘leakage’)(Jensen et al., 201027). Jackson et al. 201228 analysed in the Prevent Escape project extent and causes of escapes from marine finfish farms in six European countries; Ireland, Norway, Scotland (UK), Spain, Greece and Malta. They documented the extent, size and knowledge of the causes of escapes over a three year period. There were a total of 255 escape incidents

---

identified through questionnaires across the 6 countries (Ireland, UK, Norway, Spain, Greece, and Malta), and other data supplied by the Norwegian Fisheries Directorate and the Scottish Aquaculture Research Forum. A total of 8,922,863 fish were reported to have escaped from the 242 incidents. Sea bream accounted for the highest number of escapes at 76.7% followed by Atlantic salmon at 9.2%. Of the 113 Atlantic salmon escape events almost 75% were due to Structure failure or Operational error. Almost 50% of Cod escape incidents were due to biological causes e.g. biting of nets. The costs of escapes as calculated by value at point of first sale were very significant, estimated at approximately €47.5 million per annum on average over the study period. Of this €42.8 million was for annual cost of losses of sea bass and sea bream in the Mediterranean, representing ca. 6% of the value of both species produced by EU aquaculture, and €4.7 million for losses of salmon in northern Europe.

Mooring failure and breakdown of steel cages have previously together with net failure been the dominant cause of reported escape from Norwegian farms (Jensen et al., 2010). Approximately two thirds of reported escape incidents and number of fish escape are due to holes in the nets. Multiple reasons exist for the formation of holes. Biting by predators or caged fish, abrasion, ‘collisions’ with boats, flotsam and cage handling procedures (e.g. lifting) are among the most common causes of holes in the nets. The trend in Norway is that fish farms are moving into areas with stronger and steadier currents in order to improve water quality. As a result, forces on the net increase and net deformation increases accordingly (Lader et al. 2008). Several large-scale escapes have occurred over the past years due to contact (and thus abrasion) between the net and the sinker tube chain (Moe 2008). Experimental studies under the Prevent Escape project revealed that even at mid-range current levels without any waves present there is a strong possibility of contact between sinker tube chain and net cage independent on choice of

---

net geometry and weight systems and new and improved systems for maintaining the shape and volume of the net cage would be required (Lader et al., 2013). Apart from episodes of escapes of farmed fish, recently the escape of fertilized eggs spawned by farmed cod from sea-cages came into focus (Jørstad et al. 2008, Uglem et al. 2012). Studies from Somarakis et al. (2013) demonstrated that gilthead sea bream eggs were spawned in farms and released to the environment, although fecundities in the grow-out phase were low relative to commercial brood-stock and decreased considerably with sex ratio (i.e. with fish size in the cage). If 5–10\% of the 130 000 tons of annual sea bream production is fish of larger size, they estimated that some $3.5 \times 10^{11}$ to $7.0 \times 10^{11}$ eggs of gilthead sea bream could be released each year from farms into the Mediterranean Sea. Whilst survival rates of fertilized eggs in the vicinity of the sea-cages were low, the findings imply that the escape by spawning of sea bream may have ecological consequences, which likely depend on the sex ratio and intensity of farming within specific regions.

According to Thorstad et al., 2008 information on low-level leakage and escapes from freshwater hatcheries remains uniformly poor and the threat from such freshwater escapes is generally insufficiently recognized. Fisher et al. 2014 assumed that a significant number of escapees went unreported by the salmon farming industry partially due to the continuous leakage. Interestingly, in a recent study based on a modelling approach coupling genetic and demographic dynamics to evaluate alternative management approaches to minimizing unintended consequences of aquaculture escapees, it was demonstrated that reducing escapees through low-level leakage is more effective than reducing an analogous number of escapees from large, rare pulses.

**Behaviour of escapees**

Extended studies on the behavior of farmed species such as salmon and cod (Norwegians Fisheries Directorate: Norwegian aquacultures Statistics 2007; Moe et al., 2007; Hansen et al., 2009; Jensen et al., 2010) exist. Escaped farmed fish attempted...
to join spawning shoals post-escape, but did not appear to develop the same long-term fidelity to spawning grounds as wild cod.

Experiments from Damsgård et al. 2012 revealed that escape behaviour in Atlantic cod was not random, but linked both to net traits, and the motivation to get to a food source outside the net. The observed behaviours were significantly related to hunger level and feeding motivation, and increased when the food source was present outside the net wall, and decreased again when the food source was removed. This behavioural change was only observed in net walls with holes and damages, compared to only minor differences on nets with no food, or on control nets without damages. The Atlantic cod inspected and bit the net systematically, focusing to a large extent on loose treads or minor damage to the net. The fish were capable of causing severe net damage within a few days. Net repair type had a significant effect on inspection and biting, with both behaviours recorded as most frequent for nets repaired with the highly visible, black thread (a contrast colour to the white nets).

A previous study of escape behaviour indicated that Norwegian coastal cod might have higher escape risk compared to north east Arctic cod, and that these traits are strongly linked with individual exploratory traits (Hansen et al., 2009).

Meager et al. 2010 examined the spatial behavior on the spawning ground of escaped farmed cod and the potential to interact and hybridize with their wild conspecifics. They concluded that hybridization between farmed and wild cod is likely in the short term, and farmed females the most likely vector for genetic introgression.

P. Arechavala-Lopez et al. (2012) examined the post escape behaviour of sea bream and sea bass. In sea bream they observed in most cases a high degree of farm-site fidelity. However, a high mortality rates was also observed, probably due to the abundant predators presented beneath cages, such as bluefish (Pomatomus saltatrix), or a high fishing intensity around farms as it was shown by the high recapture rates. Moreover, a high connectivity among farms and local fishing areas was demonstrated through the movements of escapees which dispersed from the release farm. Furthermore, those escapees showed a high trophic flexibility and ability to feed on their most common natural prey in their native habitats after a short period of time in the wild. The study concluded that even a modest survival after escape might entail negative ecological consequences for wild populations but local fishermen might reduce this negative impact. Similar to their observations in sea bream, their results indicate that also sea bass moved fast and repeatedly among fish farms and the immediate mortality of escaped seabass may be high in the wild. They concluded that more knowledge regarding survival and movements of escapees following large-scale escape incidents is required to evaluate the potential for negative ecological impacts due to escaped farmed sea bass.

