



Genetic patchiness in European eel adults evidenced by molecular genetics and population dynamics modelling

José Martin Pujolar^{a,*}, Daniele Bevacqua^b, Marco Andreollo^c, Fabrizio Capoccioni^d, Eleonora Ciccotti^d, Giulio A. De Leo^b, Lorenzo Zane^a

^a Dipartimento di Biologia, Università di Padova, Via G. Colombo 3, I-35131, Padova, Italy

^b Dipartimento di Scienze Ambientali, Università degli Studi di Parma, Parco Area delle Scienze 11A, I-43100, Parma, Italy

^c Laboratoire d'Ecologie Alpine (LECA), CNRS UMR 5553, 38041 Grenoble, France

^d Dipartimento di Biologia, Università Roma Tor Vergata, I-00133, Rome, Italy

ARTICLE INFO

Article history:

Received 18 May 2010

Revised 18 November 2010

Accepted 22 November 2010

Available online 1 December 2010

Keywords:

Adult eels

Anguilla anguilla

European eel

Genetic patchiness

Population dynamics modelling

ABSTRACT

Disentangling the demographic processes that determine the genetic structure of a given species is a fundamental question in conservation and management. In the present study, the population structure of the European eel was examined with a multidisciplinary approach combining the fields of molecular genetics and population dynamics modelling. First, we analyzed a total of 346 adult specimens of known age collected in three separate sample sites using a large panel of 22 EST-linked microsatellite loci. Second, we developed a European eel-specific model to unravel the demographic mechanisms that can produce the level of genetic differentiation estimated by molecular markers. This is the first study that reveals a pattern of genetic patchiness in maturing adults of the European eel. A highly significant genetic differentiation was observed among samples that did not follow an Isolation-by-Distance or Isolation-by-Time pattern. The observation of genetic patchiness in adults is likely to result from a limited parental contribution to each spawning event as suggested by our modelling approach. The value of genetic differentiation found is predicted by the model when reproduction occurs in a limited number of spawning events isolated from each other in time or space, with an average of 130–375 breeders in each spawning event. Unpredictability in spawning success may have important consequences for the life-history evolution of the European eel, including a bet-hedging strategy (distributing reproductive efforts over time) which could in turn guarantee successful reproduction of some adults.

© 2010 Elsevier Inc. All rights reserved.

1. Introduction

One major paradox concerning the population genetics of marine organisms is the observation of small but statistically significant genetic differences among local populations despite high rates of gene flow (Burton, 1983; Hedgecock, 1994). While the apparent absence of real barriers for fish/larvae displacement in marine environments could lead to a depression of the extent of genetic differentiation between subpopulations (Ward et al., 1994), several studies have detected significant heterogeneity among recruits on a small spatial scale in marine species dispersing via pelagic larvae, including gastropods (Johnson and Black, 1982), bivalves (Hedgecock, 1994; David et al., 1997; Li and Hedgecock, 1998), echinoderms (Edmands et al., 1996; Moberg and Burton, 2000; Flowers et al., 2002) and fish (Ruzzante et al., 1996; Planes and Lenfant, 2002; Pujolar et al., 2006, 2007). Hedgecock (1994) proposed that such genetic heterogeneity is likely to result from a large variance in reproductive success of

parents. Under the sweepstakes hypothesis, chance events determine which adults are successful in each spawning event. The random variation in parental contribution to the next generation leads to unpatterned variation in genetic composition of recruits (genetic patchiness). Many individuals fail to contribute to recruitment, and in each generation the entire population is replaced by a small fraction of individuals by a sweepstakes-chance matching of reproduction activity with oceanic conditions. Reproduction of marine organisms is mediated by spatio-temporal variation in oceanographic processes that affect spawning, fertilization, larval survival and successful recruitment. Despite high fecundities, marine organisms show great variability in gamete quantity and quality (Leviton, 2008), suggesting that the potential for variance in reproductive success exists prior to fertilization.

The dependence of recruitment on oceanic conditions holds particularly true for the European eel (*Anguilla anguilla*), a catadromous fish species with a complex life cycle that includes two migrations across the North Atlantic, one feeding migration at the beginning of its life and a spawning migration at the end (Van den Thillart et al., 2009). After spawning in the Sargasso Sea, larvae (leptocephali) cross the Atlantic Ocean following the

* Corresponding author.

E-mail address: martupujolar@gmail.com (J.M. Pujolar).

Gulf Stream and North Atlantic Drift Current and metamorphose into glass eels upon reaching the edge of the continental shelf off Europe and North Africa (Kettle and Haines, 2006). Arrival of glass eels occurs in pulses or groups known as arrival waves (Boëtius and Boëtius, 1989). Glass eels complete the migration into continental growth habitats as yellow eels, and after an highly variable feeding period of 2–20 years depending on sex and geographic latitude (Vøllestad, 1992), they metamorphose into silver eels that migrate back to the Sargasso Sea, reproduce once and die (Van den Thillart et al., 2009).

The stock of the European eel has shown a decline over the last decades and is considered to be outside safe biological limits (ICES, 2008). Recruitment has shown a decline of 90–99% in different areas since the 1970s (Dekker, 2003). Most data sets also show a decline in fishing yield and landings of yellow and silver eels (Dekker, 2003), with catches reported to FAO showing a gradual drop to about 25% of the level of the mid 1960s (ICES, 2008). The crash seems to have affected in a similar fashion European, American and Japanese eel, with no single apparent cause (Dekker et al., 2003). Several hypotheses have been put forward concerning the causes of the eel stock decline, including human and natural causes operating during the different stages of the life cycle. On the one hand, there are several anthropogenic factors mainly affecting eels on their continental phase, such as overfishing, migration barriers (dams and hydroelectric power plants), habitat loss, pollution (PCBs and heavy metals) and human-introduced diseases (EVEX virus) and parasites (the swimbladder nematode *Anguillicoloides crassus*) (Van den Thillart et al., 2009). On the other hand, climatic and oceanic events might contribute to the decline as they influence silver eel migration, mating and spawning success of adults in the Sargasso Sea, egg and larvae development, and larval survival during migration to the continental feeding grounds (Knights, 2003; Friedland et al., 2007; Bonhommeau et al., 2008; Miller, 2009; Miller et al., 2009; Martin et al., 2010).

A question of fundamental importance to conservation and management is whether the European eel consists of a single panmictic population. While the existence of one single spawning ground in the Sargasso Sea could lead to individual mixing and homogenization of the gene pool, genetic differences between populations could arise if temporal or spatial segregation occurs between spawning groups followed by non-random larval dispersal (Kettle and Haines, 2006). In this sense, panmixia in the European eel is widely accepted, following the study of Dannewitz et al. (2005) that proved previously observed Isolation-by-Distance (IBD) patterns to be unstable over time when temporal replicates of the geographical sampling were included in the analysis. Recent studies focusing on the genetic composition of glass eels have observed a subtle pattern of genetic patchiness among intra-annual samples within cohorts (arrival waves) (Pujolar et al., 2006, 2007, 2009a). Panmixia on a broader scale and genetic patchiness on a local scale are not contradictory but possibly the consequence of the long migration loop of the European eel notably affected by fluctuating oceanic conditions (Pujolar et al., 2009a).

