

## Challenges in using genetics for European eel management: current status

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### ABSTRACT

Marine organisms experience a wide range of intrinsic and extrinsic influences during their life cycle, which considerably impact their population dynamics and genetic structure. Subtle interpopulation differences reflect the continuity of the marine environment, but also pose challenges to define management units. The catadromous European eel *Anguilla anguilla* (Anguillidae; Teleostei) represents no exception. Its spawning habitat in the Sargasso Sea and vast migrations across the North Atlantic Ocean qualify it fully as marine. But the synergy between hydrographic variability and a shifting climate in the ocean, and the impact of habitat degradation and overfishing in continental waters has brought this intriguing species to the brink of extinction. The protracted spawning period, variance in age-at-maturity, parental contribution and reproductive success, and the difficulty to sample the spawning region, all may mask a weak geographical genetic differentiation. Recent molecular data report evidence for as well spatial as temporal differences between populations. However, temporal heterogeneity between intra-annual recruitment waves and annual cohorts exceeds spatial differences. Despite its common name of “freshwater eel”, the European eel should be managed on a North Atlantic scale. The fishery should be curtailed, migration routes kept open and water quality restored. Eel aquaculture has to focus on efficient rearing in the short term and controlled breeding in the long term. Future research in eel should focus on (1) establishing a biological baseline from pre-decline historical collections for critical long-term monitoring of genetic composition, (2) on the occurrence of adaptive genetic polymorphisms (genes under selection), to detect adaptive divergence between populations, requiring separate management, and (3) the joint validation of demographic and genetic models following various scenarios.

**Keywords:** effective population size, genetic patchiness, Isolation-by-distance, Isolation-by-time, population genetics, reproductive variance, simulations.

## Introduction

For decades, marine research has focused amongst others on the population dynamics of marine organisms. Fisheries data are the main source of information on population composition, numbers, stability and fitness of commercial species. From the late 1980s onwards it became clear that many marine fish stocks are fully exploited or even overexploited (Myers & Worm, 2003), prompting for increased accuracy in the estimation of stock structure and size. Genetics has proven to be invaluable to discriminate independently evolving populations (Park & Moran, 1994; Ward, 2000) and to provide indirect estimates of dispersal, population size, demography and stock sustainability (Palumbi, 1994; Waples, 1998; Avise, 2004). Marine species are known to exhibit large population sizes, high fecundities and fluctuations in reproductive success (Waples, 1998; Flowers *et al.*, 2002). However, a clear gap exists between their census population size and their effective population size ( $N_e$ ), while the  $N_c/N_e$  ratio is often lower than expected (Turner *et al.*, 2002; Hauser *et al.*, 2002). Due to their biological characteristics, marine species are strongly influenced by fluctuating ocean currents and food abundance, leading to unpredictable reproductive success, high larval mortality and strong dispersal capacity (Hedgecock, 1994). Several commercial marine fisheries have strongly declined or even collapsed (Myers & Worm, 2003; Mullon *et al.*, 2005), most likely due to a synergy between climate change and anthropogenic influences, such as heavy fisheries and habitat degradation (Dulvy *et al.*, 2004).

The European eel is beyond safe biological limits (Dekker, 2003). Fisheries data indicate that the eel stock is at its historical minimum; only 1% of the 1960 recruitment level is reached at the moment. Several causes have been proposed for the decline ranging from pollution, overfishing, migration barriers, habitat destruction, parasites and diseases to global oceanic and climatic changes (Dekker, 2003; Knights, 2003). Synergy between all these factors seems the most likely cause (Wirth & Bernatchez, 2003). In practice, genetic data may help defining species integrity within the North-Atlantic, identifying the number of genetic stocks within the European eel, spatio-temporal stability of the genetic structure, influences of oceanic conditions on genetic variability, the effect of a population decline on the genetic variability and fitness of eel, and assessing whether such decline has already occurred earlier on.

The European Commission released an action plan for the European eel, which aims at strengthening the return rate of adult eels to the Sargasso Sea and includes the development of national management plans. To do so, a detailed review of the genetics of European eel is required, as well as the delineation of future research areas to improve our knowledge. This is of importance to maintain intraspecific genetic diversity, to develop sound restocking programs for brood stock enhancement and to help realizing a profitable artificial breeding of the species. The aim of the current paper is to provide an introduction on the genetic consequences of typical marine life-history traits and anthropogenic pressures, to synthesize the most recent genetic knowledge in European eel and other Anguillids, and to provide an overview of possible better use of genetics in future management decisions in this strongly declining species.

## Biological characteristics of marine organisms and genetic consequences

Marine organisms experience a wide range of intrinsic and extrinsic influences during their life cycle, which considerably impact their population dynamics and genetic structure. Subtle genetic differences reflect the continuity of the marine environment, but also pose serious challenges to define management units.

A high fecundity, external fertilization, a high dispersal potential and a high number of spawners often characterize marine fish species. However, they suffer from large fluctuations in reproductive success, depending on the match (or mismatch) of larval hatching with spring algal bloom. They experience strong effects of natural selection at early life stages, while atmospheric and oceanic influences often determine cohort strength in subsequent years.

Marine fish are thus expected to exhibit a high genetic variability, high exchange between populations (gene flow) inducing a low genetic differentiation and a high genetic population size. Marine fish are challenging to study as they exhibit a low genetic signal/noise ratio, requiring careful sampling designs to decipher subtle spatio-temporal differences (Waples, 1998). Additionally, strong fluctuations in allele frequency due to year-to-year differences and fluctuations in adult reproductive contribution (genetic patchiness) are the consequence of differential mortality and variable reproductive success. For diadromous species, selection pressure during larval migration and during freshwater/marine residence can also influence the genetic pattern of populations.