References:

The escape behavior of the European sea bass (*Dicentrarchus labrax*) is related to the conditions of the outer environment of the aquaculture net cage, in particular, natural or a constructed obstacle close to the net (Papadakis *et al.*, 2013).

Glaropoulos *et al.* 46, concluded from their experiments with gilthead sea bream under the Prevent Escape project that feeding condition is strongly related with fish’s motivation to escape. The highest escape rate observed (1%) is related to feeding condition and can be attributed to fish activity of the tank due to intensified prey searching. The frequency of escapes was increased at the latter phases of the experiment, which correlates well with related studies indicating that intensified prey search is proportional to the hunger level of fish in other species (Yacoob & Brownman, 2007; Hansen *et al.*, 2009). Furthermore prolonged starvation is required to cause major behavioral changes that can lead to an increased motivation to escape.

**Factors for introgression**

A model for how escaped farmed salmon may affect a wild population has previously been developed (Hindar *et al.*, 2006; Hindar *et al.*, 2007; Diserud *et al.* 2010; Diserud *et al.* 2012). This model is based on estimated proportions of escaped farm salmon in the spawning populations over time, the reproductive success of escaped farm salmon, and estimated survival rates for wild salmon juveniles, farmed offspring and hybrids in controlled natural and semi-natural settings (Fleming *et al.* 2000; McGinnity *et al.* 2003). The model estimates a decline in the wild salmon stock, as well as the proportions of the recruits, after each spawning, that have a wild salmon background, a farm salmon background, or a mix of the two. Factors controlling farmed to wild gene flow in Atlantic salmon populations identified are, e.g. the river specific environment and population characteristics that may influence to what extent escaped farmed salmon, and their offspring pass on their genes to wild Atlantic salmon populations. Some potential environment and population factors controlling the gene flow are water flow regime, migratory complexity and obstacles, spawning population density, phylo-geographic history and possible differences in spawning time and body size between farmed and wild salmon.

---

45 I.E. Papadakis, V.M. Papadakis, S.I. Petroutsos, A. Glaropoulos, M. Kentouri (2013), Do the visual conditions at the point of escape affect European sea bass escape behaviour? Mediterranean Marine Science 14/1, 2013, 172-178


Similarly the model framework *Offshore Mariculture Escapes Genetic/Ecological Assessment* (OMEGA) is designed to better understand possible genetic and ecological effects of escaped fish on wild fish of the same species. At the same time OMEGA should support the design of management strategies to address the potential risks to marine resources\(^54\).

Besnier *et al.* 2011\(^55\) examined the ability to detect genetic change in wild populations when gene flow stemmed from multiple farmed sources by simulating genetic change based upon data from 9 microsatellite loci in 4 wild Atlantic salmon populations receiving variable mixtures of escapees from 5 commercial Norwegian strains. As expected, neutral markers detected genetic change in wild populations when gene flow came from a single and distinct farmed strain. However, the genetic change detected in the wild population was significantly lower when gene flow was simulated from multiple farm strains simultaneously (‘concealing effect’) and the degree of concealing varied among the wild populations. While individual admixture analysis succeeded in detecting introgression, it was nevertheless strongly underestimated. They concluded that when gene flow is expected to originate from multiple farmed sources, analysis of selectively neutral genetic markers is thus likely to underestimate the true level of genetic introgression.

In analyses of genetic diversity of Chilean rainbow trout, Consuegra *et al.*\(^56\) indicated that variation in the incidences of escapees was well explained by the number and distance to nearby fish farms. Rivers close to fish farms tended to have more trout escapees than rivers located further away, demonstrating the overriding effect of propagule pressure in determining invasion success. Their study indicates the existence of at least four ancestral lineages among Chilean rainbow trout, as well as considerable hybridisation among lineages, particularly amongst free-ranging ‘wild’ trout. A positive relationship was detected between the degree of genetic admixture and genetic diversity, suggesting that the number of source (founding) trout populations has an additive effect on within-population genetic diversity, which may increase fitness and facilitate adaptation if admixture also enhances selectively important variation. More generally, the results suggest that non-native salmonids introduced into the Southern Hemisphere could benefit from admixture because local adaptations may not have yet developed and there may be initially depression.

**Identification of escapees**

A wide range of tests have been developed for identifying singly or in combination the origin of fish and fish products (Martinsohn *et al.* 2011\(^57\); Cadrin *et al.* 2013\(^58\)). Frequently, methods for the differentiation between farmed and wild fish are based on morphological,

---


morphometric (Arechavala-Lopez et al., 2011)\(^{59}\), genetic and other biological indicators such as growth pattern of scales and otoliths (Arechavala-Lopez et al., 2012)\(^{60}\), fatty acids (Fernandez-Jover et al. 2011)\(^{61}\) and trace elements (Adey et al., 2009)\(^{62}\).

**Genetic tools**

Due to the advances in genetics and genomics, particularly genetic analysis meanwhile underpins many approaches and tools to enable traceability of fish and fish products but also to measure genetic introgression (reviewed in Martinsohn 2011)\(^{63}\). In order to quantify gene flow from farmed to wild fish, S. Karlsson et al. (2011)\(^{64}\) identified a set of 60 SNPs that collectively was diagnostic in identifying Norwegian Atlantic salmon as of farmed or wild origin, regardless of their population of origin. From a large set of samples, including both historical and contemporary samples, this developed tool was evaluated and applied for quantifying genetic changes in wild salmon populations due to interbreeding with escaped farmed salmon. This design makes possible estimates of relative spawning success of escaped farmed salmon as well as estimates of selection against their offspring at the juvenile stage.

**Management practice**

As mentioned above, in Norway, the genetic interaction between wild and farmed Atlantic salmon is already taken up into a framework for risk assessment on the environmental impact of Norwegian salmon farming, that feeds into fisheries and aquaculture management\(^{65}\).

Through the integration of its research with risk assessment, AquaTrace aims at enabling a similar approach supporting sustainable aquaculture management practices for marine fish species.