Previous genetic studies on eels present many limitations. First, the low number of genetic markers used, with all recent studies being based on 6–7 microsatellite loci (Wirth and Bernatchez, 2001; Dannewitz et al., 2005; Maes et al., 2006; Pujolar et al., 2006, 2007). Second, neutral loci (microsatellites) might fail to detect differences at loci affected by selection, which could lead to underestimating adaptive variation between populations (Conover et al., 2006). Third, previous genetic studies have focused entirely on glass eel samples, which might not be an adequate representation of potential spawning stocks because eels were sampled after completion of their trans-Atlantic migration and before commencing their continental growth stage. The only exception in the use of adults is the recent study of Palm et al. (2009), which had the

caveat of using only six microsatellite loci. Despite large sample sizes, no signs of genetic differentiation were observed, with an average $F_{ST} = -0.00003$ ($p = 0.61$). To correct for the bias caused by a limited number of neutral markers, in the present study we used a total of 22 EST-derived microsatellite loci, which were previously used to successfully screen for genetic variation in glass eel arrival waves (Pujolar et al., 2009a). Expressed Sequence Tags or ESTs are single-read sequences produced from partial sequencing of a mRNA pool and have the advantage of being putatively under the influence of selection, either directly or indirectly through genetic linkage, because of their location in gene regions. Ecologically important traits are likely to have been shaped by natural selection, which means that examining patterns of molecular evolution in EST-linked microsatellites could provide a way of screening numerous genetic loci for signatures of adaptive evolution. In this sense, the recent study of Gagnaire et al. (2009) suggested that spatial distribution of allele frequencies displayed by some AFLP loci in *A. anguilla* and *Anguilla rostrata* hybrids in Iceland were possibly shaped by natural selection associated with ecological conditions. To correct for the dominance of glass eels samples in previous studies, our analysis focused entirely on adult eels of known age. We expect that by analyzing adults only, our sampling will be a better representation of the spawning stock.

Finally, understanding of the genetic structure of the European eel can greatly benefit from the development of integrated genetic/demographic models that take into consideration the complex life cycle of this catadromous species. Thus far, modelling studies on eel population dynamics have focused on quantifying the recruitment-stock relationship, thus providing new knowledge on factors affecting the continental phase (e.g. overfishing, migration barriers) that can impair spawner production at local level (Vøllestad and Jonsson, 1988; De Leo and Gatto, 1995; Dekker, 2000a; Feunteun, 2002; Bevacqua et al., 2007). In this sense, modelling studies on eel have always neglected the oceanic phase, including reproduction and an overall stock–recruitment relationship. The only exception is the model of Åström and Dekker (2007), which was aimed at estimating potential recovery time for the overall eel stock and thus disregarded the estimation of key aspects of eel demography (e.g. effective number of breeders) and omitted any evolutionary or genetic component.

The goal of this study is to bring together molecular genetic and population dynamics in order to gather new knowledge on the role of demographic processes that determine the genetic structure of the European eel. First, we analyzed the population structure of the European eel using adult specimens of known age that will allow estimating genetic differentiation among samples. Second, we developed a European eel-specific model to unravel the demographic mechanisms that can produce the level of genetic differentiation estimated by molecular markers. The demographic model considers both continental and oceanic phases of the eel life cycle and encompasses particular life history features of the European eel such as sexual dimorphism, sexual maturation, body growth, larval and adult migration, glass eel and adult survival, and reproduction. The information obtained by molecular markers and a genetic–demographic model will ultimately allow setting up an integrated conservation framework that takes into account contemporary evolution.

2. Material and methods

2.1. Sampling collection

A total of 1234 European eel *A. anguilla* individuals were caught using fyke nets in three separate geographic sites in the Mediterranean Sea (Fig. 1): (1) the low course of the Tiber river (41°48'N;

12°25'E) in the west coast of Italy/Tyrrhenian Sea. Samples were collected during May–November 2007 ($N = 422$); (2) Laguna di Lesina (41°53'N; 15°51'E) in the east coast of Italy, a brackish lagoon connected to the Adriatic Sea, with an area of 51.4 km² and characterized by variable salinity (11–33 psu) and high productivity (8–15% total organic matter). Samples were collected during October 2007–March 2008 ($N = 407$); (3) Laguna di Caprolace (41°21'N; 12°59'E) in the National Park of Circeo, a small lagoon connected to the Tyrrhenian Sea, with an area of 2.26 km² and high average salinity (40‰). Samples were collected during October 2007–March 2008 ($N = 405$). All individuals were measured for total length (L , mm) and weight (W , mg). Otolithometry was used to estimate individual age and assign each fish to a single cohort or year-class, which refers to the year of recruitment of an individual as glass eel. Otoliths were extracted from all adults, embedded in epoxy resin, and stained with 3% toluidine blue following grinding of the convex side. The number of annual otolith rings (annuli) were read starting from the first ring after the marine nucleus edge using a light stereomicroscope following the guidelines outlined in ICES (2009). Ages ranged from 2 to 13 in Tiber, 2 to 11 in Lesina and 3 to 15 in Caprolace. A sub-sample of 346 adults from the three sampling sites (Tiber, $N = 109$; Lesina, $N = 134$; Caprolace, $N = 103$) were selected for genetic analysis on the basis of good quality of otolith reading and to include sufficient numbers of individuals per group to conduct statistical analysis (Table 1). Our study included three cohorts in Tiber (2002, 2003, 2004), four cohorts in Lesina (2001, 2002, 2003, 2004) and three cohorts in Caprolace (1999, 2000, 2001).

2.2. Microsatellite analysis

Minute sections of tissue from ethanol-preserved eel finclips were digested in a lysis buffer containing 100 μ l TE Buffer, 7 μ l

Table 1

Summary of genetic variability in all samples including number of individuals (N), observed (H_o) and expected (H_e) heterozygosities and allelic richness (AR). Standard deviation in parentheses. Cohort refers to glass eel arrival year.

Sample/cohort	N	H_o	H_e	AR
Tiber	109	0.53 (0.24)	0.57 (0.26)	9.48 (5.77)
2002	33	0.54 (0.25)	0.55 (0.26)	6.16 (3.90)
2003	42	0.52 (0.27)	0.55 (0.27)	6.41 (4.02)
2004	34	0.54 (0.23)	0.59 (0.24)	6.54 (3.89)
Lesina	134	0.52 (0.22)	0.57 (0.26)	9.63 (6.22)
2001	30	0.55 (0.24)	0.57 (0.24)	6.19 (3.43)
2002	31	0.52 (0.24)	0.56 (0.26)	6.02 (3.46)
2003	46	0.50 (0.21)	0.56 (0.26)	6.25 (3.94)
2004	27	0.51 (0.27)	0.55 (0.27)	6.40 (4.34)
Caprolace	103	0.51 (0.22)	0.57 (0.25)	8.97 (6.00)
1999	33	0.50 (0.24)	0.57 (0.26)	6.33 (4.24)
2000	41	0.50 (0.24)	0.55 (0.25)	6.12 (3.56)
2001	29	0.54 (0.23)	0.57 (0.23)	6.10 (3.39)

1 M DTT (dithiothreitol) solution pH 5.2 (diluted in 0.08 M NaAC) and 2 μ l proteinase K solution (20 mg/ml) for at least 8 h at 56 °C. After incubation at 96 °C for 10 min, samples were centrifuged at 13,000 rpm for 11 min, and the supernatant was stored at –20 °C.

Genotypes were examined at a total of 22 EST-derived microsatellite loci (Pujolar et al., 2009a,b), after excluding locus 41A22, which was not in Hardy–Weinberg Equilibrium (HWE) and was suggested to include null alleles by the software Micro-checker. PCR products were obtained in a GeneAmp PCR System 2700 Thermocycler (Applied Biosystems) using the QIAGEN Multiplex PCR Kit. PCR reactions consisted of 2 μ l template DNA, 5 μ l QIAGEN Multiplex PCR Master Mix, 0.2 μ l 10 μ M forward and reverse primers, and water up to 10 μ l. PCR conditions were as follows: 15 min