Widely distributed species are nevertheless rarely fully panmictic (mating randomly), but are commonly divided into subgroups in a pattern that can be described by one of the classical population models, such as the island model, stepping-stone model or Isolation-by-Distance model (Rousset, 1997). The genetic architecture of natural populations is the outcome of factors such as population size, individual dispersal, behaviour, assortative mating, reproductive success and survival (Rousset, 1997; Avise, 2004). In populations composed of a mixture of individuals reproducing at different times within a reproductive season, temporal differentiation can supplement possible geographical partitioning. Under these conditions, gene flow is expected to be limited between early and late reproducers. A temporal -instead of spatial- restriction on gene flow hence creates a pattern of Isolation-by-Time (IBT) (Hendry & Day, 2005; Maes *et al.*, 2006). Many marine species split their reproductive effort among several events during a protracted spawning season, potentially generating an IBT pattern between consecutive spawning groups. If temporal overlap is small, a stable pattern of Isolation-by-Time can arise; its stability depending on the heritability of spawning time, the level of gene flow after dispersal and environmental conditions (Hendry & Day, 2005; Maes *et al.*, 2006). Temporal heterogeneity in the genetic composition of recruits is likely to result from a large variance in parental reproductive success driven by the unpredictability of the marine environment (Waples, 1998). Under the hypothesis of “sweepstakes reproductive success” (Hedgecock 1994), chance events determine which adults are successful in each spawning event. Hedgecock (1994) attributed the variation in reproductive success of adults to spatio-temporal variation in oceanographic conditions, occurring within and among seasons. The genetic consequences of a high variance in reproductive success is the induction of a stochastic genetic composition of recruits (genetic patchiness; Hedgecock, 1994), possibly counteracting a population structure following an IBT pattern, especially if patchiness surpasses forces restricting dispersal in time.

## **The European eel: current genetic status**

***The biology of the European eel*** - The life-history of the catadromous European eel (*Anguilla anguilla* L.) strongly depends on oceanic conditions; maturation, migration, spawning, larval transport and recruitment dynamics are completed in the open ocean (Tesch, 2003; Knights, 2003; Kettle & Haines, 2006). Despite the key biological importance of the marine phase (Knights, 2003), most research has focused on the freshwater phase of its life-history. The European eel is outside safe biological limits. Fisheries data indicate a historically low landings and recruitment level (Dekker, ICES report, 2006). A roughly estimated  $9 \times 10^6$  spawners escape from the continent to safeguard the next generation, corresponding to a 99.5% accumulated life-span fishing mortality. A restriction in fisheries pressure is thus urgently needed. The decline in eel landings has been followed by similar decrease in recruitment with a lag of 15 years (1-2 generations). The population dynamic course of the decline is nevertheless very different with a “plateau” like pattern in adults and an “erratic” pattern in glass eels (Mullon *et al.*, 2005). The slow depletion of a huge continental (sub)adult population till a threshold density could explain such plateau pattern (strongly age structured population), while the occasional high reproductive success of few breeders might have triggered the few recruitment peaks provided that the oceanic conditions were favourable. There have been several hypotheses concerning the causes of the eel stock decline during the second half

of the century. On the one hand, there are several anthropogenic factors influencing eel reproductive success, such as migration barriers (dams and hydroelectric power plants), overfishing, pollution (PCBs and heavy metals), habitat destruction, diseases (EVEX virus) and parasites (the swimbladder nematode *Anguillicola crassus*) (Lefevre *et al.*, 2002; Robinet & Feunteun, 2002). On the other hand, there is strong evidence of a correlation between climatic and oceanic events and recruitment success (Castonguay *et al.*, 1994; Dekker, 1998, Knights, 2003). It is thus likely that a synergy of negative effects has caused the decline of eel (Wirth & Bernatchez, 2003). A precautionary approach has been proposed to protect the European eel stock at a regional, national and international level (Starkie, 2003). Integrating data and knowledge of the marine part of its life-cycle and genetics is a next step in the direction of a global management strategy.

***The genetic status of the European eel*** - Early population genetic studies, based on observed differences in transferrins and liver esterases, claimed that European eel populations differed between several continental European locations (Drilhon *et al.*, 1966, 1967; Drilhon & Fine 1968; Pantelouris *et al.*, 1970), suggesting that eels in the south-eastern part of the Mediterranean formed a separate group and reproduce in this area. Later allozymatic studies failed to detect obvious spatial genetic differentiation (de Ligny & Pantelouris, 1973; Comparini *et al.*, 1977; Comparini & Rodinò, 1980; Yahyaoui *et al.*, 1983). Later studies based on mitochondrial DNA initially provided only limited insights into the geographical partitioning of genetic variability in European eel. Lintas *et al.* (1998) found so little DNA differentiation among European eel individuals from distant geographical locations, that they suggested all European eels were derived from a common genetic pool. This commonly accepted view of a panmictic genetic population structure of European eel, based on oceanographic (Sinclair, 1988; Tesch, 2003) and genetic features has recently been challenged in three independent studies (Daemen *et al.*, 2001, Wirth & Bernatchez, 2001, Maes & Volckaert, 2002). Wirth & Bernatchez (2001) and Maes & Volckaert (2002) detected a relationship between genetic and geographic distance (the so called Isolation by distance, IBD), suggesting a subtle spatio-temporal separation of spawning populations, with some degree of gene flow. Ocean currents, resulting in a differential distribution of eel larvae, have recently been suggested to explain the observed clinal genetic variation (Kettle & Haines, 2006), although the genetic architecture of European eel populations proved to be unstable over time (Dannewitz *et al.*, 2005, Figure 1). Since the life history of European eel exhibits the largest migration-loop of any anguillid taxon, the possibility remains that individuals from geographically separated regions differ in arrival time at the spawning site (Tsukamoto *et al.*, 2002; Kettle & Haines, 2006), inducing a separation in time of spawning groups. For a more detailed review on the genetics of eel, we refer to Van Ginneken & Maes (2005)

