Challenges for genetic research in European eel management

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Marine organisms experience a broad range of intrinsic and extrinsic influences during their lives, which impact their population dynamics and genetic structure. Subtle interpopulation differences reflect the continuity of the marine environment, but also pose challenges to those wishing to define management units. The catadromous European eel (*Anguilla anguilla*) is no exception. Its spawning habitat in the Sargasso Sea and long migration across the North Atlantic qualify it as marine. However, the synergy between hydrographic variability, changing climate, and the impacts of habitat degradation and overfishing in continental waters has negatively affected stock sizes. Its protracted spawning period, variance in age-at-maturity, parental contribution and reproductive success, and the difficulty in sampling the spawning region together may mask a weak geographical genetic differentiation. Recent molecular data report evidence for spatial as well as temporal differences between populations, with the temporal heterogeneity between intra-annual recruitment and annual cohorts exceeding the spatial differences. Despite its common name of "fresh-water eel", the European eel should really be managed on a North Atlantic scale. The fishery may have to be curtailed, migration routes kept open and water quality restored if it is to survive. Eel aquaculture has to focus on efficient rearing in the short term and controlled breeding in the long term. Future research on eel genetics should focus on (i) sampling and analysing spawning populations and recruitment waves to detect spatio-temporally discrete groups, and establishing a biological baseline from pre-decline historical collections for critical long-term monitoring and modelling of its genetic composition; (ii) the analysis of adaptive genetic polymorphism (genes under selection) to detect adaptive divergence between populations, perhaps requiring separate management strategies; and (iii) improving artificial reproduction to protect natural stocks from heavy exploitation, especially now the species has been categorized as endangered.

Keywords: effective population size, genetic patchiness, isolation-by-distance, isolation-by-time, modelling, population genetics, reproductive variance.

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Introduction

For decades, the population dynamics of marine organisms have been a key issue in management. Populations of marine species are often large, with great dispersal capacity and high fecundity (Waples, 1998; Flowers *et al.*, 2002). The main source of information on population composition, numbers, stability, and fitness of fish, almost all for commercially important species, is fisheries data. Because of their biological characteristics, marine species are much influenced by ocean currents and food abundance, leading to unpredictable reproductive success and heavy larval mortality, depending on the match (or mismatch) of larvae hatching within spring algal blooms (Hjort, 1914; Hedgecock, 1994). From the late 1980s on, it became clear that many marine fish stocks were fully exploited or even overexploited, leading to declines or even near-collapses in populations (Pauly *et al.*, 2002; Myers and Worm, 2003; Mullon *et al.*, 2005). Indeed, a clear gap exists between census population size (Nc) and effective population size (Ne), the Nc/Ne ratio often being lower than expected (Hauser *et al.*, 2002; Turner *et al.*, 2002). Genetics has proven to be valuable in discriminating independently evolving populations (Park and Moran, 1994; Ward, 2000) and providing indirect estimates of dispersal, population size, demography, and stock sustainability (Palumbi, 1994; Waples, 1998; Avise, 2004). The synergy between climate change and anthropogenic influences, such as heavy fisheries and habitat degradation, has played a major role in the decline of many commercial species (Dulvy *et al.*, 2003).

The life history of the catadromous European eel (*Anguilla anguilla* L.) depends on oceanic conditions; maturation, migration, spawning, larval transport, and recruitment dynamics are completed in the open ocean (Knights, 2003; Tesch, 2003; Kettle and Haines, 2006). Despite the biological importance of the marine phase (Knights, 2003), most research to date has focused on the fresh-water phase of the life history. The European eel is beyond safe biological limits (Dekker, 2003), because fisheries data indicate that the stock is at its historical minimum (1% of the 1960 recruitment level). European eels have several life history characteristics that make them particularly vulnerable to overexploitation: they are long-lived, are large, mature late, produce all their offspring at once, are subject to heavy mortality, and migrate long distances, right across the Atlantic. There is significant international trade demand for the species, both for live glass eels (from Europe to Asia) and the highly valued meat of adults. Reports have shown that poaching and the illegal trade

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are a concern for the species, suggesting that better regulation of international trade is necessary, given that further decline may be exacerbated by other anthropogenic factors such as freshwater and coastal habitat loss, pollution, parasitism, climate change, change in ocean currents, and blocking of inland migration routes (Dekker, 2003; Knights, 2003). A synergy between all these factors seems the most likely cause of the declines (Wirth and Bernatchez, 2003). In practice, genetic data may help assess species integrity within the North Atlantic, evaluate the number of genetic stocks of the European eel, clarify the spatio-temporal stability of genetic structure, define the influences of oceanic conditions on genetic variability, and evaluate the effect of population decline on genetic variability and the overall fitness of eels.

The European Commission recently produced an action plan for the European eel, which aims to strengthen the return rate of adult eels to the Sargasso Sea and includes the development of national management plans (CEC, 2005). Further, the European eel has been added recently to Appendix II of the CITES Red List of Endangered Species, implying drastic restrictions on trading. To better protect the species, it is important to maintain intraspecific genetic diversity, to develop sound restocking programmes for broodstock enhancement, and to help realize profitable artificial breeding. The aim here is to introduce briefly the genetic consequences of typical marine life-history traits and anthropogenic pressures, to synthesize the most recent genetic knowledge on the European eel, and to provide an overview of possible better use of genetics in future management decisions on this declining species.

Biological characteristics of marine organisms and genetic consequences

Marine organisms experience a wide range of intrinsic and extrinsic influences during their lives, which impact the population dynamics and the genetic structure (Waples, 1998). Because of the lack of obvious barriers to dispersal, delineation of marine biological populations requires great research effort to avoid overexploiting seemingly well-connected stocks. On the one hand, such subtle genetic differences reflect the continuity of the marine environment, on the other, they pose serious challenges to the definition of management units (Kenchington *et al.*, 2003). Marine fish are expected to exhibit great genetic variability, a high rate of exchange between populations (gene flow), inducing low genetic differentiation (low genetic signal:noise ratio), and a large genetic population size (Waples, 1998). In diadromous species, selection pressure during larval migration and freshwater/marine residence may further influence the genetic pattern of populations (Mitton, 1997).

Widely distributed species are 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, e.g the island model, the stepping-stone model, or the isolation-by-distance (IBD) model (Rousset, 1997). In populations consisting of a mixture of individuals reproducing at different times within a reproductive season, temporal differentiation may supplement geographical partitioning. Under such conditions, gene flow between early and late reproducers would be expected to be limited, possibly creating a pattern of isolation-by-time (IBT) (Hendry and Day, 2005; Maes *et al.*, 2006b). Additionally, temporal heterogeneity in the genetic composition of recruits is likely to result from 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, attributing the variation in reproductive success of adults to spatio-temporal variation in oceanographic conditions, within and among seasons. Many marine species split their reproductive effort between several events during a protracted spawning season, to maximize reproductive success (Hutchings and Myers, 1993; Maes *et al.*, 2006b).