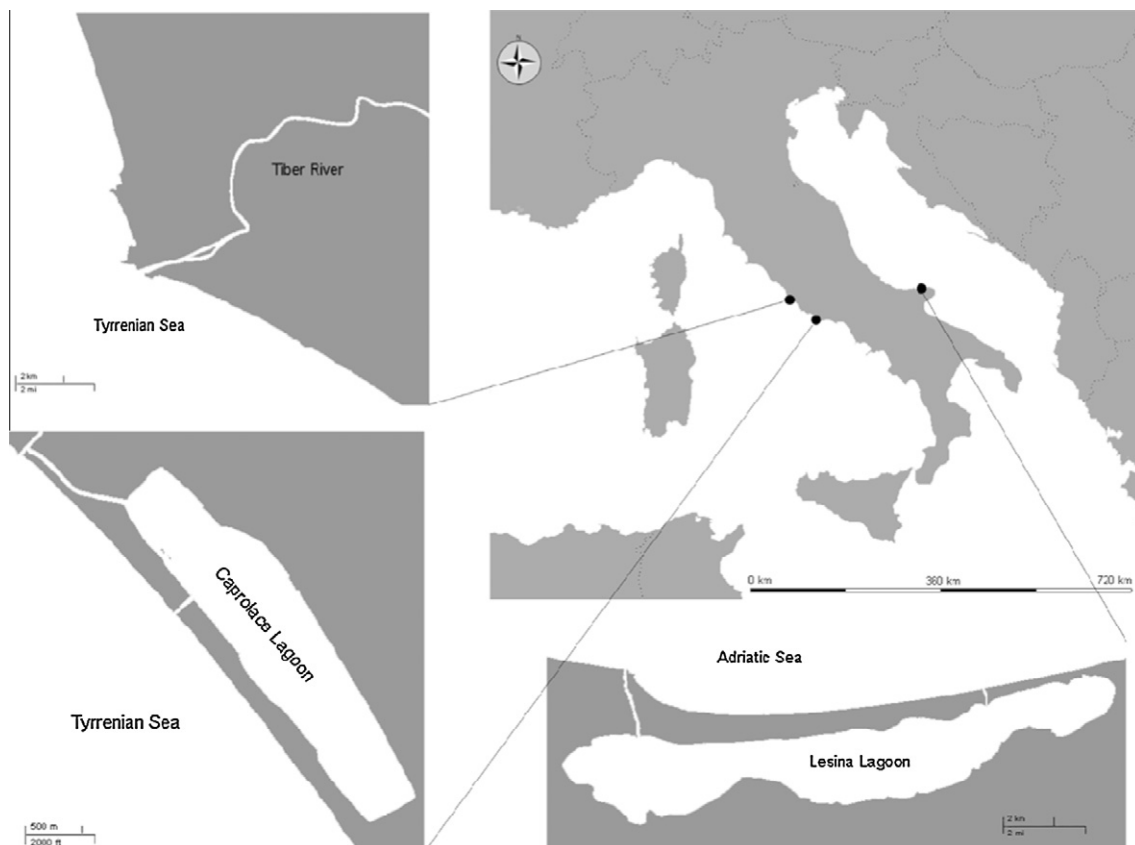


Fig. 1. Sampling locations of European eel.

at 95 °C, 35 cycles of 30 s at 94 °C, 90 s at 57 °C and 1 min at 72 °C, and final elongation for 30 min at 60 °C. PCR products were visualized in 1.8% agarose gels and screened for microsatellite polymorphism using an ABI 3130 AVANT automatic capillary sequencer (Applied Biosystems). Alleles were sized according to a Liz500 (50–500 bp) marker.

2.3. Data analysis

Within-sample genetic diversity statistics were assessed by observed (H_o) and expected (H_e) heterozygosities per locus using GENETIX version 4.05 (Belkhir et al., 2005), and allelic richness (AR) using FSTAT (Goudet, 2002). Differences in genetic diversity among samples were tested by one-way ANOVA using STATISTICA (StatSoft Inc.). Deviations from HWE, linkage disequilibrium, and differences in allele and genotype frequencies among samples were tested using GENEPOP version 3.4 (Raymond and Rousset, 1995). Presence of null alleles was tested using the program MICRO-CHECKER version 2.2.3 (Van Oosterhout et al., 2004). Population structure was studied using non-hierarchical and hierarchical F -statistics (Weir and Cockerman, 1984) calculated using GENETIX and ARLEQUIN (Schneider et al., 2000), respectively. Significance tests were assessed with 10,000 permutation tests. In all cases, significance levels were corrected for multiple comparisons using Bonferroni (Rice, 1989). Pairwise multilocus comparisons between samples were calculated by Cavalli-Sforza and Edwards (1967) chord distance and graphically represented by Multi-Dimensional Scaling using STATISTICA. Isolation-by-Distance (IBD) and Isolation-by-Time (IBT) were tested using single and partial Mantel tests (Mantel, 1967) implemented in GENETIX, by correlating linearized genetic distance $F_{ST}/(1 - F_{ST})$ versus coastal distance (between sites) and temporal distance (measured as number of years between cohorts).

Average relatedness of all individuals to each other within samples (r) at 22 microsatellite loci was calculated using ML-Relate (Kalinowski et al., 2006). The significance of the values of relatedness was tested using 1000 permutations of alleles among individuals in order to calculate the proportion of pair-wise relatedness values attributable to significant half or full-siblings compared to random sharing of alleles.

2.4. The genetic–demographic model

The model we present is based on the simple model of stock dynamics developed by Åström and Dekker (2007), but with two substantial modifications, namely (i) the inclusion of a genetic component and (ii) additional demographic parameters not considered in the original. Since Åström and Dekker (2007) acknowledged their model to be too simplistic regarding the biology of the European eel, we use a size and age-structured model that explicitly takes into account size-dependent biological features such as sexual differentiation, sexual maturation and fecundity.

The model incorporates knowledge on molecular studies that reported no evidence of spatial genetic structure (Dannewitz et al., 2005; Palm et al., 2009), which supports the panmixia hypothesis, namely that all European eel comprise a single, randomly mating population. In accordance with the lack of genetic substructuring found, the model considers no larval homing to the parental original freshwater habitat, and that larval migratory routes are random (Feunteun, 2002; Pujolar et al., 2009a). In order to simulate the arrival of glass eels that occurs throughout the year in pulses or groups (recruitment waves) as outlined by Boëtius and Boëtius (1989), mating in the spawning ground was assumed to occur in a finite number of reproductive events isolated from each other in time and/or in space. For simplicity we assumed that the overall number of breeders (N_B) equally splits in a given number

of spawning events (NSE). The number of breeders per event (NBE) depends upon NSE, i.e. $NBE = N_B/NSE$.

Breeding individuals belong to different cohorts as a result of the large variability in age-at-maturity observed between males and females. In the reproductive phase, the genotype of each breeding individual is randomly drawn from the genotype frequency distribution of its corresponding continental cohort. Initial genotype frequencies were set in Hardy–Weinberg equilibrium with a uniform allele distribution ($AR = 10$ alleles). Preliminary simulations showed no effect of allele richness on F -statistic estimates when using 5, 10 or 20 alleles per locus. Gamete exchange occurs following a Random Mating scheme in which any female is assumed to produce f eggs that are randomly fertilized by males involved in the same spawning event. Offspring genotype frequencies are calculated as the product of male and female gamete frequencies (Crow and Kimura, 1970). Mutation occurs before union of gametes under a stepwise mutation model with mutation rate $\mu = 5 \times 10^{-4}$ (Waples and Gaggiotti, 2006). Breeders die after reproduction (semelparity). Offspring produced in each spawning event remain together so that dispersal of larvae originating from a common spawning event is cohesive during the larval life stage. The fraction of all larvae surviving oceanic migration (from all spawning events/recruitment waves) constitutes the new cohort entering the continental phase, whose genotype frequency is maintained throughout all the life of the cohort, thus allowing estimation of the genetic differentiation F_{ST} among adult eels.

Eel demography was modelled under a steady-state hypothesis (i.e. constant N_B) with the aim to detect which level of breeding stock fragmentation (i.e. value of NSE and consequent NBE) could determine the F -statistic values obtained with the 22 genetic markers in this study. In addition, the model was run using F -statistic values obtained from literature including the study of Pujolar et al. (2009a) conducted using the same markers but on glass eels, plus the studies of Wirth and Bernatchez (2001), Dannewitz et al. (2005) and Maes et al. (2006) on glass eels using genomic microsatellites. Simulations were run for 200 generations until convergence to stable F -statistic values.

Duration of the oceanic larval stage is assumed to be 2 years (Åström and Dekker, 2007; Bonhommeau et al., 2010). Glass eel recruitment R at time t depends upon the abundance of silver eels N_S that migrated at time $t - 2$ and successfully spawned, which can be written as:

$$R(t) = N_S(t - 2) \cdot \sigma_S \cdot 0.5 \cdot f \cdot \sigma_L$$

where σ_S and σ_L respectively represent survival probability of silver eels and larvae during the oceanic migration, 0.5 represents a balanced sex-ratio as in Bonhommeau et al. (2009), and f is the mean per-capita fertility (number of eggs per female).