Similar observations were made on other temperate and tropical eel species. The American eel (*A. rostrata*) showed no evidence for a geographical subdivision, with the exception of clinal allozyme variation putatively imposed by selection (Williams *et al.* 1973; Koehn & Williams 1978; Williams & Koehn 1984; Avise *et al.* 1986, Wirth & Bernatchez, 2003, Figure 1). These data suggested that *A. rostrata* is genetically homogenous, forming a single randomly mating population. In the Japanese eel (*A. japonica*), no evidence was found of genetic structure over large geographic areas in studies based on mitochondrial DNA (Sang *et al.*, 1994; Ishikawa *et al.*, 2001), but clinal variation was observed at allozymes (Chan *et al.*, 1997). In *A. australis* and *A. dieffenbachii*, an allozyme based study showed a signal of differentiation between recruiting and resident populations (Smith *et al.*, 2001). In the giant mottled eel (*A. marmorata*), even several genetically isolated populations were detected using mtDNA (Ishikawa *et al.*, 2004). The distribution pattern of five populations was closely associated with the water-mass structure of oceans and major current systems, suggesting the establishment of new population specific spawning sites in different oceanic current systems as the species colonized new areas (Tsukamoto *et al.*, 2002; Ishikawa *et al.*, 2004).

## Recent advances in European eel genetics

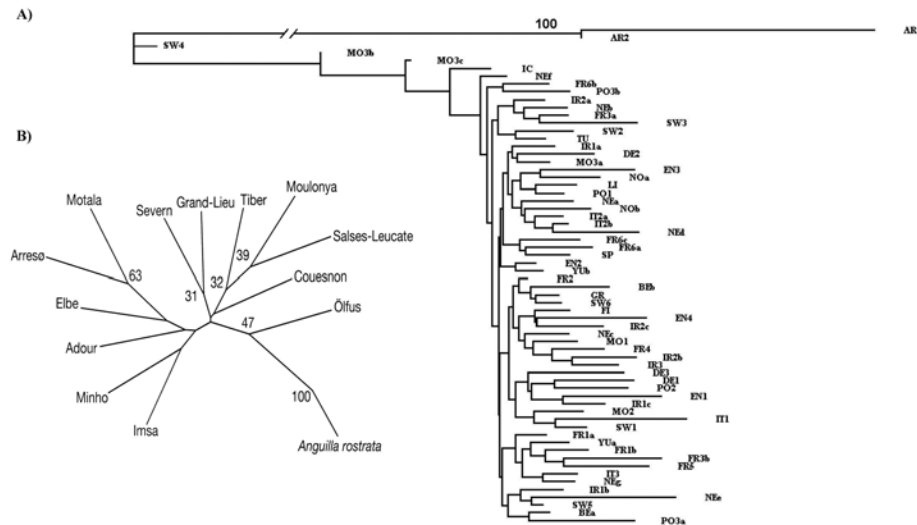
Genetic knowledge in anguillids is continuously growing and the last years, several new insights have been gained to help the management of eels. The main genetic research focus can be separated into studies of genetic species/hybrid identification, spatio-temporal genetic structure analyses, genetic variability-fitness correlations and the application of genomics in eel aquaculture.

**Genetic identification of species and hybrids** - A total of fifteen *Anguilla* species are officially recognized, although the morphological and meristic characteristics are highly unstable, even between phylogenetically distant species, and remain difficult to use for species determination. A recent reassessment of eel morphology resulted in the detection of only four unambiguous groups and the detection of much overlap in formerly accepted morphological characters (Watanabe *et al.*, 2004). Species recognition is even more problematic at the larval stage, where essential traits such as coloring and dentition characters are lacking (Tesch, 2003; Watanabe *et al.*, 2004). Due to remote translocations of non-native species for aquaculture purposes, the natural distribution of species has become disrupted. The identification of species and detection of hybrids has many applications in eel management, mainly for in the field of fish traceability, forensics of fish products, law enforcement, the detection of intercrossing between species and its evolutionary implications. Eel taxa have been identified using various molecular techniques (Comparini & Rodino, 1980; Tagliavini *et al.*, 1995; Aoyama *et al.*, 2001; Lin *et al.*, 2001; Rehbein *et al.*, 2002; Hwang *et al.*, 2004; Minegishi *et al.*, 2005) and several reliable molecular tests now exist to identify *Anguilla* spp. simultaneously in processed, historical or alcohol preserved samples without sequencing (Lin *et al.*, 2002; Watanabe *et al.*, 2004; Sezaki *et al.*, 2005; Itoi *et al.*, 2005). In a recent study, Maes *et al.* (2006) showed that reliable species identification could be performed using one single PCR reaction of nuclear markers, enabling the joint assessment of the species identity and the existence of hybrids between eel species. For species showing no external morphological differences at the adult or larval stage, which is typical for many anguillids, the reliable discrimination of such species opens many possibilities to study hybridisation and translocations. Highly traded species are often released accidentally or intentionally in the natural environment. The example of the European eel occurring for 31 % in some Japanese rivers (Zhang *et al.*, 1999; Okamura *et al.*, 2002; Tesch, 2003) highlights the need for identification of possible hybrids between morphologically almost indistinguishable eel species. As both species are even known to co-migrate (Okamura *et al.*, 2002) and form viable hybrids in aquaculture (Okamura *et al.*, 2004), the method proposed may help eel conservation and aquaculture management, by rapidly and reliably detecting translocated individuals and possible genetic admixture with indigenous species.