To ensure that the risk assessment is meaningful and valuable for management and policy purposes the proper identification and management goals and assessment endpoints is indispensable. In contrary to Atlantic salmon, a very well-studied species, this is challenging for the AquaTrace target species sea bream, sea bass and turbot as they have been much less intensely studied, in particular genetically. Management goals which could be set in the context of this risk assessment are:


[1] Aquaculture will not contribute to a permanent alteration of the fitness of wild populations;

[2] Aquaculture will not contribute to a permanent alteration of the genetic characteristics of wild populations;

[3] Aquaculture will not contribute to a permanent alteration of the genetic diversity of wild populations.

Goal [1] is, considering the current lack of scientific knowledge of all these species not feasible, while the genetic tools developed by AquaTrace might move goals [2] and [3] within reach.

Assessment endpoints could be ‘Fitness of wild fish populations of the target species’ or ‘The genetic characteristics / genetic diversity of wild fish populations of the target species’, with the former not being measurable with the current state of knowledge.

While in Norway, the legislation on aquaculture is well-defined and the Norwegian government has established a set of environmental goals for sustainability in the “Strategy for an Environmentally Sustainable Norwegian Aquaculture Industry”, the legislative framework for aquaculture activities in the EU is much less well defined. The resulting legislative ambiguity adds to the difficulties when carrying out a risk assessment on the genetic interaction of farmed and wild fish.

However, while the EU legislation so far stipulates no specific legally binding rules for EU aquaculture management, the existing legislative framework does already provide for objectives which justify the development of a risk assessment on the potential genetic interaction and impacts of aquaculture on native fish stocks.

An example is the Marine Strategy Framework Directive (MSFD; Directive 2008/56/EC), aiming at the enhancement of marine environment protection across Europe. In order to achieve its goal, the Directive establishes environmental targets and associated indicators to achieve ‘Good Environmental Status’ (GES) by 2020 European in marine regions and sub-regions on the basis of geographical and environmental criteria. GES is determined and assessed through a set of 11 descriptors, of which the following could profit from the AquaTrace risk assessment:

• Descriptor 1: “Biological diversity is maintained”.
• Descriptor 3: “The population of commercial fish species is healthy”
• Descriptor 4: “Elements of food webs ensure long-term abundance and reproduction”.

Also Art.2 - Objectives of the Common Fisheries Policy basic regulation (Regulation (EU) No 1380/2013) is relevant: “The CFP shall ensure that fishing and aquaculture activities are environmentally sustainable in the long-term and are managed in a way that is consistent with the objectives of achieving economic, social and employment benefits, and of contributing to the availability of food supplies.”

Moreover, the Communication from the Commission to the European Parliament and the Council on building a sustainable future for aquaculture (COM(2009) 162 final) stipulates under 4.1.1. “An environmentally-friendly aquaculture: The EU is committed to a high level of environmental protection and Community legislation is based on the precautionary principle. The Commission will continue to emphasise the importance of environmentally sustainable development of aquaculture in its policies and actions; continue to monitor developments in terms of escapees and if necessary, assess the added value of possible action at the EU level.”

These provisions in the EU policy framework provide a basis for the AquaTrace risk assessment, and also room for resulting management advice.
The species subject to the risk analysis

For the three target species, gilthead sea bream (*Sparus aurata*), European sea bass (*Dicentrarchus labrax*) and turbot (*Scophthalmus maximus*) detailed knowledge reviews have been carried out in the in the AquaTrace project. These reviews aimed to put together the most updated scientific knowledge on the species, in particular as regards aquaculture production and genetics of wild populations and hatchery stocks in Europe. Since the results are openly available on the project web site under [https://aquatrace.eu/species_leaflets](https://aquatrace.eu/species_leaflets), in the following chapters only a short summary of the reviews is given.

**Gilthead sea bream (*Sparus aurata*)**

**Population structure**

The population genetics of gilthead sea bream throughout its full range has not been resolved yet, as only a few studies on this subject have been published. The absence of a common methodology in the various surveys (i.e. the use of different types of markers or number of loci scored), the different types of analyses used and the different geographic sampling scales, make the comparison of data and extrapolation to the whole distribution range of the species extremely difficult. This matter is further complicated by the discrepancies in the degree of structuring pattern in such published results, with some papers reporting absence of or subtle genetic structure, and others suggesting evidence for a population structure, within and among different geographic areas.

Three types of markers have been employed for the study of gene flow and population genetic structure of this species thus far: allozymes, microsatellites and mitochondrial DNA.

Gene-enzyme or allozymes have been mainly used to study the gilthead sea bream in the Mediterranean region. They have produced a wide range of differentiation values (*F*<sub>ST</sub>=0.001-0.265), which are nowadays considered high for a marine fish, and that were interpreted as the consequence of local sea currents and conditions or aquaculture related activities. Nevertheless, these markers are linked to expressed genes that are likely under selection, thus making them unsuitable for the study of neutral variation in natural populations (i.e. population structure). Such could be the cause for the high levels of genetic differentiation detected in the above-mentioned earlier studies.

Over the years, allozymes have been slowly replaced by microsatellites, whose higher mutation rate gives higher statistical power in studying neutral genetic variation. Some 35 microsatellite loci have been specifically isolated and characterized in gilthead sea bream; another 8 loci from a related sparid, *Pagellus bogaraveo*, provided good cross-amplification. The numerous studies with microsatellite loci confirmed the role of oceanographic processes, such as marine currents and gyres, in connecting gilthead sea bream populations. Microsatellites also highlighted the effect of restocking actions and escapes from farms on wild populations. This has led to the hypothesis that aquaculture "may already have partly contributed to wild population homogenization, casting doubt on the effective neutrality of microsatellites used in the studies. Indeed the use of different sets of microsatellites, i.e. seven anonymous and three linked to three candidate genes, proved that whereas the former indicate high gene flow across geographic locations, the latter (two out of the three) correlate with habitat type, and allowed to infer a role of differential survival between habitats. A further 200 microsatellites were produced and optimized and half of them were used for producing a linkage map (see BRIDGEMAP project: [http://www.bridgemap.tuc.gr/](http://www.bridgemap.tuc.gr/)).
Redesigning and evaluating 138 microsatellites previously described, a standardized panel of 22 loci was also developed for further studies on population genetic structure and for individual identification and pedigree reconstruction.