Dekker (2000b) estimated glass eel recruitment $R = 2 \times 10^9$ and the abundance of silver eels $N_S = 3.9 \times 10^7$. Silver eel survival can be derived as:

$$\sigma_S = R(t) / (\sigma_L \cdot 0.5 \cdot N_S(t - 2) \cdot f)$$

By setting larval survival $\sigma_L = 0.0015$ (as estimated by Bonhommeau et al., 2009) and fecundity $f = 3.7 \times 10^6$ (as estimated by Barbin and McCleave, 1997), silver eel survival can be estimated as $\sigma_S = 0.018$. The fraction σ_S of migrating silver eels that survive oceanic migration and contribute successfully to reproduction in the Sargasso Sea constitute the overall breeding stock N_B .

Demographic parameters in the continent were modelled following an age and size-structured model as in De Leo and Gatto (1995). Inter-individual variability in body growth was neglected and individual body size l was assumed to linearly increase with age x following:

$$l(x) = l_0 + g \cdot x$$

where l_0 is body length at recruitment (6 cm, Melià et al., 2006) and g represents the annual growth rate (cm year^{-1}). In order to explicitly consider sexual dimorphism in body growth, with females growing faster and silvering at a larger size than males, values of g varied between sexes following Vøllestad (1992):

$$g = (l_s - l_0) \cdot x_s^{-1}$$

where l_s is average length at silvering (62 and 40 cm, respectively for females and males) and x_s is average age at silvering (9 and 6 year, respectively for females and males).

All individuals are subject to an annual mortality rate Z , sum of natural mortality rate M (0.14 year^{-1} , Åström and Dekker, 2007) and fishing mortality rate F (0.54 year^{-1} , Åström and Dekker, 2007), the latter acting only on those individuals larger than 30 cm in order to consider size selectivity of fishing gear (Bevacqua et al., 2009).

Sexual maturation probability (i.e. the probability that a yellow eel becomes silver in a given year) was assumed to be a sex dependent increasing and saturating function of l as in Bevacqua et al. (2006):

$$\gamma(l) = (1 + e^{(\lambda-l)\eta^{-1}})^{-1}$$

where λ is a semisaturation constant and η is a shape parameter inversely proportional to the slope of the curve at $l = \lambda$. Parameter λ represents the average size at which silvering occurs and was set equal to 62 and 40 cm, respectively for females and males (Vøllestad, 1992). Parameter η was set equal to 4.2 and 1.1 cm, respectively for females and males, as average of the values estimated by Bevacqua et al. (2006) upon three European eel locations. An overview of the parameters included in the demographic model is presented in Table 2.

3. Results

All samples showed similar levels of genetic diversity (Table 1), with H_o ranging from 0.50 to 0.55 and H_e ranging from 0.55 to 0.59. When pooling samples from the same geographic location, values of H_o were similar in all three locations (Tiber: 0.53, Lesina: 0.52, Caprolace: 0.51), while the same H_e values were found in all locations (0.57). Allelic richness was highly similar in all samples, ranging from 6.02 to 6.54 (Table 1). When pooling samples from the same location, AR was similar in Tiber (9.48), Lesina (9.63) and Caprolace (8.97). All differences in H_o , H_e and AR across samples were statistically not significant (one-way anova; $p > 0.05$). All loci were in Hardy–Weinberg equilibrium.

Overall genetic differentiation was low ($F_{ST} = 0.0048$) but highly significant ($p < 0.001$). Comparison of allele frequencies among samples showed significant differences at two loci after Bonferroni correction, AAN33D15 ($p = 0.001$) and AAN24A09 ($p = 0.001$), while three more loci showed significant differences but those disappeared after Bonferroni correction (AAN26N13, $p = 0.014$; AAN42O08, $p = 0.010$; AANCT 347, $p = 0.036$). A test of genetic differentiation between sample pairs showed significant differences at 19 out of 22 loci for at least one population pair, but populations involved were different across loci. When comparing allele frequencies across all loci, 23 out of 45 population pairs showed significant differences. Pairwise F_{ST} over all loci was highly significant between 15 sample comparisons (Table 3). Pairwise genetic distances (Cavalli-Sforza and Edwards, 1967) between all samples were generally small but 27 out of 45 comparisons were significant (Table 3).

A hierarchical AMOVA showed that most genetic variation was present within samples, but genetic differentiation partitioned significantly among temporal samples within locations ($F_{SC} = 0.00554$;

Table 2
Overview of parameters included in the demographic model.

Parameter	Meaning	Value	Source
N_S	Silver eel abundance	3.9×10^7	Dekker, 2000b
R	Glass eel recruitment	2.0×10^9	Dekker, 2000b
f	Mean per-capita fertility (eggs per female)	3.7×10^6	Barbin and McCleave, 1997
σ_L	Larval survival during oceanic migration	1.5×10^{-3}	Bonhommeau et al., 2009
σ_S	Adult survival during oceanic migration	1.8×10^{-2}	This work
l_0	Body length at recruitment	6 cm	Melià et al., 2006
$l_{S,F}$	Average body length at silvering (females)	62 cm	Vøllestad, 1992
$l_{S,M}$	Average body length at silvering (males)	40 cm	Vøllestad, 1992
$x_{S,F}$	Average age at silvering (females)	9 yr	Vøllestad, 1992
$x_{S,M}$	Average age at silvering (males)	6 yr	Vøllestad, 1992
M	Instantaneous natural mortality rate	0.14 yr^{-1}	Åström and Dekker, 2007
F	Instantaneous fishing mortality rate	0.54 yr^{-1}	Åström and Dekker, 2007
η_F	Shape parameter of the silvering probability curve (females)	4.2 cm	Bevacqua et al., 2006
η_M	Shape parameter of the silvering probability curve (males)	1.1 cm	Bevacqua et al., 2006

Table 3
Pairwise F_{ST} estimates (above diagonal) between samples and genetic distances (D_{CE} , below diagonal) using Cavalli-Sforza and Edwards (1967). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ after Bonferroni correction.

D_{CE}	F_{ST}									
	1	2	3	4	5	6	7	8	9	10
1. Tiber 2002	0	0.005*	0.004	0.006*	0.013***	0.002	0.015***	0.004	0.011**	0.007*
2. Tiber 2003	0.017	0	0.003	0.003	0.009**	0.001	0.008*	0.003	0.003	0.002
3. Tiber 2004	0.021*	0.019	0	0.003	0.003	0.002	0.004	0.001	0.001	0.000
4. Lesina 2001	0.022*	0.019	0.020*	0	0.003	0.007*	0.008*	0.003	0.002	0.004
5. Lesina 2002	0.026*	0.022*	0.025*	0.024*	0	0.006*	0.008*	0.000	0.003	0.004
6. Lesina 2003	0.017	0.014	0.018	0.019	0.020*	0	0.006	0.001	0.004	0.006*
7. Lesina 2004	0.024*	0.021*	0.025*	0.022*	0.023*	0.020*	0	0.007*	0.006*	0.005
8. Caprolace 1999	0.022*	0.016	0.021*	0.022*	0.022*	0.017	0.024*	0	0.003	0.002
9. Caprolace 2000	0.020*	0.016	0.018	0.017	0.022*	0.016	0.021*	0.017	0	0.004
10. Caprolace 2001	0.025*	0.018	0.024*	0.018	0.023*	0.020*	0.020*	0.019	0.017	0

Table 4

Tests for Isolation-by-Distance (IBD) and Isolation-by-Time (IBT) using coastal geographical, temporal (in days) and genetic ($F_{ST}/1 - F_{ST}$) distances across samples. IBD was tested considering all samples, those samples including similar cohorts (Tiber + Lesina) and samples from the Tyrrhenian Sea (Tiber + Caprolace). IBT was additionally tested at each location individually. Pearson's correlation coefficient r , degrees of freedom (df) and p -values result from Mantel's (1967) correlation test.