There are only two species in the North-Atlantic Ocean, the European (*A. anguilla*) and the American eel (*A. rostrata*). Based on the number of vertebrae, the American eel (vertebrae ranging from 103-110, mean 107.1) can be distinguished from the European eel (vertebrae ranging from 110-119, mean 114.7) (Boëtius 1985). Genetically both species can be discriminated reliably with the allozyme locus *MDH-2\** (Williams & Koehn (1984) and mitochondrial DNA markers (Avise *et al.*, 1986), with only a small fraction of genetic exchange detected in Iceland (Avise *et al.*, 1990). Recently, Mank & Avise (2003) reassessed these conclusions with highly polymorphic microsatellites markers, but surprisingly found no indications for hybridisation. This result prompted for further investigations on the paradigm of complete isolation of European and American eels and reopened the debate of the existence and maintenance of a hybrid zone at more than 6,000 km from the spawning site. Most recently two studies re-analyzing samples originating from Iceland found evidence for a higher proportion of hybrids (Albert *et al.*, 2006, Maes *et al.*, unpubl. data), even suggesting the existence of backcrosses between hybrids. The nature and origin of such hybrid zone has still to be discovered.

**Spatio-temporal genetic structure of European eel** - The study of genetic diversity within a species is of importance to define reproductively isolated stocks, to define fisheries quotas to preserve

genetic diversity, to enable the sound management of fisheries stocks and to assess the level of gene exchange between neighbouring populations. A recent and extensive genetic study on European eel increased significantly the geographical sampling (42 sites) and included crucial temporal replicates (at 12 sites) into their analyses to check for consistency in the observed spatial pattern (Dannewitz *et al.*, 2005). Surprisingly, no stable spatial genetic structuring was detected anymore, while temporal variance in allele frequency exceeded well the geographical component (Figure 1). Possible sampling bias due to life stage mixing and a lower effective population size than expected could explain these conflicting results (Dannewitz *et al.*, 2005).

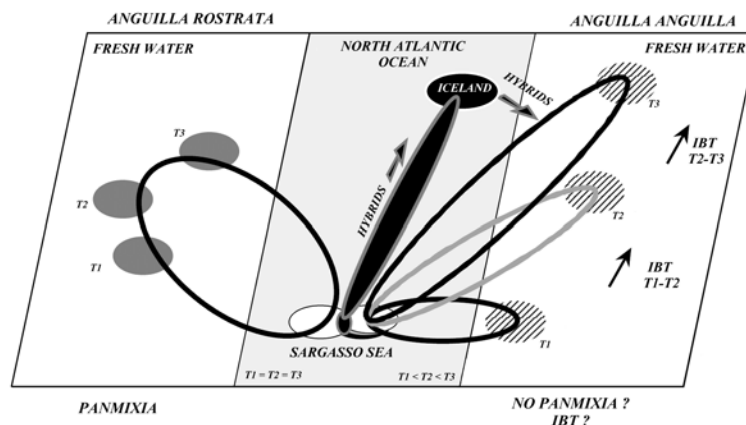


**Figure 1:** Genetic evidence based on microsatellites in favor of and against the Panmixia hypothesis using a) combined geographical and temporal (Dannewitz *et al.*, 2005) or b) exclusively geographical (Wirth & Bernatchez, 2001) samples across Europe.

Two complementary studies also highlighted the importance of temporal variation in genetic composition. Maes *et al.* (2006) detected a subtle IBT pattern between years, namely between cohorts differing two to three years. Since a heritable component inducing IBT in eel remains questionable, a transient pattern of IBT can arise mainly under the influence of environmental factors. They suggested a possible scenario for the spatio-temporal genetic structure of the European eel, taking into account its catadromous life-strategy, which is largely affected by a long migration loop and oceanic conditions. The European eel shows a protracted asynchronous spawning window in the Sargasso Sea; it is induced by differential departure times for the spawning migration and compounded by differential migrational distances for geographically distinct groups (Tesch, 2003; Kettle & Haines, 2006). Once at the Sargasso Sea, only a subset of the adults will spawn successfully and contribute to the next generation by a sweepstakes-chance matching of reproductive activity with oceanic conditions (Hedgecock, 1994; Pujolar *et al.*, 2006). Together with a differential composition at the start of the spawning migration, the random variation in parental contribution results in a heterogeneous genetic composition of recruits. Since gene flow is limited between groups differing the most in spawning time, mixing may be largely restricted to neighbouring spawning groups, producing a continuously increasing genetic differentiation in time. The large scale IBT observed in European eel most likely originates from a cumulative effect of a subtle adult genetic background and random parental success occurring each spawning season, which yields most differences between rather than within years. In summary, they detected a double pattern of variance in the genetic structure of European eel. (1) A broad scale Isolation-by-Time pattern of spawning cohorts separated by 2-3 years, possibly a consequence of the long migration loop in anguillids and strong variance in annual adult reproductive contribution; (2) a smaller scale variance in reproductive success (genetic patchiness) within cohorts among seasonally separated spawning groups, most likely originating from variable oceanic and climatic forces (Pujolar *et al.*,

2006). The protracted spawning season as well as the variance in age-at-maturity might represent a 'bet-hedging strategy', by spreading reproductive effort over time to protect eggs and larvae against unpredictable environmental conditions and food availability in the Sargasso Sea (Boyce *et al.*, 2002; Flowers *et al.*, 2002; Dulvy *et al.*, 2003, Kettle & Haines, 2006).

The European eel has been studied for over hundred years and hypotheses concerning its population structure were tested using newly developed techniques every time they appeared. Although many new insights were discovered in North Atlantic eels (see Figure 2 for a summary), the black box remains tightly closed for researchers. Many factors of its catadromous life-strategy increase the chance of panmixia, such as the variable age at maturity, the highly mixed spawning cohorts, the protracted spawning migration, the sex biased latitudinal distribution and the unpredictability of oceanic conditions. Only by tracking migrating adults and genetic monitoring their offspring through time, a reliable assessment of the factors influencing the population structure of the European eel will be possible.