The use of mitochondrial DNA (mtDNA) analysis is limited to two studies on the non-coding control region. Despite its relatively high mutation rate, this marker did not reveal any differentiation between Mediterranean and Atlantic samples. These results were recently confirmed by the analyses of individuals from Northern and Central coastal areas of the Atlantic Ocean, where only 15 haplotypes were identified in the 76 individuals analysed. Such result was explained by the recent northwards expansion of the species into Irish waters.

Other high polymorphic nuclear makers like random amplified polymorphic DNA (RAPD), expressed sequence tags (EST) and amplified fragment length polymorphism (AFLP) were used to explore the genetic structure of the species, also in relation to adaptive processes, still delivering non homogeneous results. It is expected that the development of large numbers of single nucleotide polymorphisms (SNP) covering the full genome will lead to a better understanding of population structure and patterns of adaptation, as observed in the transferrin gene.

**Breeding practice**

Commercial hatcheries export eggs and fingerlings to fish farms all over the Mediterranean region. However, in spite of the clear need for systematic monitoring and tracking of aquaculture stocks in order to avoid the loss of genetic variation, there is still no appropriate control and traceability on the origin of this material.

Several cultured populations and aquaculture broodstocks of the species were genetically assessed and compared to wild populations. Important genetic information on broodstocks were collected in Italy, Greece, Spain and Croatia. However, the results do not allow a generalized interpretation on the impact of domestication on the genetic variability of farmed gilthead sea bream.

Some of these studies revealed strong founder effects and a loss of genetic diversity, at least as far as most genetic parameters sensitive to short-term events are concerned. Aquaculture broodstocks are also often affected by a reduction in effective population size ($N_e$) and by a heterogeneous composition from a geographical point of view. Given the genetic characterisation of the broodstock, wild and reared fish proved to be distinguishable, but no universal domestication markers are yet available.

Genetic analysis revealed the origin of broodstock from at least two different populations in Israel and a mixed and highly heterogeneous origin of Italian broodstocks and a massive presence of Atlantic individuals among the breeders. On a broader geographic scale, the analysis of French, Spanish, Italian and Greek samples revealed a significant genetic divergence between farmed samples and local wild populations and among different farms.

The application of genetic markers proved to be very powerful to estimate the effective contribution of breeders in each spawning: microsatellite-based traceability methods allowed accurate reconstruction of the pedigree.

Offspring analysis from several mass-spawning events demonstrated the consistently low effective population size, a differential average contribution by male and female parents and a high variance in family size. To maximize the possible genetic variance within each group and minimize inbreeding and genetic drift, investigations on the selection of individuals to establish broodstocks were performed.
Besides microsatellites, 76 single-nucleotide polymorphisms (SNP) were developed for individual identification and parentage assignment within breeding programmes. Several preliminary studies for genetic improvement based on quantitative trait loci (QTL) have been carried out, revealing good potential for marker assisted selection. At the same time they contribute to the understanding of the genetic determinism of important commercial traits in gilthead sea bream, as for morphometric traits, body weight and stress response.

Performances of triploid sea bream were investigated in land based conditions: as a protandric hermaphrodite, triploids developed in sterile males but did not sex inverse to females and their growth was similar to diploid males, at least until the latter began to feminize.

**Genetic resources and selective breeding**

Considerable genomic resources have been developed in fish, including in gilthead sea bream. Despite a rapid development the impact on breeding programmes remains limited. Since 2001 the characterization of the *Sparatus aurata* genome became the object of EU funded projects. They led to the development of large numbers of microsatellites, which were used to construct a linkage map. Also two physical maps were produced, a radiation hybrid and BAC map. The mapped BAC clones cover about 75% of the sea bream genome. The linkage (genetic) and physical maps were integrated and compared with other model organisms. They turned out to be useful to understand gene expression and to facilitate full genome sequencing. In particular they identified genes determining important phenotypes (Marine Genomics Europe - MGE project; BRIDGEMAP project) and those associated with stress and disease resistance useful for marker-assisted selection (MAS) (AQUAFIRST project) to improve immunity to infectious pathogens (IMAQUANIM project).

A QTL analysis for disease resistance based on 151 microsatellite loci detected two significant QTLs for resistance to fish pasteurellosis.

More recently a first step towards whole-genome sequencing was undertaken, applying next generation sequencing based on the comparative BAC map. Gene expression studies were performed in parallel with the implementation of DNA libraries. The first gene expression profiling of gilthead sea bream was aimed at identifying key genes involved in early developmental stages and stress related genes. More recently, a microarray, enriched by subtractive hybridization with stress and immune-relevant genes, was used to assess stress response exploring liver expression profile.

At present the culture of most gilthead sea bream is still based on non-domesticated stocks, although there are some programmes based on structured selective breeding. This related usually to classical mass or family-based selection. Genetic response in sea bream ranges from 5 to 10% per generation according to industrial reports. The protandric hermaphroditism of the species certainly makes selective breeding programmes more difficult. However, considering an inter-generation interval of 4 years for males and 6 years for females, successful breeding programmes would require long term projects with stable facilities and funding. This is a difficult target for project funded research or by a single commercial company. Only support from and collaboration among government, industry and academia will allow long term breeding programmes and lead to improved phenotypes (*GENIMPACT* project).

The identification of genetic markers directly linked to suitable phenotypes, enables selection with a greater response and better accuracy. This is particular useful for traits, such as disease resistance, which can't be measured on the fish. Panels of genetic tools to assist breeding programmes have been developed.
Escapees and restocking - interaction with wild stocks

With a production of over 150,000 tonnes/year, and an estimated 300–500 million sea bass and sea bream combined held in sea-cages at one time only in the Mediterranean region, the risk of escapes into the wild is very high. Escapees from farms, gametes released by cultured fish at sea and the intentional release of fry/juveniles of unknown origin in restocking programmes in more or less confined coastal lagoons, all have contributed to the presence of farmed samples in the natural environment. These fish may affect wild gilthead sea bream populations through predation and competition for food, space and cross breeding opportunities, the spread of parasites and diseases, and interbreeding.