Sample	Mantel test	r	df	p
All samples	Coastal distance	0.121	1.43	0.412
	Temporal distance	0.020	1.43	0.911
Tiber + Lesina	Coastal distance	-0.060	1.19	0.798
	Temporal distance	0.208	1.19	0.365
Tyrrhenian	Coastal distance	0.010	1.13	0.970
	Temporal distance	-0.445	1.13	0.097
Tiber	Temporal distance	0.009	1.10	0.995
Lesina	Temporal distance	-0.866	1.10	0.333
Caprolace	Temporal distance	0.745	1.40	0.089

$p < 0.001$), while no significant differentiation was observed between geographic locations ($F_{CT} = -0.00074$; $p = 0.754$). Similarly, when comparing Tyrrhenian (Tiber and Caprolace) versus Adriatic (Lesina), temporal differentiation was an order of magnitude higher than geographic differentiation.

Overall Mantel tests for either IBD or IBT showed a lack of correlation between genetic and geographic distance ($r = 0.121$, $p = 0.412$) and between genetic and temporal distance ($r = 0.020$; $p = 0.911$) (Table 4). No significant correlations were observed when excluding the Caprolace location (in order to compare similar cohorts) or when only the two Tyrrhenian locations were used in the Mantel test. Simple Mantel tests at each location separately showed again no correlation between genetic and temporal distance. Concordantly, a Multi-Dimensional Scaling analysis using Cavalli-Sforza and Edwards (1967) chord distance showed no clear temporal/spatial grouping of samples (Fig. 2), with cohorts and locations appearing mixed in the tree. Furthermore, none of the groupings showed a robust bootstrap support.

Mean relatedness of all individuals to each other was low and similar across samples (Table 5), ranging from 0.027 (Caprolace 1999) to 0.036 (Tiber 2002 and Tiber 2003). Despite that, half and full-siblings were observed in all samples, with a percentage that ranged from 0.20 (Tiber 2002) to 2.70 (Caprolace 2000).

The model was able to estimate the size of the reproductive events that can explain the values of genetic differentiation

observed in our study after simulations were run for 200 generations to reach convergence of F -statistic values. The model provided a F_{ST} value equal to 0.0048 when the average effective number of eels per spawning event (NBE) is set to 130 individuals. The total number of breeders equals 130 times the number of spawning events ($N_B = 130 * NSE$). When the model was run with F_{ST} values obtained from literature, NBE was slightly higher: 241 individuals when using $F_{ST} = 0.0024$ (Pujolar et al., 2009a), 307 individuals when using $F_{ST} = 0.0018$ (Maes et al., 2006), 322 individuals when using $F_{ST} = 0.0017$ (Wirth and Bernatchez, 2001) and 375 individuals when using $F_{ST} = 0.0014$ (Dannewitz et al., 2005). Thus, when considering all studies, NBE ranged between 130 and 377 individuals. Considering a total of 3.9×10^7 migrating silver eels per year (Dekker, 2000b) and a silver eel survival of $\sigma_S = 0.018$ (Table 2), the overall number of breeders could be around 700,000 individuals split into around 2000–5000 spawning events of 130–375 individuals per event.

4. Discussion

This is the first study that reveals a pattern of genetic patchiness in maturing adults of the European eel. A highly significant genetic differentiation was observed among samples from different cohorts reconstructed using age data from three distinct samples (Tiber, Lesina, Caprolace) despite the low observed F_{ST} values. An unpatterned genetic heterogeneity (genetic patchiness) was apparent as samples did not cluster together according to sampling location or cohort, and consequently no pattern of either Isolation-by-Distance or Isolation-by-Time was detected. The observation of genetic patchiness in adults is likely to result from a limited (finite) parental contribution to each spawning event as suggested by our modelling approach. Our findings do not preclude that other mechanisms not explicitly simulated in the model might contribute to produce the observed genetic structure, including a non-random mating scheme or the effect of varying oceanic conditions on larval survival.

4.1. Comparison with previous genetic studies

The genetic heterogeneity observed in adults in our study contrasts with the homogeneity found in the study of Palm et al.

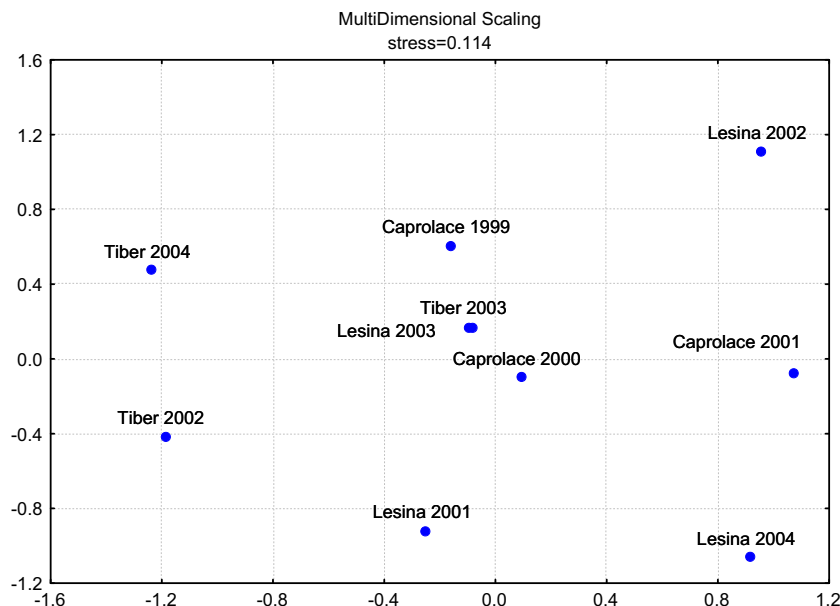


Fig. 2. Multi-Dimensional Scaling of pairwise genetic distances among all samples based on Cavalli-Sforza and Edwards (1967) chord distance.

Table 5

Values of average and maximum relatedness (r) within samples, including percentage of pair-wise relatedness values attributable to significant half-siblings or full-siblings compared to random sharing of alleles. Standard deviation in parentheses.

Sample	% Half/full-siblings	Average r (SD)	Maximum r
Tiber			
2002	0.20	0.036 (0.061)	0.367
2003	1.47	0.036 (0.064)	0.410
2004	1.68	0.031 (0.057)	0.338
Lesina			
2001	1.17	0.031 (0.061)	0.500
2002	0.43	0.035 (0.088)	0.950
2003	1.64	0.034 (0.058)	0.325
2004	0.93	0.028 (0.057)	0.500
Caprolace			
1999	0.69	0.027 (0.050)	0.349
2000	2.70	0.034 (0.062)	0.500
2001	0.49	0.031 (0.055)	0.363

(2009), which is the only other study to date conducted in adults of known age. Despite the small sampling overlap (both studies include individuals from Lesina-2001), Palm et al. (2009) observed no significant temporal genetic variation which might be related to the different constraints of the markers used. In this sense, Palm et al. (2009) employed six genomic microsatellite loci (only four in HWE equilibrium), while our study was conducted using 22 microsatellite loci derived from EST sequences. When comparing the levels of genetic variation in adults between studies, genomic microsatellites showed a higher level of genetic variability (expected heterozygosity of $H_e = 0.72$ and 83.3% of loci showing H_e values >0.70 , Palm et al., 2009) than EST-derived microsatellites ($H_e = 0.55$ – 0.59 , 39.1% showing H_e values >0.70). Similarly, a microsatellite survey in rice, wheat and barley showed non-random distribution and frequencies of genomic and EST-derived microsatellites (La Rota et al., 2005), suggesting that genomic microsatellites have the potential for a larger polymorphism than those derived from coding regions, which are length-constrained by purifying selection.