**Figure 2:** Diagram showing a scenario for the contemporary genetic structure of both North-Atlantic species based on the occurrence of one (*A. rostrata*) or several (*A. anguilla*) temporally separated migration loops, with a limited amount of hybridisation between both species (modified from Tsukamoto *et al.*, 2002).

**The relation between genetic variability and fitness in eel** - Genetic diversity is the product of thousands of years of evolution, yet irreversible losses can occur rapidly (Nielsen & Kenchington, 2001). The genetic diversity characteristic of a species is essential for its long-term survival to adapt to climate change, and loss of populations, with the subsequent a loss of adaptive variation. Organisms exhibiting low levels of genomic variability may suffer from a reduced fitness, through the negative effect of inbreeding depression and genetic load of lethal allelic variants. Inbreeding depression can be seen by decreased fertility and fecundity, increased offspring mortality, fluctuating asymmetries and morphological variation and disease susceptibility. For fisheries management, the amount of genetic variability within populations is crucial to assess the quality of stocks, the potential productivity /growth of a population and the sustainability (extinction spiral).

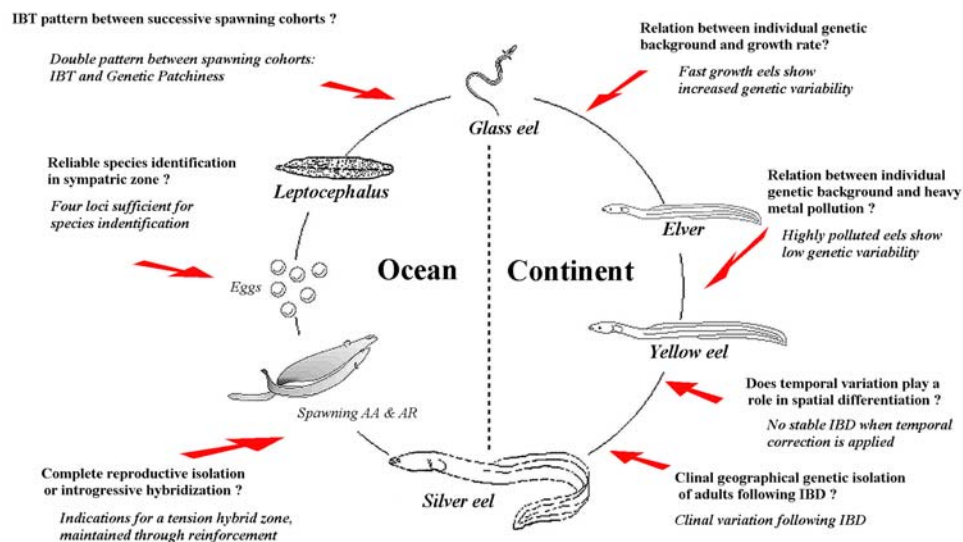
Pujolar *et al.* (2005) and Maes *et al.* (2005) assessed whether the genetic background of European eel is linked with two fitness traits, namely growth and detoxification success. Summarizing both studies, there was strong evidence for Heterozygosity-Fitness Correlations (HFC), that might be explained either by an effect of direct allozyme overdominance or associative overdominance. Selection affecting some of the allozyme loci would explain the greater strength of the HFCs found at allozymes in comparison with microsatellites, and the lack of correlation between individual heterozygosity at allozymes and at microsatellites. Associative overdominance (where allozyme loci are merely acting as neutral markers of closely linked fitness loci) might provide an alternative explanation for the HFCs. HFCs do however have consequences on the population structure and persistence of the European eel. The positive consequence of the eel's catadromous life history is that locally polluted rivers will only have a low impact on the entire population, due to the lack of spatial genetic structure at the local level. Nevertheless, selection on

each generation will erode genetic variability in a different way locally, possibly slowly decreasing overall genetic variability. Differential selective pressures might induce differences between spawning cohorts in time and space, possibly increasing the temporal differentiation pattern described above (Maes *et al.*, 2006). Similarly, fast growing individuals will mature early and may be more heterozygous than slow growing individuals. Unfortunately, eutrophic systems producing large females with much fat reserve are also the most polluted habitats. During spawning migration, pollutants will strongly decrease the fitness of such individuals, weakening the whole populations. If population size decreases even more together with genetic variability, less fit individuals will be left over for spawning, weakening even more the entire species.

**Genomics for the fisheries and aquaculture of Anguillids** - Genomics is the study of an organism's genome and the functioning of its genes. It deals with the systematic use of genome information, associated with other data (mainly phenotypic), to provide answers in biology, medicine, and industry. The field of genomics in fish is expanding and several fishes are being sequenced (see the GenBank database). Genomics can help understanding basic biological questions, especially in challenging species like eels. Applications include the understanding of adaptation, improvement of aquaculture and the discovery of novel genes coding for characteristic life-history traits.

The main future application area of genomics in eels is artificial reproduction, enabling the sustainable management of declining species. The only eel species where genomic tools are being developed is the Japanese eel (*Anguilla japonica*), one of the most important species in aquaculture because of its high economic value, particularly in East Asia. After the artificial maturation, the production of viable leptocephali and even glass eels, genetic improvement is expected to gain importance in eel aquaculture when the routine production of artificially produced glass eels can be realized (Nomura *et al.*, 2006). Nomura *et al.* (2006) mapped a number of microsatellite loci in relation to the centromere of the eel chromosomes. In the future, a high-resolution linkage map will be constructed, satisfying an essential requisite in identifying commercially important quantitative traits in the aquaculture species, and their application in marker-assisted selection (MAS).

Most recent results have been able to answer several evolutionary questions during the life-cycle of the European eel (Figure 3). The implementation of these results into management strategies will require additional work, summarized in the following section.