Different types of escapes should be considered. The first one is the escape of live fish as consequences of technical or operational failures, due to storms, holes in the netting caused by wear and tear, abrasion on cage ropes, predators or biting by farmed fish within the cage, or spills during handling of fish. Little is known on the scale of sea bream escapes from sea-cage culture, as only few official data on escapes are available, as there is no mandatory reporting of escapes in most countries, for the lack of ad hoc legislation/regulations.

A second form of escape involves the release of fertilized eggs spawned by females cultured in sea-cages. Due to the longer culture period (up to 40 months) used in the last decade to face market demands for larger individuals, sea bream reach sexual maturity and undergo sex inversion in culture cages, becoming potential spawners. Histological follow-up of breeders associated with a site-intensive ichthyoplankton survey and genetic identification of sparid eggs demonstrated that sea bream eggs were indeed spawned in farms and released in the environment. An estimated 3.5 \times 10^{11} to 7.0 \times 10^{11} eggs are released into the Mediterranean Sea annually (PREVENT ESCAPE project).

The increase in gilthead sea bream production that took place in Greece during the past decade and the subsequent decrease in the market price led to structural and functional changes in the rearing process that resulted in extended farming durations (up to 40 months) and production of bigger sized fish. Gilthead sea bream is a protandrous hermaphrodite species and the expansion of the on-growing period led to the production of 2 to 4 year-olds female fish, capable of reaching sexual maturation. In fact, there is evidence that sex reversal and production of both male and female gametes occur within cages under the current industrial rearing method. Within the PREVENT ESCAPE project, the main objective was to determine the extent and timing of spawning of sea bream at an industry-wide scale. S. Somarakis et al. (2013)\textsuperscript{66} provided data showing that female sea bream reach final maturation, ovulation and spawning in sea cages during the normal spawning period. Egg production decreases with sex ratio (i.e. mean fish size).

Molecular tagging of commercial sea bream broodstock might provide a suitable tool to monitor these effects, as demonstrated by simulations and field surveys on salmonids.

However, the genetic impact of the accidental or intentional release of individuals from aquaculture facilities (culture cages, ponds and hatcheries) on wild local populations is difficult to estimate, both for the lack of information on the number of escapees and because the fitness in the wild of escapees and of introgressed offspring is largely unknown. Farmed and wild individuals can be recognized by external characteristics; the former are marked by culture conditions, in particular stocking density and feeding

conditions. Wild sea bream differ morphologically from farmed individuals in several aspects: they have a lower body height, sharper snout, a more squat and compact shape, stronger fins and bigger, sharper teeth. Different laboratory techniques can be used to discriminate farmed from wild sea bream; those techniques using differences in microchemical or genetic composition are more useful in tracing escapees, as they have a higher accuracy and can detect escapees long after the escape event.

European sea bass (*Dicentrarchus labrax*)

Population structure
A range of genetic markers have been employed over the years to unravel the population structure of sea bass: allozymes, mitochondrial DNA, RAPDs (Random Amplified Polymorphic DNA), microsatellites and, most recently, SNPs (Single Nucleotide Polymorphisms). Clear genetic distinctions were found between the northeastern Atlantic, eastern Mediterranean and western Mediterranean populations. The sharply defined border between the Atlantic and Mediterranean population is situated at the Almerian-Oran oceanic front whereas the break-up between the eastern and western Mediterranean populations is around the Siculo-Tunisian Strait, both representing areas of reduced genetic connectivity and hybridization. This pattern is believed to be related to climate change and sea water fluctuations during the Pleistocene and has been observed in several species. Additionally, the three main metapopulations are phenotypically distinct, with recorded differences in morphometry, growth, sex ratio and body composition.

Within the eastern Mediterranean Sea, subpopulation structure was found corresponding with the various sea basins: the Adriatic, Ionian and Aegean seas and the Levantine basin. Within the western basin, no population structure was found. Instead, western Mediterranean sea bass are thought to be introgressed (hybridized between eastern Mediterranean and Atlantic populations) and might represent a hybrid swarm. This is the result of many generations of backcrossing to the parental populations from the Atlantic and eastern Mediterranean and mating among hybrids.

Within the Atlantic Ocean sea bass populations were often considered homogeneous as most studies could not identify genetic population structure. Ultimately, a recent broad-scale study using microsatellites and SNP makers reports subtle genetic differentiation in southern, central and northern Atlantic populations. The observed pattern could be described as isolation by distance caused by high levels of gene flow between adjacent (sub)populations.

Next to the observed geographical patterns, genetic differences have been found between adults inhabiting coastal lagoons and offshore using allozymes and microsatellites. These are believed to be caused by differential larval behavior; following offshore spawning, part of the larvae moves inshore whereas the rest remains offshore for feeding. These two groups will then aggregate again for offshore spawning.

It is expected that in the near future studies based on many genome-wide SNP markers will provide a better insight into the neutral and adaptive genetic diversity present between sea bass populations, especially in the Atlantic Ocean. This would form the first reliable base upon which to build a sustainable management plan for natural sea bass populations as well as traceability and enforcement tools.

Breeding practice
Compared to terrestrial animals, fish have only been domesticated recently and farmed stocks are still very similar to their wild counterparts. This is especially the case in sea
bass, as only few companies practice selective breeding and most farms still rely on wild brood stock for reproduction.

Not many studies have been carried out to elucidate the genetic composition of farmed sea bass, but the few ones available reported most populations to be in Hardy-Weinberg Equilibrium and largely outbred, whereas others found a reduction in genetic diversity.

In the mid-1980s domestication and selective breeding of sea bass was spearheaded by some breeders in France, Spain, Italy and Israel. Today, breeding practices are very heterogeneous: from yearly replenishment with wild stock, to a closed cycle without selection over selection with or without scientific follow-up.

There is a great potential for selective breeding; already after a few generations large improvements can be expected in at least some economically important traits. This is because heritability for most of these traits is moderate to high, correlations between desired traits are often positive, and fecundity is high, allowing strong selection intensity. Traits that are economically interesting and thus typically selected for in sea bass include: growth, sex ratio (aiming for female-only stocks), disease resistance, product quality, feed conversion and yield.