While our study detected no increased differences over time, suggesting that heterogeneity among adult samples was not attributable to an IBT pattern, Maes et al. (2006) detected a subtle IBT pattern among glass eels. A marginally significant correlation was observed between samples differing by 2–3 years between microsatellite genetic distance and daily distance ($r = 0.184$, $p = 0.043$) but not yearly distance ($r = 0.095$, $p = 0.134$) or using allozyme genetic distance (daily distance: $r = 0.005$, $p = 0.450$; yearly distance: $r = 0.077$, $p = 0.166$). Nevertheless, both our study and Palm et al. (2009), using aged adults and a more extensive inter-annual sampling, fail to verify the IBT pattern. In our study, no significant correlation was observed between genetic distance and temporal distance measured as number of years between cohorts covering the period 1999–2004 ($r = 0.020$; $p = 0.911$). In the study of Palm et al. (2009) a comparison of six cohorts with large sample sizes ($N > 100$) from the period 1990–2001 showed a clear lack of correlation between genetic and yearly distance ($r = 0.00013$; $p = 0.24$). A population structure following a true IBT pattern can arise in populations composed of individuals reproducing at different times within the spawning season. When reproductive times are highly heritable, a restriction of gene flow might occur between groups, generating a pattern of IBT (Hendry and Day, 2005). Studies of flowering plants and salmonids showing highly heritable reproductive times provide evidence of IBT, although its strength and consistency in nature need further specific testing (Fillatre et al., 2003; Hendry and Day, 2005). In this sense, the European eel exhibits a protracted spawning season, with field studies suggesting that spawning is restricted to a few months in late winter and spring, while otolith studies of glass eels imply that spawning occurs almost all throughout

the year (McCleave, 2008; Bonhommeau et al., 2010), but the heritable component of reproduction remains unknown. The failure to detect increased differences over time using adult data suggests that there is no apparent heritable component in spawning time within the reproductive season of the European eel. Nevertheless, a seasonal separation might be blurred by a large variance in age-at-maturity in adults and mixing of larvae during the migration from the Sargasso Sea to the European feeding grounds.

Our study detects a higher differentiation in adults ($F_{ST} = 0.0048$) than in glass eels using the same 22 EST-derived microsatellite markers ($F_{ST} = 0.0024$; Pujolar et al., 2009a). Possible different levels of relatedness among individuals at the two stages might account for the increase in F_{ST} . Nevertheless, all adult cohorts showed the same pattern of relatedness previously observed in glass eels, namely a majority of unrelated individuals mixed with 0.2–2.7% half or full-siblings. The increase in F_{ST} might also be explained by selection. However, many loci in our study showed significant genetic differentiation between sample pairs but loci involved were different across populations, which suggests that selection is not the mechanism responsible for the temporal genetic differentiation found. Alternatively, we propose that the higher genetic heterogeneity observed in adult samples is a consequence of genetic patchiness. Adults cohorts are composed by a mix of glass eels arrival waves, each of them resulting from low effective population sizes. As a consequence of the temporal variation in the genetic composition of the recruits, cohorts will differ from each other following a pattern of genetic patchiness shaped by random genetic drift.

4.2. Oceanic influence on adults

A differential contribution of spawning adults to the next generation is likely to explain the pattern of temporal genetic patchiness among adult cohorts. Hedgecock (1994) proposed that in marine species with external fertilization random events determine which adults are successful in each spawning event, in which many individuals fail to contribute to recruitment and a small fraction of individuals replaces the entire population. How many and which individuals contribute to reproduction is determined by chance, and results in a reduction in effective population size and in consequent changes in allele frequencies over time due to random genetic drift, leading to the variation in genetic composition of samples observed in genetic patchiness. Such a reproductive sweepstakes scenario is in concordance with the predictions of our genetic-demographic model, which suggests an average effective number of eels per spawning event between 130 and 375 individuals.

Spatio-temporal variation in climatic and oceanographic processes can explain the large variance in reproduction success of adults as they influence key life history events associated with the marine phase of the European eel life cycle. The long spawning migration, the mating and spawning process in the Sargasso Sea, the development of eggs and larvae, and the larval migration to the continental feeding grounds are all under the influence of oceanic factors and climate variation. Large-scale environmental fluctuations such as the North Atlantic Oscillation (NAO) have been linked to significant changes in the physical and biological structure of the North Atlantic Ocean, affecting thermal, wind and mixing parameters in areas of the Sargasso Sea that correspond spatially to the spawning grounds of the European eel (Knights, 2003; Miller, 2009; Miller et al., 2009). Environmental changes within the spawning and early larval development areas of eels in the Sargasso Sea might reduce the number of successful breeders and surviving larvae (Friedland et al., 2007; Bonhommeau et al., 2008). In a recent study using otolith microchemistry, Martin et al. (2010) found significant differences in trace element signatures between glass eels/elvers collected in European locations and leptocephali caught in the Sargasso Sea, suggesting that the former were born in a spawn-

ing site linked to favourable transport and/or feeding conditions, while the latter may have been sampled in a less favourable region in the Sargasso Sea, with a low probability of reaching continental growth areas. Perturbation of the spawning success might be exacerbated by the detrimental effects of continental factors such as pollution or parasites leading to a reduced fitness and/or fecundity of spawners (Palstra et al., 2006; Sjöberg et al., 2009).

4.3. A bet-hedging strategy to guarantee successful reproduction

Wide variation in spawning success may have important consequences for the life-history evolution of the European eel. Crow and Kimura (1970) argued that loss of genetic diversity due to genetic drift would be negligible in an ideal (Wright–Fisher) population showing binomial or Poisson variance in progeny number. Marine species with external fertilization may have variances in progeny number much greater than binomial or Poisson variance as a result of high fecundities, high pre-reproductive mortality and the unpredictability of the marine environment (Hedgcock, 1994). If so, genetic drift may have important effects at the genetic level (e.g. patchiness) even if census sizes are large. In the case of the European eel, long-term swim tunnel experiments have given some indirect information on the possible constraints on the migration success of silver eels. An observed small fat consumption by locomotion (<40% of the initial fat reserve) could guarantee covering the 6000 km distance from the European feeding grounds to the Sargasso Sea and the final spawning activity (Van den Thillart et al., 2009). Despite high numbers of migrating silver eels (3.9×10^7 ; Dekker, 2000b) and energy reserves being sufficient for the migration, the genetic–demographic model could only explain the differentiation found if an average of 130–375 eels constituted each spawning event. The disparity between escaping silver eels and eels effectively contributing to reproduction might be attributed to ocean-atmospheric processes occurring in the Sargasso Sea that could hamper spawning success. Asynchronies between spawning activity and suitable environmental conditions could lead to reproductive failure by a significant fraction of individuals. It has been proposed that distinct temperature fronts are used by adults as cues to locate the boundaries of the spawning area within the Sargasso Sea (McCleave, 1993). Shifts in the latitude or intensity of those fronts might cause adults to mislocate the spawning ground, resulting in a reduction of the number of successful breeders (Friedland et al., 2007).

It has been hypothesized that life history traits of marine free-spawning populations have partially evolved as a result of the unpredictability of spawning success (Flowers et al., 2002). In species with high pre-spawning mortalities, selection might favour allocation of more resources to growth/maintenance than reproduction, resulting in a longer life-span and a wider variance in maturity-at-age. The latter would allow distributing reproductive efforts over time (i.e. bet hedging), which could be essential for increasing the chances of spawning success in species with a high dependence on unpredictable environmental conditions like the case of the European eel. In this sense, age-at-maturity is highly variable in eels, with metamorphosis into silver eels occurring between 2 and 20 years (Vøllestad, 1992). Silvering of adults does not only occur at different ages but is also variable across seasons, which translates into a protracted escapement of silver eels to the Sargasso Sea throughout the year. The spawning migration of the European eel generally takes place in autumn between August and December but can begin as early as July and continue until spring, and be delayed until as late as April if climatic conditions for migration are unfavourable (Acou et al., 2008). A bet hedging tactic to ensure spawning of some adults could be a compensatory mechanism for the large variance in parental contribution to reproduction. Hence, the number of adults contributing to a single spawning event might

be low (130–375 individuals as predicted in our model) but the total number of successful breeders (700,000 individuals) might be large enough to guarantee sufficient recruitment abundance.

5. Conclusions

The genetic composition of adults observed in our study was the diagnostic signature expected from sweepstakes reproductive success, namely that annual cohorts of recruits should be differentiated following a pattern of genetic patchiness. This prediction is based on the hypothesis that chance matching of reproductive activity with environmental conditions suitable for spawning success will result in genetic drift because a limited number of adults contribute to each spawning event.