Good knowledge of stock structure, demographic dynamics and stability, and the sustainability of harvested stocks is therefore required for effective management of natural resources (Kenchington *et al.*, 2003). Such information is vital in assessing and establishing management units, or in determining whether unique (neutral or adaptive) genetic variation is restricted to specific regions. For example, a local fishery might collapse if genetic variation is reduced massively. Recent advances in biotechnology have produced exciting high-resolution techniques that can be applied to long-standing problems in fisheries science and conservation biology. Marine conservation genetics presents many challenges, because of the high rate of gene flow, the openness of populations, and the lack of obvious barriers to dispersal (Waples, 1998). The population dynamics of marine species are sometimes so complex that the results of genetic studies become increasingly difficult to interpret to infer the demographic stability of harvested stocks. For instance, the occurrence of overlapping generations, plasticity in life-history traits, and low Ne/Nc ratios make it difficult to interpret the biological significance of genetic parameters such as the level of genetic differentiation (e.g. F_{ST}) and effective population size values (e.g. Ne), despite their statistical significance (Waples, 1998).

The European eel: current genetic status

Early population genetic studies, based on differences in transferrins and liver esterases, resulted in claims that European eel populations differed between continental European locations (Drilhon *et al.*, 1966, 1967; Pantelouris *et al.*, 1970), suggesting a southeastern Mediterranean reproductive area. Later allozymatic studies failed to detect obvious spatial genetic differentiation (De Ligny and Pantelouris, 1973; Comparini *et al.*, 1977; Comparini and Rodinò, 1980; Yahyaoui *et al.*, 1983). Mitochondrial DNA initially provided only limited insight into the geographical partitioning of genetic variability in the European eel, suggesting a single common gene pool (Lintas *et al.*, 1998). This commonly accepted view of a panmictic genetic population structure, based on oceanographic (Sinclair, 1988; Tesch, 2003) and genetic features, was, however, recently challenged by three independent studies (Daemen *et al.*, 2001; Wirth and Bernatchez, 2001; Maes and Volckaert, 2002). Wirth and Bernatchez (2001) and Maes and Volckaert (2002) detected a relationship between genetic and geographic distance (the so-called IBD), suggesting a subtle spatio-temporal separation of spawning populations, with some degree of gene flow. Ocean currents, causing differential distribution of eel larvae, have also been suggested partly to explain the observed clinal genetic variation (Kettle and Haines, 2006). However, the unstable genetic architecture of European eel populations over time may be linked to it (Dannewitz *et al.*, 2005).

Recent advances in European eel genetics

Genetic knowledge in anguillids continues to grow and, over the past few years, several new insights have been gained to help management of eels. Research focus can be separated into studies on the genetic species and hybridization status, spatio-temporal genetic structure, genetic variability–fitness correlations, and the application of genomics in eel aquaculture.

Genetic identification of species and hybrids

In all, 15 species of *Anguilla* are formally recognized. However, the morphological and meristic characteristics are highly unstable, even between phylogenetically distant species, and they remain difficult to use for species identification (Tesch, 2003). 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 identification is problematic at the larva stage, where essential traits such as colour and dentition characters are lacking (Tesch, 2003; Watanabe *et al.*, 2004). Owing to the remote translocations of non-native species for aquaculture, the natural distribution of several species has been disrupted. That introduced European eels represent up to 31% of the eel population of some Japanese rivers and are known to co-migrate and form viable hybrids in aquaculture with the native Japanese eel (*A. japonica*) (Zhang *et al.*, 1999; Okamura *et al.*, 2002, 2004; Tesch, 2003), highlights the need for rapid identification of species and possible interspecific hybrids. This issue has many repercussions on eel management, mainly for fish traceability, fish product forensics, law enforcement, the detection of intercrossing between species, and the evolutionary implications of hybridization. Until now, eel taxa have been identified by various molecular techniques (Comparini and Rodinò, 1980; Tagliavini *et al.*, 1995; Aoyama *et al.*, 2001; Lin *et al.*, 2002; Rehbein *et al.*, 2002; Hwang *et al.*, 2004; Minegishi *et al.*, 2005), and several reliable molecular tests are available to identify *Anguilla* spp. simultaneously in processed, historical or alcohol-preserved samples without the need for sequencing (Lin *et al.*, 2002; Watanabe *et al.*, 2004; Itoi *et al.*, 2005; Sezaki *et al.*, 2005; Gagnaire *et al.*, in press). Recently, Maes *et al.* (2006a) showed reliable species identification using a single PCR reaction of nuclear markers, permitting the joint assessment of species identity and the presence of hybrids between eel species. Such efficient molecular tools to monitor and document harvesting and marketing of food products are highly suited to identifying illegal fishing, fraud, and criminal activities.

The two North Atlantic eel species can be separated based on the number of vertebrae. The American eel (*A. rostrata*) has a vertebral count ranging from 103 to 110 (mean 107.1) compared with the 110–119 (mean 114.7) of the European eel (Boëtius and Harding, 1985). Using genetics, both species are reliably discriminated with the allozyme locus *MDH-2** (Williams and Koehn, 1984) and mitochondrial DNA markers (Avise *et al.*, 1986), with only a small fraction of genetic exchange in Iceland (Avise *et al.*, 1990). Recently, Mank and Avise (2003) reassessed these conclusions using highly polymorphic microsatellites markers, but surprisingly found no obvious indications for hybridization. This result prompted further investigation of the paradigm of complete isolation of European and American eels and reopened the debate on the existence and maintenance of a hybrid zone more than 6000 km from the spawning site. Very recently, two studies re-analysing samples originating from Iceland found evidence of a greater proportion of hybrids in Iceland than before (Maes, 2005; Albert *et al.*, 2006), even suggesting backcrosses between hybrids. The nature and origin of such a hybrid zone remains to be discovered.

Spatio-temporal genetic structure

The study of genetic diversity within a species is of importance in defining reproductively isolated stocks, in giving fisheries management advice, in preserving genetic diversity, in facilitating sound management of fisheries stocks, and in assessing the level of gene exchange between neighbouring populations. A recent genetic study on European eels increased significantly the geographic sampling (42 sites), and included temporal replicates (at 12 sites) to check for consistency in the spatial pattern observed (Dannewitz *et al.*, 2005). Surprisingly, no stable spatial genetic structuring was detected, but temporal variance in allele frequency exceeded by far the geographic component. Possible sampling bias attributable to mixing of life stages and a lower effective population size than expected could explain these conflicting results (Dannewitz *et al.*, 2005).