Next to heritability, also genotype-by-environment interactions and genetic correlations between traits are important. The genotype-by-environment interaction is relevant as selection could be carried out in hatcheries and fish sold to grow-out farms that operate under different conditions. Additionally, selective breeding is becoming more practicable as progressively tools for artificial fertilization, cryopreservation and genetic parentage assignment (e.g. using microsatellites) are becoming available.

A next step in the process towards MAS (Marker Assisted Selection) is the identification and mapping of QTL (Quantitative Trait Loci). QTL are stretches of DNA linked to or within genes that underlie a (commercially important) quantitative trait and are therefore useful to construct genetic tools for MAS. In sea bass, some QTL for such traits were identified: one for length and depth, two for body weight, six for morphometric traits and three suggestive QTLs for stress response, explaining between 8 and 38% of phenotypic variance. Such research merits to be extended in order to have a more targeted approach to selection.

In a recent study the potential for performance in aquaculture of five different natural strains and ten strain crosses was evaluated in a multi-site common garden experiment. Significant genetic variation between strains was found but the effects of heterosis as well as G x E interaction appeared weak for most traits. Furthermore, no perfect strain could be identified and the authors argue that one generation of selective breeding would cancel out potential differences due to initial strain selection.

Triploidization is a kind of chromosome set manipulation whereby by nature diploid organisms are manipulated to become triploids, possessing one additional chromosome set. Triploidization has the advantage that it induces sterility in both sexes and reduces the risk of genetic contamination when escaped fish get in contact with wild conspecifics. In theory, triploidization could also increase growth because all energy is allocated to somatic growth rather than the development of gonads. Triploids were obtained in sea bass by retention of the second polar body with a heat-, cold- or hydrostatic pressure shock with success rates as high as 100%. In contradiction to expectations, some observed that juvenile diploids and triploids had similar growth rates but that adult triploids grew slower although they reached similar fork lengths. Better growth in diploids as compared to triploids has been observed in a study up to an age of 4 years, but triploids showed higher gutted yield than diploids, especially in females. Nevertheless, in a 2009 study, better growth was found in older triploid females in comparison to diploid females or diploid and triploid males. Also, some other studies saw that for 30-day-old larvae the
length of triploids was significantly higher than diploids. Today, triploidization is not commercially applied as companies fear consumers’ reactions.

Genomic resources and selective breeding

Sea bass are diploid with a genome size of 1,525 Mb and 24 chromosome pairs, covering a range of sizes. Genomic resources are very well developed and include: a medium density linkage map, a radiation hybrid map and a transcriptome with 65,000 expressed sequence tags configured into 19,734 unique transcripts. There are 368 polymorphic microsatellite markers, 200 polymorphic AFLP markers, 20,000 SNPs, mined from three chromosomes of a single male and 35 validated SNPs. The EU project REPROSEL validated another 61 SNPs and 14 SSRs markers for paternity analysis to better control inbreeding in selection programs. A microarray platform for jaw deformity was developed. Furthermore, the sea bass genome is fully sequenced and assembled to a high level (SeaBass Genome Consortium). A full genome sequencing analysis of Mediterranean and Atlantic populations is in progress and might reveal complementary diversity, structural, adaptive and architectural information. Despite this rich variety of genomic tools mainly developed for population genetic research, integrated biological and physiological studies and their application in selective breeding are generally lacking.

Escapees and restocking - interaction with wild stocks

Escaped sea bass have been observed exploiting natural food resources and moving between nearby farms, local fishing grounds and coastal habitats. But due to poor and scattered management of aquaculture, little is known about the real extent of this phenomenon in the Mediterranean Sea. Nonetheless, sea bass escapes are believed to be frequent and potentially problematic for natural populations as farms are often situated in or near natural breeding grounds. In a recent effort made to understand and prevent escapees, a survey identified that in 15 reported escape events, over 1 million sea bass escaped\(^\text{67}\). On top of that, it is estimated that the ratio of cultured to wild sea bass in the Mediterranean is close to 1:1.

Although sea bass are not intensively domesticated or selected, mixing has potentially adverse effects as Mediterranean farms often use Atlantic breeders or exchange breeders between eastern and western basins. Despite the small amount of data gathered so far, there is genetic evidence that escapees survive in the wild, both in the Atlantic Ocean and Mediterranean Sea. In the eastern Mediterranean, sea bass with a typical ‘western’ genetic makeup were detected. This could be explained by the fact that in the early 1980s many farms used broodstock, eggs or fingerlings from the western Mediterranean basin, where the first farms were located. In the northeastern Atlantic ocean, two individuals with a Mediterranean haplotype were found (but Atlantic nuclear pattern), probably as a result of hybridization with escapees from farms in northern France. But more studies are needed to understand the extent of hybridisation between wild and cultured fish, in order to assess the impact and consequences of fish farming on natural stocks.

---

**Turbot (Scophthalmus maximus)**

**Population structure**

Different types of molecular markers have been used on population genetic studies of wild and cultured stocks of turbot. Initially, European populations were analyzed with allozyme markers, but the development of co-dominant DNA markers has greatly expanded over the last decade, particularly of anonymous microsatellites. Population variation at mitochondrial DNA sequences has also been described. Anonymous microsatellites are used for studies on structural genomics, for advanced studies of the population genetics, and for cross-species amplification in other flatfish. Recently, functional genomic approaches have provided large numbers of gene-linked microsatellite and SNP markers. They are helpful tools for the study of adaptive variation through genome scans.

Low population genetic diversity at allozyme loci compared to other flatfishes with similar habitat and life history features suggested historical population bottlenecks. Phylogeographical analyses based on mitochondrial DNA variation pointed to a moderate diversity with a stock shared between the Mediterranean Sea and the Atlantic Ocean and characterised by a late Pleistocene expansion. Microsatellite diversity is high and comparable to other marine fishes. Heritability estimates for growth rate and genetic breeding programs suggest that genetic variability for quantitative traits is high and does not point to a bottleneck in the recent past.