**Figure 3:** Summarizing figure of the main evolutionary questions and their answers in European eel.



## Genetic research perspectives in the European eel

Conclusions drawn from molecular studies are of crucial importance to infer the panmictic status of the European eel. Future genetic research could focus on several of the following issues, to help clarify the evolution of European eel and the integration of genetics in management:

**Monitoring spawning adults and larvae in the Sargasso Sea** - Despite numerous efforts, our knowledge of the spawning biology and migration routes of North-Atlantic eels has remained extremely sparse. To date no observations have been made of adult eels in the Sargasso Sea, and their eggs have yet to be identified in the area (Tesch, 2003). Recent studies of related species, *A. japonica* and *Anguilla dieffenbachii*, based on data storage tags, showed that eels on spawning migration generally swim at depths up to just a few hundred meters (Aoyama *et al.*, 1999; Jellyman & Tsukamoto, 2005). Based on the distribution of newly hatched larvae, the spawning grounds of the Japanese eel (*A. japonica*) have recently been reconfirmed by genetic identification techniques (Tsukamoto, 2006). It thus remains difficult to make firm conclusions about the genetic structure of European eel. One basic problem is that eels are not sampled at the level of reproductive units but rather at the foraging areas, where mixing of different populations could severely complicate interpretation of patterns of genetic structure. The optimal solution would be to sample spawning eels across the Sargasso Sea and analyse them using genetic markers. Due to the extreme difficulty, the second best option would be to take samples of newly hatched eel larvae from different localities. They would be representative of the genotypes of their parents and, if sampled soon after hatching, the problem of mixing of different spawning groups would be minimized.

A more precise identification of spawning and nursery areas, and environmental conditions, along with a greater knowledge of the ecosystem where spawning takes places (in particular plankton composition) would considerably improve the basis for understanding the causes of decline, in particular the possibility of climatic factors being responsible. Moreover, this information could be highly important for developing the artificial propagation of eel for aquaculture, which would be of both considerable economic interest, and would decrease fishing pressure on wild eels. Finally, knowledge of the genetic structure is essential to evaluate if e.g. harvest of eels in one specific region affects the total eel population (in case the species is panmictic) or subpopulations within the total population (in case genetically distinct eel populations exist). A Danish research cruise ([www.Galathea3.dk](http://www.Galathea3.dk)) envisages the sampling of as well adults as larvae in early 2007. The specific aims of the study are: 1. Estimating the distribution of eel larvae in the Sargasso Sea in relation to oceanographic features (currents, fronts, eddies, pycnoclines) and comparing to earlier findings from the 20<sup>th</sup> century. 2. Sampling of newly hatched eel larvae for population genetic analyses. 3. Identification of European and American eel eggs using novel DNA analyses. 4. Observation of spawning eels in the Sargasso Sea and pelagic trawling to catch mature eels. 5. Satellite Tagging using newly developed small satellite pop-up transmitters (storing information on migration depth, temperature, salinity and light).

**Combining recruitment monitoring and genetic analyses** - Considering the drastic decline in fisheries catches since 1960, the European Union has declared an emergency plan to conserve and increase the European eel population size. Measures mainly propose the reduction of fishery, the monitoring of recruitment, preservation of migration routes and the estimation of population size. Temporal fine scale analysis of successive recruitment waves of the European eel at a Southern European location (continuous recruitment) for instance would enable to validate both mechanisms (IBT and genetic patchiness) and to confirm the stochastic/deterministic nature of the IBT pattern in eel. It represents a crucial prerequisite to understand the relative contribution of oceanic and anthropogenic influences to the sharp decline of the European eel. Such standardized small-scale analysis of recruiting juveniles may provide additional answers about the spatio-temporal partitioning of genetic variation and the presence/absence of a genetically determined spawning time (Pujolar *et al.*, 2005b). We expect the number of successful families to be low if there is a

mismatch between algal bloom and larval production, resulting in a lower genetic variability and higher relatedness values. The presence of temporally separated discrete spawning groups would also be visible if present.

***Analysis of historical tissue collections and fisheries induced changes*** - The analysis of long – term time series of historical material of European eel may increase the confidence of genetic estimates of population sizes. A first step would be the use of aged adults, so that back calculations till 30-40 years ago can be performed. More importantly, to assess the influence of heavy fisheries and yearly/decadal fluctuating oceanic conditions, the analysis of historical material covering the last century is needed. This is now possible due to newly developed genetic techniques for ancient DNA and will enable the reliable calculation of a pre- and post industrial fishery genetic population size. This knowledge is of crucial importance to preserve genetic variation, known to correlate with fitness components in eel (Maes *et al.*, 2005; Pujolar *et al.*, 2005) and to define sound management issues.

Strong population declines in recruiting or adult individuals can trigger as well phenotypic as genetic changes as population dynamic effects (Law, 2000). Possible phenotypic changes under influence of overfishing or environmental changes include shifts in age and size at maturity, lower reproductive success, higher mortality, changes in growth in juveniles and adults, lowering of fecundity/fertility and possibly changes in sex-ratio. If such changes are heritable, this may lead to almost irreversible genetic changes in life-history traits. Changes in genetic composition (lowering of genetic variability) through the population decline of a species may induce unpredictable consequences, such as lower evolutionary potential and a higher mortality due to high genetic load in marine organisms (Frankham, 2002; Bierne *et al.*, 2000). Recent recommendations from the EU (WGAGFM, 2005) ask for an urgent assessment of fisheries and climatologically induced changes in declining marine stocks. A suitable strategy to detect climatic and anthropogenic influences is the joint analysis of phenotypic and genetic data from contemporary populations compared to a reference situation (before the population decrease). There is thus the need for reliable calculations of effective population sizes and possible adaptive responses in exploited marine organisms using archival material (Nielsen *et al.*, 1997; Myers & Worm, 2003). It is clear that fisheries pressure is exerted at several stages in the life-history of this catadromous species, possibly inducing considerable changes in phenotypic and genetic characteristics on the long term. There are several indications of phenotypic changes in the European eel stock throughout the last half-century, although a thorough analysis of otoliths has never been published so far. First, the size of adults (females and males) yellow/silver eels has been significantly increasing through time (W. Dekker, pers. comm.). This could be due to their older age-at-maturation or faster growth than before. It is known that density-dependant factors trigger as well sexual differentiation and growth as survival. A lower continental stock density might induce a faster growth, lower mortality and possibly shifts in sex-ratio. A faster maturation might nevertheless produce less fit females with suboptimal reproductive success. Subsequent maternal effects might possibly induce less strong offspring, resulting in smaller glass eel cohorts. Such differences in glass eel length has been observed in the last 50 years (Dekker, 2004), although environmental factors (eg North Atlantic Oscillation Index) might result in similar trends (Knights, 2003).