Two complementary studies further highlighted the importance of temporal variation in genetic composition (Maes *et al.*, 2006b; Pujolar *et al.*, 2006). Maes *et al.* (2006b) detected a subtle IBT pattern between years, most likely attributable to the influence of environmental factors. They suggested the following scenario for spatio-temporal genetic structure of the European eel. The protracted asynchronous spawning window of European eels in the Sargasso Sea is induced by differential departure times for the spawning migration and is compounded by differential migrational distances of geographically distinct groups (Tesch, 2003; Kettle and Haines, 2006). Once at the Sargasso Sea, only a subset of the adults spawn successfully and contribute to the next generation by a lottery matching of reproductive activity with oceanic conditions (Hedgecock, 1994; Pujolar *et al.*, 2006). Besides the differential composition of the spawning group at the start, the variation in parental contribution may result in a heterogeneous genetic composition of recruits. As gene flow is limited between groups differing the most in spawning/arrival time, mixing may be largely restricted to neighbouring spawning groups, producing a continuously increasing genetic differentiation with time. The large-scale IBT observed in European eels most likely originates from a cumulative effect of a subtle adult genetic background and variable parental success during each spawning season, which yields most differences between rather than within years (Maes *et al.*, 2006b; Pujolar *et al.*, 2006). The protracted spawning season as well as the variance in age-at-maturity might therefore 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 and Haines, 2006; Maes *et al.*, 2006b).

Relation between genetic variability and fitness

Genetic diversity is the product of thousands of years of evolution, yet irreversible losses may occur rapidly (Kenchington *et al.*, 2003). It is essential for long-term survival, to adapt to climate change and anthropogenic pressure leading to the loss of populations, with the likely subsequent loss of adaptive variation. For fisheries management, the extent of genetic variability within populations is crucial in assessing the quality of stocks, the potential productivity or growth of a population, and the sustainability of fisheries.

Maes *et al.* (2005) and Pujolar *et al.* (2005) assessed whether the genetic background of European eels could be linked to two fitness traits, early growth and pollutant bioaccumulation. Summarizing both studies here, there was strong evidence for heterozygosity-fitness correlations (HFC), likely explained either by an effect of direct allozyme overdominance or associative overdominance. The positive consequence of the catadromous life history of eels is that locally polluted rivers will only have a low impact on the entire population, because of the lack of spatial genetic structure at a local level. Nevertheless, selection during each generation may erode local genetic variability differentially, slowly reducing overall genetic variability. Differential selective pressures might induce variation between spawning cohorts in time and space, possibly increasing the temporal differentiation pattern described by Maes *et al.* (2006b) and Pujolar *et al.* (2006).

Genomics for fisheries and aquaculture management of anguillids

Genomics is the large-scale study of an organism's genome and the functioning of its genes, most often associated with other data (mainly phenotypic). The science is generally divided into functional, environmental, and comparative genomics. Comparative genomics essentially consists of comparing the DNA content and arrangement between different organisms with the aim of understanding evolution and how organisms adapt to different environments and life. Functional genomics takes this one stage further, by taking a DNA sequence and working out what its function is, how it is regulated, and in what biochemical pathways it is involved. Environmental genomics deals with the understanding of the functional significance of genetic variation in natural biological communities. The field of genomics in fish is expanding, and several fish have been or are being sequenced (see the GenBank database at http://www.ncbi.nlm.nih.gov). Genomics can help to understand many basic biological questions, especially in challenging species such as eels. Applications include the understanding of adaptation, improvement of aquaculture, and the discovery of novel genes coding for characteristic life-history traits.

The most promising application of genomics in eels is artificial reproduction, which gives real perspectives for sustainable management. Genomic tools are being developed for the Japanese eel, one of the most important species in aquaculture because of its great economic value in East Asia. After success in artificial maturation, the production of viable leptocephali and even glass eels (Kagawa *et al.*, 2005), 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). In future, a high-resolution linkage map may be constructed, satisfying an essential prerequisite to identification of commercially important quantitative traits, and their application in marker-assisted selection (MAS).

Genetic research perspectives and management of the European eel

The European eel has been studied for more than 100 years, and hypotheses concerning its population structure have been tested using newly developed techniques every time they appeared. Most recent genetic results have answered several evolutionary challenges along the life cycle of the European eel (Figure 1). Many factors of its catadromous life strategy on the one hand 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. Implementation of these results, summarized below, will require additional work. For a more detailed review of population genetics and the life cycle of eels (genus *Anguilla*), we refer the reader to Van Ginneken and Maes (2005).

Several management and scientific measures have been proposed by the European Union to understand and reverse the decline of eel populations. They include assessing and reducing the impact of the fishery, monitoring recruitment, preserving migration routes (removing migration barriers), assessing the impact of restocking (preserving potential local populations), assessing anthropogenic influences (pollution, parasites), and estimating the spawning population size (CEC, 2005; ICES, 2006). When considering the genetic complements of these measures, one immediately sees a need to assess the spatio-temporal population structure by sampling the spawning grounds (in the Sargasso Sea), to carry out an integrated analysis of census population size (Nc) and to determine the relationship between historical and current effective population sizes (Ne), to analyse genetic markers located in functional regions to unveil possible adaptive variation under natural and anthropogenic conditions, and to gain understanding of molecular mechanisms involved in important traits for aquaculture and artificial reproduction.

Earlier conclusions drawn from molecular studies are not only important in inferring the panmictic status of the eel, but also to preserve the genetic resources in European eels and to define additional research priorities. For each priority, one can define a specific management objective and the time-frame during which changes or reversal may be achieved (Table 1). It is obvious, for instance, that genetic diversity may be lost rapidly (i.e. genetic erosion), and that it recovers very slowly within populations (ICES, 2005). Future genetic research may therefore focus on the conservation issues listed above, to help clarify the evolution of European eel and the integration of genetics into management. We propose three major lines of research: assessment of the spawning population structure and effective population size, including adaptive genetic variation in management plans, and improving artificial reproduction through aquaculture genomics.