Most natural populations are in Hardy-Weinberg equilibrium at all loci (coding and non-coding regions), pointing to random mating. Genetic differentiation was detected between the Baltic and North Sea populations, between Black Sea and Mediterranean populations, between the Western and Eastern Mediterranean basin, between the Irish shelf and the North Sea – English Channel – Gulf of Biscay, but not so much between North Sea – English Channel – Gulf of Biscay - Cantabric populations However, closer evaluation of the North Sea suggests a break between the populations of the southern and northern North Sea along the Friesian front. The differentiation between North Sea and Baltic Sea turbot involves a hybrid zone in the transition area between both seas. Significant differentiation at specific loci suggests divergent selection associated with environmental variables.

**Breeding practice**

Genetic analysis of domesticated stocks in Europe and the PR China with allozymes and microsatellites has shown in some cases lower genetic variation compared to wild populations. This could be related to management practices and the heterogeneity in the number and types of molecular markers applied. A few studies have focused on genetic differentiation between farmed and wild populations, as well as among domesticated stocks. It has been suggested that genetic drift could be partly involved in the foundation and management of broodstocks, although the genetic representativeness of commercial populations could be also affected by sampling variance in number of loci and individuals among studies. Microsatellite panels are used to support broodstock management and breeding programs through parentage and kinship analyses, and genealogical monitoring. Most recently, genome scan analysis of broodstock and natural populations is in progress in order to monitor domestication and directional selection, and to target interesting gene regions and quantitative trait loci (QTL) for selection. In general, there is a clear need for monitoring of the genetic structure of broodstock in order to optimize their management in aquaculture, hence avoiding the loss of genetic variation.

Several studies have been carried out using the turbot genetic map to identify genomic regions and markers associated with quantitative traits (QTL). These studies have contributed to an enhanced understanding of the genetic architecture of important...
economic traits and have provided new markers for marker assisted selection (MAS). Here single markers explain 13–23% of the phenotypic variance in many of the morphometric traits evaluated.

**Genomic resources and selective breeding**

Compared to other vertebrates and many fish species, the turbot genome of 800 Mb is small, being one of the smallest genomes among farmed fish. It is organized in 2n=44 chromosomes without sex-linked chromosome heteromorphism. Structural and functional genomics resources have facilitated the genetic dissection of complex production traits, such as sex, growth and disease resistance. Most of this research has been carried out in Europe, in collaboration with the turbot industry, and in the PR China.

Genomic resources developed specifically for turbot include DNA and RNA libraries, sequenced and stored in databases for their exploitation. Different cDNA libraries have been developed using traditional Sanger, and Next Generation Sequencing Roche 454 and Illumina platforms from embryonic, immune-related, growth-related and reproductive tissues, which enabled discovering a great deal of expressed sequence tags (EST). From these databases different oligo-microarray versions have been designed to evaluate gene expression profiles related to immune response for disease resistance. Also, gonad development expression profiles have been analyzed by cDNA-AFLPs.

Panels of reference families to construct genetic maps and eventually to establish association with productive traits are available in turbot. The first generation consensus genetic map based on anonymous microsatellites was organized in 26 linkage groups (LG) and centromeres were mapped by using diploid gyngenetic progeny. EST databases have been mined for many gene-linked microsatellite and SNP markers for the construction of gene-enriched genetic maps. These maps have been used to identify QTL and candidate genes for sex determination, growth rate and resistance to pathologies.

The identification of a major QTL for sex determination at turbot linkage group 5 (LG5) is used to obtain all-female populations in industrial projects under a Spanish patent (ES2354343A1). Anonymous and EST-linked markers have also been used to analyze population genetic structure and to identify candidate genes subjected to divergent selection.

In addition, a 5X BAC genomic library containing ~46,000 clones of ~100 kb useful for physical mapping and sequencing of regions of the turbot genome has been constructed. This library has been used as a source of DNA probes for FISH hybridization to assign linkage groups of the genetic map to chromosomes of the karyotype. Efforts have been carried out to integrate all available turbot genomic information to date and even for its transfer to the congeneric species *S. rhombus*. Comparative mapping against model fish (*Ensembl Genome Browser*, at [http://www.ensembl.org](http://www.ensembl.org)) has been used as a complementary strategy to capture genomic information from non-model fish species, especially to search for candidate genes at QTL associated with productive traits.

Adaptation to the farm environment is well established in the turbot since 1990s. Cultured specimens are much heavier and longer than wild fish of the same age. Turbot females largely outgrow males (10 to 20% at 800 g), and accordingly, it would be advantageous to obtain female monosex progeny. Gynogenesis could be an option, but a ZW sex-determining system was demonstrated by using both crosses between sex reversed breeders and genetic sex markers. Thus, to achieve all-female populations some companies are obtaining WW superfemales using marker assisted selection and hormone sex reversal. Minor genes and/or interaction with environmental factors as temperature were suspected.
The development of a microsatellite-based traceability tool has been essential to support breeding programs. Also, sperm cryopreservation is commonly used to facilitate the design of crosses in breeding programs. Low heritability for survival of turbot was reported, with positive genetic correlations between growth and survival. Growth-related traits are the main targets of breeding programs and they demonstrated medium-high heritabilities in turbot, thus rendering a good response to selection (about 10-20% per generation). These programs are being applied by the main turbot companies in Europe and they have progressed to the fifth generation of selection. Several studies have been developed to identify genomic regions associated with productive traits in turbot, particularly growth, resistance to pathologies, and sex-determination. This information is being evaluated for their application in marker assisted selection programs.

Because of growth delay by early sexual maturation, turbot producers may be interested in producing sterile stocks by polyploidisation. Induced triploidy in turbot can be obtained by applying short cold shocks after fertilization, and triploidy is now well established for large egg volumes for production. Triploids have demonstrated a better growth performance than diploids for fish of more than 3 years old (sexual maturity achieved) and also a significantly higher proportion of females, which supports its industrial application.

**Escapees and restocking - interaction with wild stocks**

There are no studies on genetic interactions between farmed and wild turbot. Although no information on escape events is available, escapees are likely to be rare in land-based facilities, especially in those with water recirculation system, and are probably higher in floating cages or in open-circuit pumped seawater. Restocked turbot, although usually bred from wild parents, might also present challenges to the native turbot and ecosystem. As adaptation of farmed juveniles to the wild could be good, there is a need to investigate those aspects of primary importance to estimate the magnitude and the importance of genetic impacts of farmed turbot on wild populations. Genetic introgression and admixture of wild and domestic genomes is theoretically possible, although it has not been demonstrated up to date. If individuals from monosex female stocks, which are nowadays in an early stage of development, are soon produced, their impact on wild populations must be investigated. Gynogenetic individuals are able to mature. Therefore, the use of triploids to ensure genetic confinement of all genetically modified individuals should be explored, because they show additional interesting properties to improve turbot production.