A direct implication of our findings is that sweepstakes reproductive success can limit effective population numbers to fractions of actual abundances. If only a subset of the adults contribute to reproduction, the effective size of the European eel might be considerably lower than the census size. Together with anthropogenic (overfishing, pollution, parasites) and oceanic factors, a low effective population size might have contributed to the current decline in the abundance of the European eel. A precautionary approach to fisheries should be implemented (as currently advised, ICES, 2008) in order to avoid the loss of genetic diversity and maximize genetic potential to cope with changing anthropogenic and environmental pressures. As foreseen by EU regulation 1100/2007, a reduction in eel exploitation would allow enhancing of the European eel spawning stock. It is important that (1) monitoring is continued and enhanced, including monitoring of temporal changes in genetic composition and genetic diversity, and (2) development of an expanded genetic/demographic model that tests a wider number of alternative demographic scenarios as we used a simple model limited by the lack of data on the mating behaviour of the species.

Acknowledgments

This work has been funded by an Italian Research Program grant to LZ and by the University of Padova grant CPDA 085158/08 to LZ. We thank the CNR Lesina, the Parco Nazionale del Circeo and the Tiber fishermen for support in sampling.

References

- Acou, A., Laffaille, P., Legault, A., Feunteun, E., 2008. Migration pattern of silver eel (*Anguilla anguilla*) in an obstructed river system. *Ecol. Freshw. Fish.* 17, 432–442.
- Åström, M., Dekker, W., 2007. When will the eel recover? A full life-cycle model. *ICES J. Mar. Sci.* 64, 1491–1498.
- Barbin, G.P., McCleave, J.D., 1997. Fecundity of the American eel *Anguilla rostrata* at 45 N in Maine, USA. *J. Fish Biol.* 52, 840–847.
- Belkhir, K., Bors, P., Goudet, J., Bonhomme, F., 2005. GENETIX 4.05: Logiciel sous Windows pour la génétique des populations. Laboratoire Genome & Population, CNRS-UPR, Université de Montpellier II, Montpellier, France.
- Bevacqua, D., Melià, M., Crivelli, A.J., De Leo, G.A., Gatto, M., 2006. Timing and rate of sexual maturation of European eel in brackish and freshwater environments. *J. Fish Biol.* 69, 200–208.
- Bevacqua, D., Melià, M., Crivelli, A.J., Gatto, M., De Leo, G.A., 2007. Multi-objective assessment of conservation measures for the European eel (*Anguilla anguilla*): an application for the Camargue lagoons. *ICES J. Mar. Sci.* 64, 1472–1482.
- Bevacqua, D., De Leo, G.A., Gatto, M., Melià, M., 2009. Size selectivity of fyke nets for European eel *Anguilla anguilla*. *J. Fish Biol.* 74, 2178–2186.
- Boëtius, I., Boëtius, J., 1989. Ascending elvers, *Anguilla anguilla*, from 5 European localities—analyses of pigmentation stages, condition, chemical-composition and energy reserves. *Dana* 7, 1–12.
- Bonhommeau, S., Chassot, E., Rivot, E., 2008. Fluctuations in European eel (*Anguilla anguilla*) recruitment resulting from environmental changes in the Sargasso Sea. *Fish. Oceanogr.* 17, 32–44.
- Bonhommeau, S., LePape, O., Gascuel, D., Blanke, B., Tréguier, A.M., Grima, N., Vermard, Y., Castonguay, M., Rivot, E., 2009. Estimates of mortality and duration of the trans-Atlantic migration of European eel *Anguilla anguilla* leptocephali using particle tracking model. *J. Fish Biol.* 74, 1891–1914.
- Bonhommeau, S., Castonguay, M., Rivot, E., Sabatie, R., LePape, O., 2010. The duration of migration of Atlantic *Anguilla* larvae. *Fish Fisheries* 11, 289–306.