Spawning population structure and size

The genetic structure of natural marine populations is best understood by identifying, sampling, and analysing discrete reproductive aggregations (Waples, 1998). Our knowledge of the spawning biology and migration routes of North Atlantic eels remains poor. Precise localization of spawning grounds, nurseries, and retention zones, along with a greater knowledge of the ecosystem where spawning takes place, would help management decisions considerably. To date no observations have been made of adult eels in the Sargasso Sea, and their eggs have yet to be identified there (Tesch, 2003). In the Pacific Ocean, based on the distribution of newly hatched larvae, the spawning grounds of the Japanese eel have been reconfirmed by genetic identification techniques (Tsukamoto, 2006). For the European eel, there is no biological material available from the reproductive areas for genetic research. The continental populations constitute mixed feeding aggregations, complicating interpretation of patterns of genetic structure (Dannewitz *et al.*, 2005; Maes *et al.*, 2006b; Pujolar

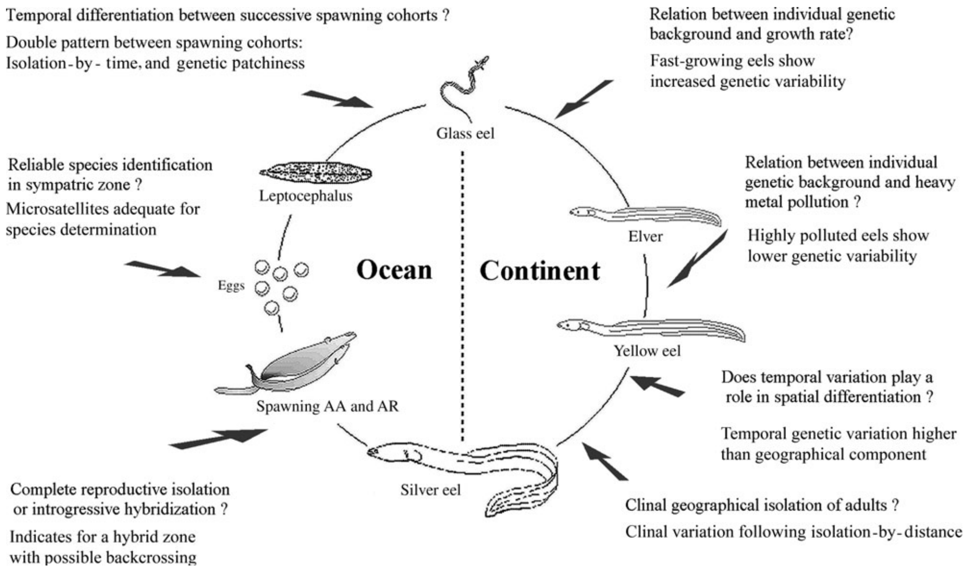

Figure 1. European eel: life cycle of the main recent evolutionary questions.

et al., 2006). Sampling putative populations on the continental shelf remains challenging, because of the confounding effect of overlapping generations in adults and the site-dependent age structure. The most effective solution is to sample spawning eels and newly hatched larvae across the Sargasso Sea, and to analyse them with a representative set of genetic markers. This would allow a reassessment of the spatial and temporal segregation found so far and a reliable calculation of the size of the spawning stock (Ne). The development of precise, performing genetic markers (such as SNPs) for application on highly degraded or old DNA, would also provide new opportunities to compare today's genetic patterns with the patterns some 100 years ago, based on the degraded larval samples of Schmidt (1923).

Additionally, analysis of successive recruitment waves of European eels at sites with year-round recruitment would permit better understanding of the fine-scale genetic composition of glass eels and possibly pinpoint discrete spawning groups. A sharp break or clinal pattern in relatedness and genetic differentiation may point to reproductively isolated aggregations (Maes *et al.*, 2006b). In turn, stochastic variance in genetic composition might point to genetic patchiness, most likely under the influence of annual and seasonal oceanic and climatological fluctuations (such as the North Atlantic

Table 1. European eel: management objectives related to the loss of genetic diversity (modified from Kenchington *et al.*, 2003).

Consideration	Example management objective	Time-scale (generations)
Genetic integrity at the species level	Avoid species translocations for restocking or aquaculture	1
	Trace species identity of endangered fish (products)	
Genetic diversity within and among populations	Maintain population size in river sheds	>100
	Decrease glass eel fishery and export	
	Increase silver eel escapement to contribute to spawning stock	
Population structure and relative abundance	Avoid large-scale translocations within Europe and between continents	>100
	Detect possible local adaptation between river basins	
	Maintain relative size of populations	
Effective population size and demographic stability	Maintain large number of individual populations	>10
	Minimize environmental degradation (pollution, habitat fragmentation)	>10
	Assess influence of parasites (e.g. *Anguillicola*) and pathogens (e.g. virus infection — EVEX) on reproductive potential	>10
Evolutionary potential	Minimize fisheries-induced selection	>10
	Avoid directional adaptation to anthropogenic and environmental changes	>10

Oscillation; Knights, 2003; Friedland *et al.*, 2007). They are thought to influence the reproductive success of adults and the survival rate of larvae (Dekker, 2004; Pujolar *et al.*, 2006).

Accurately estimating the effective (genetic) population size (Ne) is another aim in developing an appropriate conservation strategy for eels. Ne predicts the rate of loss of neutral genetic variation, the fixation rate of deleterious and favourable genetic variants, and the rate of increase of inbreeding experienced by a population (Frankham *et al.*, 2002). Importantly, the Ne of a population is often several orders of magnitude smaller than the census size (Nc) of the population, owing to unequal sex ratios, variance in reproductive success, and assortative mating. In marine fish (including eels), Ne/Nc ratios may be expected to be more extreme than in other vertebrates because of the high female fecundity that allows large census numbers to be obtained from minimal numbers of breeding animals. Indirect methods for estimating Ne based on molecular marker data have been developed to facilitate the inference of population size, a very difficult task in marine fish with their lack of confined geographic boundaries. When considering census population data of European eels, which indicate that the species is in serious decline over most of its range, it is essential to maintain the spawning stock(s) at sufficiently large levels to ensure that effective population sizes (Ne) as well as absolute population sizes (Nc) are optimized above safe limits. European eels are long-lived animals with reproductive ages roughly ranging from 6 to 60 years (Tesch, 2003). To assess fully the temporal fluctuation in population size (Ne), a long-term analysis over several generations would be ideal. An analysis of time-series of historical material may increase the confidence in genetic estimates of population sizes. This should be done over a period as long as possible to avoid the shifting-baselines trap (Pauly, 2007). Realistically, the past 100 years should suffice, because anthropogenic impact seems to have been greatest during that period (e.g. endocrine disruption of spawning, overfishing, river management). Such an analysis is now feasible thanks to the development of appropriate genetic techniques for ancient DNA (Nielsen *et al.*, 1997). For example, reliable estimates of population size have been calculated for several fish species in a pre- and post-industrial fishery (Nielsen *et al.*, 1997; Hauser *et al.*, 2002; Turner *et al.*, 2002). This knowledge is of great importance in managing genetic variation, which is known to correlate with fitness components in eel (Maes *et al.*, 2005; Pujolar *et al.*, 2005), and to define sound management strategies.