**Next steps**

In a next step, the steering committee of the AquaTrace consortium will discuss the proposed outlines regarding

a) **Management goals** (proposed definitions for discussion):

‘Aquaculture will not contribute to a permanent alteration of the fitness / genetic characteristics / genetic diversity of wild fish stocks’

very much aligned to the relevant management goal set out by the Norwegian government for their aquaculture and derived from the below listed European legislation as so far no specific and legally binding management goal for EU aquaculture had been formulated.

  - Descriptor 1: “Biological diversity is maintained”.
  - Descriptor 3: “The population of commercial fish species is healthy”
• Descriptor 4: “Elements of food webs ensure long-term abundance and reproduction” 68

• Common Fisheries Policy - Art. 2 Objectives: “The CFP shall ensure that fishing and aquaculture activities are environmentally sustainable in the long-term and ….” 69

• Communication from the Commission to the European Parliament and the Council on building a sustainable future for aquaculture - 4.1.1. “An environmentally-friendly aquaculture: The EU is committed to a high level of environmental protection and Community legislation is based on the precautionary principle. The Commission will

- Continue to emphasise the importance of environmentally sustainable development of aquaculture in its policies and actions;
- Continue to monitor developments in terms of escapees and if necessary, assess the added value of possible action at the EU level.” 70

b) Assessment endpoints (proposed entities and attributes that are at risk and which are expression of the management goals for discussion):

‘Fitness of wild fish populations of the target species’

‘The genetic characteristics / genetic diversity of wild fish populations of the target species’

c) Scope of the risk assessment (for discussion):

• Three marine species for which aquaculture has an economic importance in Europe: Wild and farmed European sea bass (Dicentrarchus labrax), gilthead sea bream (Sparus aurata) and turbot (Scophthalmus maximus)

• Geographical extent: Areas of the sampling (to be described for each of the three species)

• Analysed time period: time span from historical and contemporary samples (to be described for each of the three species)

• Extent of the genetic and fitness analyses

d) Hazard description (for discussion, to be further elaborated):

Genetic interactions of escapees with wild conspecifics, may compromising the fitness and biodiversity of local fish populations (see e.g. Genimpact 71). Thus, the risk to be assessed is the probability and severity of genetic interactions (hazard) compromising fitness and biodiversity.


The subsequent risk assessment work will follow the outlines below:

**Methods, data sources and analytical procedures**

For marine aquaculture a precautionary approach towards the environment was proposed by GESAMP Working Group 31 on Environmental Impacts of Coastal Aquaculture which proposed to extend the application of the risk assessment process developed by WHO to environmental risks.\(^{72}\)

The risk assessment may follow general principles of environmental/ecological risk assessment procedures and methods proposed for coastal aquaculture.\(^{73,74}\)

Identify suitable effect indicators (or proxies) for assessment of the degree of introgression across spatial and temporal scales (“to which extent has the genetic integrity been altered?”) and consequences

**Risk assessment** for each the 3 marine species

**Description for endpoint**

- assessments of release and exposure
- Estimate nature and likelihood of effects on the assessment endpoints
- + input from other species if necessary to fill gaps and for assumptions (such as Atlantic salmon)
- Areas and extent of uncertainty

The risk analysis tries to quantifying both the probability of introgression and severity of its consequences. In biological systems it is normally very difficult to quantify these factors precisely and therefore, alternative broad qualitative categories may be used, by scoring the probability and consequences from low to high. This can either, be based on some semi-quantitative assessment or on expert opinion. There are suggestions for how such environmental alterations can be scored scientifically, e.g. related to their scale of impact in the ecosystem, and whether the impact is reversible or not (e.g. GESAMP categories, ICES(2006)).

**Risk communication** (JRC and WP11 partners)

In form of a white paper (structure to be defined).\(^{76}\)

- What do we know
- What knowledge we have gained with the project
  - What is the risk (severity and probability)
  - What would be an acceptable level of risk
  - What are the associated uncertainties, knowledge gaps and their implications
  - Options for risk mitigation both for magnitude and/or probability.

---


\(^{75}\) ICES CM 2006/RMC:04 Ref. LRC ACFM ACE ACME

\(^{76}\) White paper: persuasive document describing problems and how to solve them - crossbreed of a magazine article and a brochure, taking the objective and educational approach of an article and weaves in persuasive corporate messages typically found in brochures (M. A. Stelzner, Learn all about white papers, WhitePaperSource Publishing, 2008); Commission White Paper: a document containing proposals for Community action in a specific area. When a White Paper is favourably received by the Council, it can lead to an action programme for the Union in the area concerned (http://europa.eu/legislation_summaries/glossary/white_paper_en.htm)
- How much can the results be relevant for other species, e.g. sturgeon?
- Taking work forward - recommendation: Provision to the EU Commission for policy development and further research and monitoring needs with a view on risk mitigation and filling gaps.
Europe Direct is a service to help you find answers to your questions about the European Union.
Freephone number (*): 00 800 6 7 8 9 10 11
(*) Certain mobile telephone operators do not allow access to 00 800 numbers or these calls may be billed.

A great deal of additional information on the European Union is available on the Internet.
It can be accessed through the Europa server http://europa.eu/.

How to obtain EU publications

Our priced publications are available from EU Bookshop (http://bookshop.europa.eu),
where you can place an order with the sales agent of your choice.

The Publications Office has a worldwide network of sales agents.
You can obtain their contact details by sending a fax to (352) 29 29-42758.
JRC Mission

As the Commission’s in-house science service, the Joint Research Centre’s mission is to provide EU policies with independent, evidence-based scientific and technical support throughout the whole policy cycle.

Working in close cooperation with policy Directorates-General, the JRC addresses key societal challenges while stimulating innovation through developing new methods, tools and standards, and sharing its know-how with the Member States, the scientific community and international partners.

Serving society
Stimulating innovation
Supporting legislation