- Burton, R.S., 1983. Protein polymorphism and genetic differentiation of marine invertebrate populations. *Mar. Biol. Lett.* 4, 193–206.
- Cavalli-Sforza, L.L., Edwards, A.V.F., 1967. Phylogenetic analysis models and estimation procedures. *Evolution* 32, 550–570.
- Conover, D.O., Clarke, L.M., Munch, S.B., Wagner, G.N., 2006. Spatial and temporal scales of adaptive divergence in marine fishes and the implications for conservation. *J. Fish Biol.* 69, 21–47.
- Crow, J.F., Kimura, M., 1970. *An Introduction to Population Genetics Theory*. Burgess, Minneapolis.
- Dannewitz, J., Maes, G.E., Johansson, L., Wickström, H., Volckaert, F.A.M., Jarvi, T., 2005. Panmixia in the European eel: a matter of time. *Proc. Roy. Soc. Lond. B Biol. Sci.* 272, 1129–1137.
- David, P., Perdieu, M.A., Jarne, P., 1997. Fine-grained spatial and temporal population genetic structure in the marine bivalve *Spisula ovalis*. *Evolution* 51, 1318–1322.
- Dekker, W., 2000a. Impact of yellow eel exploitation on spawner production in Lake IJsselmeer, the Netherlands. *Dana* 12, 25–40.
- Dekker, W., 2000b. A procrustean assessment of the European eel stock. *ICES J. Mar. Sci.* 57, 938–947.
- Dekker, W., 2003. Did lack of spawners cause the collapse of the European eel *Anguilla anguilla*? *Fish. Manage. Ecol.* 10, 365–376.
- Dekker, W., Casselman, J.M., Cairns, D.K., Tsukamoto, K., Jellyman, D., Lickers, H., 2003. Worldwide decline of eel resources necessitates immediate action. *Fisheries* 28, 28–30.
- De Leo, G.A., Gatto, M., 1995. Trends in vital rates of the European eel (*Anguilla anguilla*). *Can. J. Fish. Aquat. Sci.* 52, 1351–1367.
- Edmands, S., Moberg, P.E., Burton, R.S., 1996. Allozyme and mitochondrial DNA evidence of population subdivision in the purple sea urchin *Strongylocentrotus purpuratus*. *Evolution* 56, 1445–1453.
- Feunteun, E., 2002. Management and restoration of European eel population (*Anguilla anguilla*): an impossible bargain. *Ecol. Eng.* 18, 575–591.
- Fillatre, E.K., Etherton, P., Heath, D.D., 2003. Bimodal run distribution in a northern population of sockeye salmon (*Oncorhynchus nerka*): life history and genetic analysis on a temporal scale. *Mol. Ecol.* 12, 1793–1805.
- Flowers, J.M., Schoeter, S.C., Burton, R.S., 2002. The recruitment sweepstakes has many winners: genetic evidence from the sea urchin *Strongylocentrotus purpuratus*. *Evolution* 56, 1445–1453.
- Friedland, K.D., Miller, M.L., Knights, B., 2007. Oceanic changes in the Sargasso Sea and declines in recruitment of the European eel. *ICES J. Mar. Sci.* 64, 519–530.
- Gagnaire, P.A., Albert, V., Jonsson, B., Bernatchez, L., 2009. Natural selection influences AFLP intraspecific genetic variability patterns in Atlantic eels. *Mol. Ecol.* 18, 1678–1691.
- Goudet, J., 2002. FSTAT, a program to estimate and test gene diversities and fixation indices (v. 2.9.3.2.). <<http://www2.unil.ch/opgen/softwares/fstat.htm>>.
- Hedgecock, D., 1994. Does variance in reproductive success limit effective population size? In: Beaumont, A.R. (Ed.), *Genetics and Evolution of Aquatic Organisms*. Chapman & Hall, London, pp. 122–134.
- Hendry, A.P., Day, T., 2005. Population structure attributable to reproductive time: isolation by time and adaptation by time. *Mol. Ecol.* 14, 901–916.
- ICES, 2008. Report of the Working Group on Eels (WGEE), 3–9 September 2008, Leuven, Belgium. ICES CM 2008/ACFM:15. International Council for the Exploration of the Seas, Copenhagen.
- ICES, 2009. Report of the Working Group on the Age Reading of European and American eel (WKAREA), 20–24 April 2009, Bordeaux, France. ICES CM 2009/ACFM:48. International Council for the Exploration of the Seas, Copenhagen.
- Johnson, M.S., Black, R., 1982. Chaotic patchiness in an intertidal limpet *Saphonaria* sp. *Mar. Biol.* 70, 157–164.
- Kalinowski, S.T., Wagner, A.P., Taper, M.L., 2006. ML-relate: a computer program for maximum estimation of relatedness and relationship. *Mol. Ecol. Notes* 6, 576–579.
- Kettle, A., Haines, K., 2006. How does the European eel *Anguilla anguilla* retain its population structure during its larval migration across the North Atlantic Ocean. *Can. J. Fish. Aquat. Sci.* 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. *Sci. Total Environ.* 310, 237–244.
- La Rota, M., Kantely, R.V., Yu, J.K., Sorrells, M.E., 2005. Nonrandom distribution and frequencies of genomic and EST-derived microsatellite markers in rice, wheat and barley. *BMC Genomics* 6, 23.
- Levitan, D.R., 2008. Gamete traits influence the variance in reproductive success, the intensity of sexual selection, and the outcome of sexual conflict among congeneric sea urchins. *Evolution* 62, 1305–1316.
- Li, G., Hedgecock, D., 1998. Genetic heterogeneity, detected by PCR-SSNP, among samples of larval Pacific oysters (*Crassostrea gigas*) supports the hypothesis of large variance in reproductive success. *Can. J. Fish. Aquat. Sci.* 55, 1025–1033.
- McCleave, J.D., 1993. Physical and behavioral controls on the oceanic distribution and migration of leptocephali. *J. Fish Biol.* 43, 243–273.
- McCleave, J.D., 2008. Contrasts between spawning times of *Anguilla* species estimated from larval sampling at sea and from otolith analysis of recruiting glass eels. *Mar. Biol.* 155, 249–262.
- Maes, G.E., Pujolar, J.M., Hellems, B., Volckaert, F.A.M., 2006. Evidence for isolation by time in the European eel *Anguilla anguilla*. *Mol. Ecol.* 15, 2095–2107.
- Mantel, N., 1967. The detection of disease clustering and generalised regression approach. *Cancer Res.* 27, 209–220.
- Martin, J., Deverat, F., Pécuyer, C., Als, T.D., Feunteun, E., Réveillac, E., 2010. An otolith microchemistry study of possible relationships between the origins of leptocephali of European eels in the Sargasso Sea and the continental destinations and relative migration success of glass eels. *Ecol. Freshw. Fish* 19, 627–637.
- Melià, P., Bevacqua, D., Crivelli, A.J., De Leo, G.A., Panfili, J., Gatto, M., 2006. Age and growth of the European eel *Anguilla anguilla* in the Camargue lagoons. *J. Fish Biol.* 68, 876–890.
- Miller, M.J., 2009. Ecology of anguilliform leptocephali: remarkable transparent fish larvae of the ocean surface layer. *Aqua-BioSci. Monogr. (ABSM)* 2, 1–94.
- Miller, M.J., Kimura, S., Friedland, K.D., Knights, B., Kim, H., Jellyman, D.J., Tsukamoto, K., 2009. Review of ocean-atmospheric factors in the Atlantic and Pacific oceans influencing spawning and recruitment of anguillid eels. In: Haro, A., Avery, T., Beal, K., Cooper, J., Cunjak, R., Dadswell, M., Klauda, R., Moffitt, C., Rulifson, R., Smith, K. (Eds.), *Challenges for Diadromous Fishes in a Dynamic Global Environment*, vol. 69. American Fisheries Society Symposium Publication, pp. 231–249.
- Moberg, P.E., Burton, R.S., 2000. Genetic heterogeneity among adult and recruit red sea urchins, *Strongylocentrotus franciscanus*. *Mar. Biol.* 136, 773–784.
- Palm, S., Dannewitz, J., Prestegard, T., Wickström, H., 2009. Panmixia in the European eel revisited: no genetic difference between maturing adults from southern and northern Europe. *Heredity* 103, 82–89.
- Palstra, A.P., van Ginneken, V.J.T., Murk, A.J., van den Thillart, G.E.E.J.M., 2006. Are dioxin-like contaminants responsible for the eel *Anguilla anguilla* drama? *Naturwissenschaften* 93, 145–148.
- Planes, S., Lenfant, P., 2002. Temporal, al change in the genetic structure between and within cohorts of a marine fish, *Diplodus sargus*, induced by a large variance in individual reproduction success. *Mol. Ecol.* 11, 1511–1524.
- Pujolar, J.M., Maes, G.E., Volckaert, F.A.M., 2006. Genetic patchiness among recruits of the European eel *Anguilla anguilla*. *Mar. Ecol. Prog. Ser.* 307, 209–217.
- Pujolar, J.M., Maes, G.E., Volckaert, F.A.M., 2007. Genetic and morphometric heterogeneity among recruits of the European eel *Anguilla anguilla*. *Bull. Mar. Sci.* 81, 297–308.
- Pujolar, J.M., De Leo, G.A., Ciccotti, E., Zane, L., 2009a. Genetic composition of Atlantic and Mediterranean recruits of the European eel (*Anguilla anguilla*) based on EST-linked microsatellite loci. *J. Fish Biol.* 74, 2034–2046.
- Pujolar, J.M., Maes, G.E., Van Houdt, J.K.J., Zane, L., 2009b. Isolation and characterization of EST-linked microsatellite loci for the European eel, *Anguilla anguilla*. *Mol. Ecol. Res.* 9, 233–235.
- Raymond, M., Rousset, F., 1995. GENEPOP (version 1.2): a population genetics software for exact tests and ecumenicism. *J. Hered.* 86, 248–249.
- Rice, W.R., 1989. Analyzing tables and statistical tests. *Evolution* 43, 223–225.
- Ruzzante, D.E., Taggart, C.T., Cook, D., 1996. Spatial and temporal variation in the genetic composition of a larval cod (*Gadus morhua*) aggregation: cohort contribution and genetic stability. *Can. J. Fish. Aquat. Sci.* 53, 2695–2705.
- Schneider, S., Roessli, D., Excoffier, L., 2000. ARLEQUIN: a software for population genetics data analysis ver 3.0. Genetics and Biometry Lab, Dept. of Anthropology, University of Geneva, Switzerland.
- Sjöberg, N.B., Petersson, E., Wickström, H., Hansson, S., 2009. Effects of the swimbladder parasite *Anguillicola crassus* on the migration of European eel silver eels *Anguilla anguilla* in the Baltic Sea. *J. Fish Biol.* 74, 2158–2170.
- Van den Thillart, G.E.E.J.M., Rankin, J.C., Dufour, S., 2009. Spawning migration of the European eel: reproduction index, a useful tool for conservation management. Springer, Dordrecht, The Netherlands.
- Van Oosterhout, C.V., Hutchinson, W.F., Wills, D.P.M., Shipley, P., 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol.* 4, 535–538.
- Vøllestad, L.A., 1992. Geographic variation in age and length at metamorphosis of maturing European eel: environmental effects and phenotypic plasticity. *J. Animal Ecol.* 61, 41–48.
- Vøllestad, L.A., Jonsson, B., 1988. A 13-year study of the population dynamics of the European eel *Anguilla anguilla* in a Norwegian river: evidence for density-dependent mortality, and development of a model for predicting yield. *J. Animal Ecol.* 57, 983–997.
- Waples, R.S., Gaggiotti, O.E., 2006. What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Mol. Ecol.* 15, 1419–1439.
- Ward, R.D., Woodwark, M., Skibinski, D.O.F., 1994. A comparison of genetic diversity levels in marine, freshwater and anadromous fishes. *J. Fish Biol.* 44, 213–232.
- Weir, B.S., Cockerman, C.C., 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution* 38, 1358–1370.
- Wirth, T., Bernatchez, L., 2001. Genetic evidence against panmixia in the European eel. *Nature* 409, 1037–1040.