Finally, the accurate interpretation and extrapolation of genetic results in eels requires an assessment of demographic scenarios through the development of new population dynamics models. Such models have been the basis of fisheries research for a long time, but here we ask for a joint assessment of demographic, hydrodynamic, and genetic parameters. Simulating a range of scenarios of reproductive success, migration, survival, dispersal, age structure, maturation, fisheries pressure, and anthropogenic stress, preferably from an ecosystem perspective, looks a promising field. Subsequent validation with empirical genetic and population dynamic data may confirm the key factors.

Adaptive genetic variation for fisheries management

Heavy fishing and other anthropogenic influences, such as pollution and barriers of migration, will not only impact the census size and the effective population size of eels. Large declines in mature adults and recruiting individuals may trigger phenotypic and adaptive genetic changes over generations of harvesting (Law, 2000). Such phenotypic changes may include shifts in age- and size-at-maturity, less reproductive success, greater mortality, changes in growth patterns of juveniles and adults, lower fecundity and fertility, and changes in the sex ratio. If changes are heritable, this may lead to almost irreversible genetic changes in life-history traits (Law, 2000). Recent recommendations from the EU (ICES, 2005) urge the assessment of fisheries and climatologically induced changes in declining marine stocks. A suitable strategy would be a joint analysis of phenotypic and genetic data from contemporary populations, compared with a reference situation (preferably before the population decrease). There is clearly the need for reliable investigations of possible adaptive responses in exploited marine organisms using archival material (Nielsen *et al.*, 1997; Myers and Worm, 2003). Although some evidence exists for phenotypic changes in the European eel stock throughout the past 50 years (increasing adult size and decreasing glass eel size since the 1960s), the evolutionary interpretation of overfishing is complicated by there being too few age-specific data, such as on age-at-maturation and growth rate (Dekker, 2004). The long-term genetic consequences of heavy fishing at the adaptive molecular level, such as a decrease or shift in genetic variability at important functional genes related to maturity and growth, have not been assessed yet.

Further, the presence of only a small level of geographical genetic differentiation at neutral molecular markers may lead to seriously underestimating quantitative and adaptive differentiation between populations. Indeed, apart from analysing neutral genetic variation to assess the demographic independence and stability of fisheries stocks, knowledge of geographic and temporal scales of adaptive genetic variation is crucial to species conservation (Conover *et al.*, 2006). Local adaptation is one of the most significant components of intraspecific biodiversity, and the relevance of local adaptation to fisheries management can be divided into two main issues, each differing in temporal scale (ICES, 2006). 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, with respect to the connectivity among stocks/populations and their resilience and response to environmental change and harvesting. Local adaptation and the maintenance of biodiversity on the long term for sustainable fisheries management has yet to be implemented into management strategies (ICES, 2006). Unfortunately, the understanding of these phenomena is particularly difficult in marine organisms. The spatial and temporal scale of adaptive divergence has been assumed to be very large. However, evidence of geographically structured local adaptation in physiological, morphological, and functional genetic traits has become apparent (Giger *et al.*, 2006; Nielsen *et al.*, 2006). The proportion of quantitative trait variation at the among-population level (Q_{ST}) has repeatedly been shown to be much higher than for neutral markers (F_{ST}) (Cousyn *et al.*, 2001; Conover *et al.*, 2006). As both metrics of genetic variation are poorly correlated, knowledge of neutral variation does not provide much information about adaptive variation (McKay and Latta, 2002; see Conover *et al.*, 2006, for a review). Given the important link between population genetics and dynamics, and the strong potential for selection in species with large population sizes, the application of both selected and neutral markers is obviously needed to resolve the stock structure of marine fish effectively.

Up to now, all genetic analyses of eels have been performed with a limited number of neutral genetic markers. The observation of a temporal genetic component exceeding the geographic component suggests the possibility of genetic differences under selective influences. The development and study of markers under selection (such as expressed sequence tags, ESTs, and single nucleotide polymorphisms, SNPs, in candidate genes) would allow detection of the genetic variation underlying environmentally dependent fitness traits in eels. Additionally, genetic markers in functional regions could support understanding of specific life-history traits, such as mortality during recruitment, age-at-maturity (and its relationship to fishing pressure), and the relationship between heterozygosity–fitness–environment (level of pollution, parasite infection, temperature, salinity) (Maes *et al.*, 2005; Pujolar *et al.*, 2006). We propose starting to study the adaptive/evolutionary potential of eels through the analysis of contemporary populations and archived otoliths, to discover local populations and genetic changes in life-history traits, by jointly screening for such genetic changes at a phenotypic and a molecular level. With its high levels of gene flow and large expected Ne, the potential for selection in the European eel is huge (Conover *et al.*, 2006; Maes *et al.*, 2006b). Partitioning contemporary and historical phenotypic variance in a plasticity and genetic component can be very useful, because heritable genetic changes might be difficult to reverse and could weaken the species even further (Hutchings *et al.*, 2007).

Artificial reproduction and aquaculture genomics

Current fishing pressure on European eels could be decreased considerably if artificial reproduction were possible (but see Palstra *et al.*, 2005, and references therein). Despite many attempts over the past 30 years, it remains impossible to produce economically profitable quantities of eels in aquaculture. Recently, methodologies developed to produce eel larvae of *A. japonica* have been tested in Europe on *A. anguilla*, resulting in fertilized eggs, embryonic development, and occasional hatching (Palstra *et al.*, 2005). Success, however, remains low, calling for further study of the husbandry of eels, and of reproductive and general eel biology. Original insights on physiology and endocrinology may be expected from advanced genomic tools. For instance, Miyahara *et al.* (2000) produced 196 expressed sequence tags (ESTs) from a spleen library of Japanese eels, and Kalujnaia *et al.* (2007) was able to identify, through subtractive hybridization and microarrays, a large number of genes down- and up-regulated during osmoregulation in gill, kidney, and intestinal tissue. As new tools become available in related anguillids (e.g. Japanese eel; Nomura *et al.*, 2006) and related genome-rich species, promising insights in functional and comparative genomics are expected in the near future. EST sequencing and linkage maps may be other feasible genomic approaches, representing the first steps towards identifying important genes and quantitative trait loci (QTL), the basis for Marker assisted selection. Although larvae of Japanese eel have only been bred with great effort, Nomura *et al.* (2006) have managed to prepare a low-density linkage map based on 43 microsatellite markers, and many more are being developed (K. Nomura, pers. comm.). Given the numerous genetic markers known to cross-amplify between *Anguilla* species (Maes *et al.*, 2006a), once progeny become available for European eels, reliable paternity screening, gene expression and microarray analyses, and a linkage map become realistic goals. Quantitative traits such as growth rate, food conversion, postponed maturity, stress tolerance, and parasite resistance strongly correlate with the possibilities of artificial rearing. One long-term issue where QTL may be of great help is in the management of feed supply. Currently, wild-caught fishmeal is an important ingredient of dry feeding pellets, but it is expected to shift to a proportionally larger vegetarian diet.