***Relevance of adaptive variation/local adaptation to fisheries management of eel*** - Local adaptation is one of the most significant components of intra-specific biodiversity. The relevance of local adaptation to fisheries management can be divided into two main issues that differ with respect to temporal scale. First, local adaptations and population structure affect short-term demographics through effects on local recruitment patterns. Second, local adaptations and genetic heterogeneity affect long-term population dynamics, both with respect to connectivity among stocks/populations and their resilience and response to environmental change and harvesting. Whereas the application of genetic methods to determine stock/population structure is starting to gain practical use (e.g. Nielsen *et al.*, 2001; ICES, 2005), the second consideration of effects of

local adaptation and maintenance of biodiversity on long-term sustainable fisheries management has yet to be implemented into management strategies (WGAGFM, 2006).

Genetic analyses in eel have unfortunately all been performed with a limited number of neutral loci, requiring confirmation with an adequate number of markers to increase the power of analyses. Additionally, the presence of a temporal component of genetic variation stronger than the geographic component, raises the interesting question if the observed genetic differences are due to selective events rather than non-random mating. Indeed, although intraspecific genetic structure is very subtle in many eel species, neutral genetic variation might well underestimate adaptive variation over a broad environmental range. Natural selection could produce differences by selective mortality during larval transport or during the continental phases of the life cycle. The development and study of novel markers under selection (such as Expressed Sequence Tags (ESTs) and Single Nucleotide Polymorphisms (SNPs) in candidate genes) would enable the detection of genetic variation underlying environmentally dependent fitness traits. SNPs are considered the markers of the future, due to their unambiguous scoring, short fragment size (suitable for ancient DNA), neutral/adaptive characteristics and uniform polymorphism across the genome (Syvänen, 2001). Additionally, the use of microsatellites associated to expressed sequences (EST-SSRs) could be very interesting for eels. EST-linked microsatellites might help to obtain indications on the effect of mortality during recruitment and to acquire more knowledge about the relation between heterozygosity-fitness-environment (level of pollution, parasite infection, temperature, salinity). These data are extremely important to allow the implementation of safe and sound conservation plans aimed at preserving genetic biodiversity of European eel, including adaptive variation.

***Population dynamic and genetic simulations of complex life-histories*** - A future crucial step would be to jointly assess demographic and genetic parameters by simulating different scenarios of reproductive success, migration, survival, dispersal, age structure, maturation fisheries pressure and anthropogenic stress. By subsequently validating such models with empirical genetic and population dynamic data, more knowledge will be gained on the factors likely to affect the most the European eel population (oceanic vs continental).

***Aquaculture genomics and cross species applications*** - The current fishery pressure on the European eel stock is mostly due to the lack of artificial reproduction (but see Palstra *et al.*, 2006 and references therein). Since 30 years, researchers have been unable to produce economically profitable quantity of eels in aquaculture. European eel reproduce once in their lifetime in the Sargasso Sea, 6000-km away from the Atlantic Coast. It is this extreme situation that explains the difficulties for artificial reproduction of eel. Integrating additional oceanic knowledge into management strategies, together with the reduction of fisheries, might help to define sustainable management issues, until artificial reproduction is successful. Recently the Japanese methodologies for producing eel larvae were tested in Europe on *A. anguilla* resulting in fertilized eggs, embryonic development, and occasional hatching (Palstra *et al.*, 2006). There is not yet a similar success rate as that of the Japanese researchers to obtain acceptable hatching and feeding larvae. New knowledge of fish reproduction and eel biology is increasing and opens new avenues for research. Recently, a platform to exchange ideas and to develop the required synergy between eel scientists from different research fields has been started and will be hosted by The European Aquaculture Society (EAS). This will stimulate research with the focus on solving the current problems related to reproduction of European eel.

Besides further physiological and endocrinological research, genomic tools derived from the Japanese eel are a promising way to complement such research. Given the fact that already numerous genetic markers are known to cross-amplify between *Anguilla* species (Maes *et al.*, 2006), additional genomic resources are expected to be usable in European eel. The knowledge of specific quantitative traits such as growth rate, parasite resistance, age at maturation etc may well, besides increasing the possibilities of artificial rearing, provide new crucial insights into the biology of anguillids.

## Limitations of eel genetics

Although genetics can provide a tremendous amount of useful information, this field of research has also its limitations, especially in species with a poorly understood and complex biology. European eels are long-lived animals with reproductive ages roughly ranging from 6 to 60 years. To fully assess the temporal fluctuation in population size ( $N_e$ ), a long-term analysis is crucially needed. The analysis of historical otoliths collections is a must to reliably assess the stability of the stock, but this has not been done yet, weakening current genetic knowledge. The calculation of  $N_e$  is also most reliable in those cases where values have been calculated from several generations apart. Even with otoliths of 60-100 years, the number of generations testable still remains quite low, requiring several replicates and different techniques to validate our results. Simulations are however a good option to test empirical data. Due to the eel's catadromous life-strategy, samples taken for genetic studies mostly originate from the continental population, consisting of already mixed spawning aggregations. The lack of good samples from the breeding place is seriously impeding a powerful analysis, due to the low differentiation observed between continental populations. The high genetic diversity within this species also requires large number of individuals to be analysed, to avoid to strong sampling bias (Waples, 1998). The lack of experimental breeding possibility has restrained the possibilities to test contradicting results or inheritance patterns.