Conclusions

For many generations, scientists have dedicated time and energy to studying the catadromous European eel. Although much has been covered since Aristotle's theory of spontaneous generation, the quest to understand fully the fascinating life cycle of this fish species has taken us back to the Sargasso Sea, where everything started. As eels have been brought to the brink of extinction, new data will hopefully generate crucial insights on the reproductive cycle. Besides improving the success of artificial reproduction, it is our opinion that an integrated analysis of data and knowledge from the marine part of its life cycle, including hydrodynamics, ecotoxicology, archived material, and neutral vs. adaptive genetic variation, are the next steps in developing a global eel management strategy.

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References

Albert, V., Jonsson, B., and Bernatchez, L. 2006. Natural hybrids in Atlantic eels (*Anguilla anguilla, A. rostrata*): evidence for successful reproduction and fluctuating abundance in space and time. Molecular Ecology, 15: 1903–1916.

Aoyama, J., Nishida, M., and Tsukamoto, K. 2001. Molecular phylogeny and evolution of the freshwater eel, genus *Anguilla*. Molecular Phylogenetics and Evolution, 20: 450–459.

Avise, J. C. 2004. Molecular Markers, Natural History and Evolution. Sinauer Associates, Sunderland, MA, USA.

Avise, J. C., Helfman, G. S., Saunders, N. C., and Hales, L. S. 1986. Mitochondrial DNA differentiation in North-Atlantic eels: population genetic consequences of an unusual life-history pattern. Proceedings of the National Academy of Sciences of the USA, 83: 4350–4354.

Avise, J. C., Nelson, W. S., Arnold, J., Koehn, R. K., Williams, G. C., and Thorsteinsson, V. 1990. The evolutionary genetic status of Icelandic eels. Evolution, 44: 1254–1262.

Boëtius, J., and Harding, E. F. 1985. A re-examination of Johannes Schmidt's Atlantic eel investigations. Dana, 4: 129–162.

Boyce, M. S., Kirsch, E. M., and Servheen, C. 2002. Bet-hedging applications for conservation. Journal of Biosciences, 27 (Suppl. 2): 385–392.

CEC. 2005. Proposal for a council regulation: establishing measures for the recovery of the stock of European eel. European Commission COM(2005) 472 final, 2005/0201 (CNS). 11 pp.

Comparini, A., Rizzotti, M., and Rodino, E. 1977. Genetic control and variability of Phosphoglucose Isomerase (PGI) in eels from the Atlantic Ocean and the Mediterranean Sea. Marine Biology, 43: 109–116.

Comparini, A., and Rodinò, E. 1980. Electrophoretic evidence for 2 species of *Anguilla* leptocephali in the Sargasso Sea. Nature, 287: 435–437.

Conover, D. O., Clarke, L. M., Munch, S. B., and Wagner, G. N. 2006. Spatial and temporal scales of adaptive divergence in marine fishes and the implications for conservation. Journal of Fish Biology, 69 (Suppl. C): 21–47.

Cousyn, C., De Meester, L., Colbourne, J. K., Brendonck, L., Verschuren, D., and Volckaert, F. 2001. Rapid, local adaptation of zooplankton behavior to changes in predation pressure in the absence of neutral genetic changes. Proceedings of the National Academy of Sciences of the USA, 98: 6256–6260.

Daemen, E., Cross, T., Ollevier, F., and Volckaert, F. A. M. 2001. Analysis of the genetic structure of European eel (*Anguilla anguilla*) using microsatellite DNA and MtDNA markers. Marine Biology, 139: 755–764.

Dannewitz, J., Maes, G. E., Johansson, L., Wickström, H., Volckaert, F. A. M., and Jarvi, T. 2005. Panmixia in the European eel: a matter of time. Proceedings of the Royal Society of London Series B, 272: 1129–1137.

Dekker, W. 2003. Did lack of spawners cause the collapse of the European eel, *Anguilla anguilla*? Fisheries Management and Ecology, 10: 365–376.

Dekker, W. 2004. Slipping through our hands: population dynamics of the European eel. PhD thesis, University of Amsterdam.

De Ligny, W. D., and Pantelouris, E. M. 1973. Origin of European eel. Nature, 246: 518–519.

Drilhon, A., Fine, J. M., Amouch, P., and Boffa, G. A. 1967. Les groupes de transferrines chez *Anguilla anguilla*. Etude de deux populations d'origine géographique différente. Comptes rendus de l'Academie de Sciences de France, 265: 1096–1098.

Drilhon, A., Fine, J. M., Boffa, G. A., Amouch, P., and Drouhet, J. 1966. Les groupes de transferrines chez l'anguille. Différences phénotypiques entre l'anguille de l'Atlantique et les anguilles de mediterrannée. Comptes rendus de l'Academie de Sciences de France, 262: 1315–1318.

Dulvy, N. K., Sadovy, Y., and Reynolds, J. D. 2003. Extinction vulnerability in marine populations. Fish and Fisheries, 4: 25–64.

Flowers, J. M., Schroeter, S. C., and Burton, R. S. 2002 The recruitment sweepstakes has many winners: genetic evidence from the sea urchin *Strongylocentrotus purpuratus*. Evolution, 56: 1445–1453.

Frankham, R., Ballou, J. D., and Briscoe, D. A. 2002. Introduction to Conservation Genetics. Cambridge University Press, Cambridge, UK.

Friedland, K. D., Miller, M. J., and Knights, B. 2007. Oceanic changes in the Sargasso Sea and declines in recruitment of the European eel. ICES Journal of Marine Science, 64: 519–530.

Gagnaire, P. A., Tsukamoto, K., Aoyama, J., Minegishi, Y., Valade, P., and Berrebi, P. RFLP and semi-multiplex PCR-based identification of four eel species from the South Western Indian Ocean region. Journal of Fish Biology, in press.

Giger, T., Excoffier, L., Day, P., Champigneulle, A., Hansen, M., Powell, R., and Largiadèr, C. 2006. Life history shapes gene expression in salmonids. Current Biology, 16: 281–282.

Hauser, L., Adcock, G. J., Smith, P. J., Ramirez, J. H. B., and Carvalho, G. R. 2002. Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (*Pagrus auratus*). Proceedings of the National Academy of Sciences of the USA, 99: 11742–11747.

Hedgecock, D. 1994. Temporal and spatial genetic structure of marine animal populations in the California Current. CalCOFI Report, 35: 73–81.

Hendry, A. P., and Day, T. 2005. Population structure attributable to reproductive time: isolation by time and adaptation by time. Molecular Ecology, 14: 901–916.

Hjort, J. 1914. Fluctuations in the great fisheries of northern Europe. Rapports et Procès-Verbaux des Réunions du Conseil Permanent International pour l'Exploration de la Mer, 20: 1–227.

Hutchings, J. A., Swain, D. P., Rowe, S., Eddington, J. D., Puvanendran, V. and Brown, J. A. 2007. Genetic variation in life-history reaction norms in a marine fish. Proceedings of the Royal Society of London Series B, 274: 1693–1699.

Hutchings, J. A., and Myers, R. A. 1993. Effect of age on the seasonality of maturation and spawning of Atlantic cod, *Gadus morhua*, in the northwest Atlantic. Canadian Journal of Fisheries and Aquatic Sciences, 50: 2468–2474.

Hwang, D. F., Jen, H. C., Hsieh, Y. W., and Shiau, C. Y. 2004. Applying DNA techniques to the identification of the species of dressed toasted eel products. Journal of Agricultural and Food Chemistry, 52: 5972–5977.

ICES. 2005. Report of the Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM), 3–6 June 2005, Silkeborg, Denmark. ICES Document CM 2005/F: 01. 47 pp.

ICES. 2006. Report of the Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM), 24–27 March 2006, Newport, Ireland. ICES Document CM 2006/MCC: 04. 59 pp.

Itoi, S., Misako, N., Kaneko, G., Kondo, H., Sezaki, K., and Watabe, S. 2005. Rapid identification of eels *Anguilla japonica* and *A. anguilla* by polymerase chain reaction with single nucleotide polymorphism-based specific probes. Fisheries Science, 71: 1356–1364.

Kagawa, H., Tanaka, H., Ohta, H., Unuma, T., and Nomura, K. 2005. The first success of glass eel production in the world: basic biology on fish reproduction advances new applied technology in aquaculture. Fish Physiology and Biochemistry, 31: 193–199.

Kalujnaia, S., McWilliam, I., Feilen, A., Nicholson, J., Hazon, N., and Cramb, G. 2007. Novel genes discovered by transcriptomic approach to the salinity study in European eel *Anguilla anguilla*. Comparative Biochemistry and Physiology A—Molecular and Integrative Physiology, 146 (Suppl. S): S94–S94.

Kenchington, E., Heino, M., and Nielsen, E. E. 2003. Managing marine genetic diversity: time for action? ICES Journal of Marine Science, 60: 1172–1176.

Kettle, A. J., and Haines, K. 2006. How does the European eel (*Anguilla anguilla*) retain its population structure during its larval migration across the North Atlantic Ocean? Canadian Journal of Fisheries and Aquatic Sciences, 63: 90–106.

Knights, B. 2003. A review of the possible impacts of long-term oceanic and climate changes and fishing mortality on recruitment of anguillid eels of the northern hemisphere. Science of the Total Environment, 310: 237–244.

Law, R. 2000. Fishing, selection, and phenotypic evolution. ICES Journal of Marine Science, 57: 659–668.

Lin, Y. S., Poh, Y. P., Lin, S. M., and Tzeng, C. S. 2002. Molecular techniques to identify freshwater eels: RFLP analyses of PCR-amplified DNA fragments and allele-specific PCR from mitochondrial DNA. Zoological Studies, 41: 421–430.

Lintas, C., Hirano, J., and Archer, S. 1998. Genetic variation of the European eel (*Anguilla anguilla*). Molecular Marine Biology and Biotechnology, 7: 263–269.

Maes, G. E. 2005. Evolutionary consequences of a catadromous life-strategy on the genetic structure of European eel (*Anguilla anguilla* L.). PhD thesis, Catholic University Leuven, Belgium.

Maes, G. E., Pujolar, J. M., Hellemans, B., and Volckaert, F. A. M. 2006b. Evidence for isolation-by-time in the European eel (*Anguilla anguilla* L.). Molecular Ecology, 15: 2095–2107.

Maes, G. E., Pujolar, J. M., Raeymaekers, J. A. M., Dannewitz, J., and Volckaert, F. A. M. 2006a. Microsatellite conservation and Bayesian individual assignment in four *Anguilla* species. Marine Ecology Progress Series, 319: 251–261.

Maes, G. E., Raeymaekers, J. A. M., Pampoulie, C., Seynaeve, A., Goemans, G., Belpaire, C., and Volckaert, F. A. M. 2005. The catadromous European eel *Anguilla anguilla* (L.) as a model for freshwater evolutionary ecotoxicology: relationship between heavy metal bioaccumulation, condition and genetic variability. Aquatic Toxicology, 73: 99–114.

Maes, G. E., and Volckaert, F. A. M. 2002. Clinal genetic variation and isolation by distance in the European eel *Anguilla anguilla* (L.). Biological Journal of the Linnaean Society, 77: 509–521.

Mank, J. E., and Avise, J. C. 2003. Microsatellite variation and differentiation in North Atlantic eels. Journal of Heredity, 94: 310–314.

McKay, J. K., and Latta, R. G. 2002. Adaptive population divergence: markers, QTL and traits. Trends in Ecology and Evolution, 17: 285–291.

Minegishi, Y., Aoyama, J., Inoue, J. G., Miya, M., Nishida, M., and Tsukamoto, K. 2005. Molecular phylogeny and evolution of the freshwater eels genus *Anguilla* based on the whole mitochondrial genome sequences. Molecular Phylogenetics and Evolution, 34: 134–146.

Mitton, J. B. 1997. Selection in Natural Populations. Oxford University Press, Oxford.

Miyahara, T., Hirono, I., and Aoki, T. 2000. Analysis of expressed sequence tags from a Japanese eel *Anguilla japonica* spleen cDNA library. Fisheries Science, 66: 257–260.

Mullon, C., Fréon, P., and Cury, P. 2005. The dynamics of collapse in world fisheries. Fish and Fisheries, 6: 111–120.