## Using genetics for European eel management

Based on the Québec declaration of concern (Dekker, 2003) and the recommendation provided in the ICES-WGAGFM report of 2004 (WGAGFM, 2004), we summarize options to preserve the genetic resources in European eel and propose additional research priorities.

**Table 1.** Management objectives to address generic concerns related to the loss of genetic diversity in eel (modified from WGAGFM 2003).

Consideration	Example management objective	Time scale (in generations)
1. Species genetic integrity	1. Avoid species translocations for restocking or aquaculture	1
2. Genetic diversity among populations	2. Maintain populations in river sheds	> 100
3. Population structure and relative abundance	3. Maintain relative size of populations	> 100
4. Within-population genetic diversity	4.1. Maintain large number of individual populations	
	4.2. Minimize environmental degradation (pollution, habitat fragmentation)	
	4.3 Assess influence of <i>Anguillicola</i> and virus infection (EVEX) on reproductive potential	
5. Evolutionary potential	5.1. Minimize fisheries-induced selection	> 10
	5.2. Detect possible local adaptation	> 10

The next attention points can now be updated using new genetic knowledge and adjusted with future research priorities:

- 1) *“The genetic structure of natural populations can be best undertaken by identifying and sampling discrete reproductive aggregations”*. This is likely to happen early next year, during the Galathea campaign, during which as well adults as larvae will be caught.
- 2) *“To effectively sample putative populations on the continental shelf remains difficult, due to the confounding effect of overlapping generations in adult migrants”*. This makes that the population genetic structure of the species is as not yet fully elucidated. The routine ageing of adults in genetic studies should cope with this problem. This would also increase the comparability of results in other species and with historical data.
- 3) *“The absence of measured genotypic differentiation or small level of genetic differentiation at the molecular level does not preclude significant quantitative or adaptive differentiation”*. Reciprocal “common garden” experiments, where the phenotypes of mutual strain transfers between environments can be compared, are not feasible yet, as parental effects need to be excluded by performing such analyses on artificially produced offspring. With recent and future advances in Japanese and European eels, the production of first generation offspring becomes more feasible, enabling experiments in the “short” term.
- 4) *“Existing census data indicates that the eel is in serious decline over most of its range. It is essential that the spawning stock/stocks of eel be maintained at sufficiently large levels to ensure that effective population sizes ( $N_e$ ) as well as absolute population sizes are optimised beyond safe limits”*. There seems to be an unequal contribution of silver eels, biased towards Northern Europe. Considering recent results on the complete failure of *Anguillicola* infected and polluted eels to reach the Sargasso Sea in migration simulation experiments (Palstra *et al.*, 2006), the silver eels escaping Northern Europe might well have zero contribution to the next generation. If continental populations (mostly polluted or infected with *Anguillicola*) have not reproductive success, the marine stock component contributing to spawning should be assessed.
- 5) *“One of the few ways of monitoring the genetic health of stock size is by examining allele frequencies at a large number of highly variable microsatellite loci over time (a loss of a substantial number of alleles would be indicative of a reduction in  $N_e$ )”*. An inventory should be made of the fisheries institutes and museums that have good historical otolith collections, which could be used for temporal phenotypic and genetic analysis. Such database should be constructed and managed by a central unit to extract the maximum of information on the changes in the European eel stock during last century.
- 6) *“The sex ratio of the eel is strongly affected by the environment (temperature, stocking density and chemicals) and shifts in sex ratio occur due to anthropogenic factors (e.g. farmed and restocked eels have a large proportion of males; endocrine disruptors affect the reproductive system), could strongly reduce effective population size ( $N_e$ )”*. Such information still seems incomplete and should provide indications of temporal changes in sex-ratio.
- 7) Due to the difficulty to test specific complex hypotheses with currently available genetic tools, there is the need to combine demographic and population genetic simulations/modellisation to test empirical data. This novel research approach would enable to pinpoint the most crucial life-stage phase to be conserved and the genetic consequences of conservation plans.
- 8) Finally, we propose the study of the adaptive/evolutionary potential of eels. The analysis of otoliths to discover genetic changes in life-history traits, besides genetic changes at the molecular level (growth and maturity genes) could provide crucial insight into the repercussions on the entire species of the heavy decline of the last century. Given the phenotypic plasticity of the species, heritable genetic changes are difficult to reverse and could weaken the species even more. Environmental and anthropogenic selection can be very strong in species with a high effective population size (and low genetic drift) and sweepstakes events, providing the opportunity to selection to quickly change the genetic constitution of recruiting eels to cope with current pressures.

Generation after generation, scientists have dedicated their time and energy to study the catadromous European eel (*Anguilla anguilla* L.). Although a long way has been covered since

Aristotle's theory of spontaneous generation, the endless quest to unveil the fascinating life cycle of this mysterious creature will ultimately have to take us back to the Sargasso Sea, where everything started. For 3 million years this species succeeded in maintaining its characteristic life style with a remote spawning in the tropical North Atlantic Ocean and a juvenile foraging life phase till partial maturation in freshwater systems on the European continent. However, the dramatic decline of eel brings this species to the brink of extinction. Not much time remains to pinpoint the real causes of this decline and consequently to prevent the irreversible loss of this mysterious species.

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