Myers, R. A., and Worm, B. 2003. Rapid worldwide depletion of predatory fish communities. Nature, 423: 280–283.

Nielsen, E. E., Hansen, M. M., and Loeschcke, V. 1997. Analysis of microsatellite DNA from old scale samples of Atlantic salmon *Salmo salar*: a comparison of genetic composition over 60 years. Molecular Ecology, 6: 487–492.

Nielsen, E. E., Hansen, M. M., and Meldrup, D. 2006. Evidence of microsatellite hitch-hiking selection in Atlantic cod (*Gadus morhua* L.): implications for inferring population structure in nonmodel organisms. Molecular Ecology, 15: 3219–3229.

Nomura, K., Morishima, K., Tanaka, H., Unuma, T., Okuzawa, K., Ohta, H., and Arai, K. 2006. Microsatellite-centromere mapping in the Japanese eel (*Anguilla japonica*) by half-tetrad analysis using induced triploid families. Aquaculture, 257: 53–67.

Okamura, A., Yamada, Y., Mikawa, N., Tanaka, S., and Oka, H. P. 2002. Exotic silver eels *Anguilla anguilla* in Japanese waters: seaward migration and environmental factors. Aquatic Living Resources, 15: 335–341.

Okamura, A., Zhang, H., Utoh, T., Akazawa, A., Yamada, Y., Horie, N., Mikawa, N. *et al.* 2004. Artificial hybrid between *Anguilla anguilla* and *A. japonica*. Journal of Fish Biology, 64: 1450–1454.

Palstra, A. P., Cohen, E. G. H., Niemantsverdriet, P. R. W., van Ginneken, V. J. T., and van den Thillart, G. E. E. J. M. 2005. Artificial maturation and reproduction of European silver eel: development of oocytes during final maturation. Aquaculture, 249: 533–547.

Palumbi, S. R. 1994. Genetic-divergence, reproductive isolation, and marine speciation. Annual Review of Ecology and Systematics, 25: 547–572.

Pantelouris, E. M., Arnason, A., and Tesch, F. W. 1970. Genetic variation in the eel. 2. Transferrins, haemoglobins and esterases in the eastern North Atlantic. possible interpretations of phenotypic frequency differences. Genetic Research, 16: 177–184.

Park, L. K., and Moran, P. 1994. Developments in molecular techniques in fisheries. Reviews in Fish Biology and Fisheries, 4: 272–299.

Pauly, D. 2007. Coral: a pessimist in paradise. Nature, 447: 33–34.

Pauly, D., Christensen, V., Guenette, S., Pitcher, T. J., Sumaila, U. R., Walters, C. J., Watson, R., *et al.* 2002. Towards sustainability in world fisheries. Nature, 418: 689–695.

Pujolar, J. M., Maes, G. E., Vancoillie, C., and Volckaert, F. A. M. 2005. Growth rate correlates to individual heterozygosity in European eel, *Anguilla anguilla* L. Evolution, 59: 189–199.

Pujolar, J. M., Maes, G. E., and Volckaert, F. A. M. 2006. Genetic patchiness among recruits in the European eel, *Anguilla anguilla*. Marine Ecology Progress Series, 307: 209–217.

Rehbein, H., Sotelo, C. G., Perez-Martin, R. I., Chapela-Garrido, M. J., Hold, G. L., Russell, V. J., Pryde, S. E. *et al.* 2002. Differentiation of raw or processed eel by PCR-based techniques: restriction fragment length polymorphism analysis (RFLP) and single strand conformation polymorphism analysis (SSCP). European Food Research and Technology, 214: 171–177.

Rousset, F. 1997. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. Genetics, 145: 1219–1228.

Schmidt, J. 1923. Breeding places and migration of the eel. Nature, 111: 51–54.

Sezaki, K., Itoi, S., and Watabe, S. 2005. A simple method to distinguish two commercially valuable eel species in Japan *Anguilla japonica* and *A. anguilla* using polymerase chain reaction strategy with a species-specific primer. Fisheries Science, 71: 414–421.

Sinclair, M. 1988. Marine Populations. An Essay on Population Regulation and Speciation. University of Washington Press, Seattle.

Tagliavini, J., Gandolfi, G., Cau, A., Salvadori, S., and Deiana, A. M. 1995. Mitochondrial-DNA variability in *Anguilla anguilla* and phylogenetic relationships with congeneric species. Bollettino Di Zoologia, 62: 147–151.

Tesch, F. W. 2003. The Eel. Blackwell Science, Oxford, UK.

Tsukamoto, K. 2006. Spawning of eels near a seamount. Nature, 439: 929–929.

Turner, T. F., Wares, J. P., and Gold, J. R. 2002. Genetic effective size is three orders of magnitude smaller than adult census size in an abundant, estuarine-dependent marine fish (*Sciaenops ocellatus*). Genetics, 162: 1329–1339.

Van Ginneken, V., and Maes, G. E. 2005. The European eel (*Anguilla anguilla*, Linnaeus), its lifecycle, evolution and reproduction: a literature review. Reviews in Fish Biology and Fisheries, 15: 367–398.

Waples, R. S. 1998. Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. Journal of Heredity, 89: 438–450.

Ward, R. D. 2000. Genetics in fisheries management. Hydrobiologia, 420: 191–201.

Watanabe, S., Aoyama, J., and Tsukamoto, K. 2004. Reexamination of Ege's (1939) use of taxonomic characters of the genus *Anguilla*. Bulletin of Marine Science, 74: 337–351.

Williams, G. C., and Koehn, R. K. 1984. Population genetics of the North Atlantic catadromous eels (*Anguilla*). *In* Evolutionary Genetics of Fishes, pp. 529–560. Plenum Press, New York.

Wirth, T., and Bernatchez, L. 2001. Genetic evidence against panmixia in the European eel. Nature, 409: 1037–1040.

Wirth, T., and Bernatchez, L. 2003. Decline of North Atlantic eels: a fatal synergy? Proceedings of the Royal Society of London Series B—Biological Sciences, 270: 681–688.

Yahyaoui, A., Bruslé, J., and Pasteur, N. 1983. Etude du polymorphisme biochimique de deux populations naturelles (Maroc Atlantique et Rousillon) de civelles et anguillettes d'*Anguilla anguilla* L. et de deux échantillons d'élevages. IFREMER Actes de Colloques, 1: 373–390.

Zhang, H., Mikawa, N., Yamada, Y., Horie, N., Okamura, A., Utoh, T., Tanaka, S., and Motonobu, T. 1999. Foreign eel species in the natural waters of Japan detected by polymerase chain reaction of mitochondrial cytochrome B region. Fisheries Science, 65: 684–686.

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