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RESEARCH ARTICLE

No apparent genetic bottleneck in the demographically declining European eel using molecular genetics and forward-time simulations

J. M. Pujolar · D. Bevacqua · F. Capoccioni · E. Ciccotti · G. A. De Leo · L. Zane

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Abstract The stock of the European eel is considered to be outside safe biological limits, following a dramatic demographic decline in recent decades (90–99% drop) that involves a large number of factors including overfishing, contaminants and environmental fluctuations. The aim of the present study is to estimate the effective population size of the European eel and the possible existence of a genetic bottleneck, which is expected during or after a severe demographic crash. Using a panel of 22 EST-derived microsatellite loci, we found no evidence for a genetic bottleneck in the European eel as our data showed moderate to high levels of genetic diversity, no loss of allele size range or rare alleles, and a stationary population with growth values not statistically different from zero, which is confirmed by finding comparable value of short-term and long-term effective population size. Our results suggest that the observed demographic decline in the European eel did not entail a genetic decline of the same magnitude. Forward-time simulations confirmed that large exploited marine fish populations can undergo genetic bottleneck episodes and experience a loss of genetic variability. Simulations indicated that the failure to pick up the signal of a genetic bottleneck in the European eel is not due to lack of power. Although anthropogenic factors lowered the continental stock biomass, the observation of a stable genetic effective population size suggests that the eel crash was not due to a reduction in spawning stock abundance. Alternatively, we propose that overfishing, pollution and/or parasites might have affected individual fitness and fecundity, leading to an impoverished spawning stock that may fail to produce enough good quality eggs. A reduced reproduction success due to poor quality of the spawners may be exacerbated by oceanic processes inducing changes in primary production in the Sargasso Sea and/or pathway of transport across the Atlantic Ocean leading to a higher larval mortality.

Keywords Anguilla anguilla · Bottleneck · Effective population size · European eel · Forward-time simulations

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J. M. Pujolar (✉) · L. Zane
Dipartimento di Biologia, Università di Padova,
Via G. Colombo 3, 35131 Padova, Italy
e-mail: martipujolar@gmail.com

D. Bevacqua · G. A. De Leo
Dipartimento di Scienze Ambientali, Università degli Studi di Parma, Viale Usberti 11A, 43100 Parma, Italy

F. Capoccioni · E. Ciccotti
Dipartimento di Biologia, Università Roma Tor Vergata,
00133 Rome, Italy

Introduction

Recent studies have revealed that evolutionary changes can occur on contemporary time scales, often within decades, as suggested by reports of rapid evolution spanning a variety of studies, traits, taxa and time frames (Hendry and Kinnison 1999; Ashley et al. 2003; Stockwell et al. 2003). Rapid evolution in contemporary populations is usually associated with disturbance events due to anthropogenic pressures (overexploitation including overfishing and overharvesting, habitat degradation, habitat fragmentation, introduction of exotic species, introduced diseases and parasites) or caused by environmental factors (natural disasters, climate fluctuations) (Stockwell and Ashley

2003). Reduced population size due to over-harvesting can cause the loss of genetic diversity (heterozygosity and allelic diversity), which can occur in populations with large census sizes (e.g., marine fish) because effective population size is often much smaller than the census size (Hauser and Carvalho 2008; Palstra and Ruzzante 2008). For instance, in the New Zealand snapper *Pagrus auratus* microsatellite heterozygosity and alleles per locus declined after initiation of a fishery, despite an estimated standing population well over three million fish (Hauser et al. 2002). This suggests that even very large exploited marine fish populations might be in danger of losing genetic diversity (Allendorf et al. 2008; Hauser and Carvalho 2008).

The strong dependence on anthropogenic and environmental factors holds particularly in the case of the European eel *Anguilla anguilla*, a catadromous highly migratory species with a complex life cycle that moves between marine and continental environments. After spawning in the Sargasso Sea, larvae (leptocephali) are transported to the shores of Europe and North Africa following the Gulf Stream and North Atlantic Drift. Upon reaching the continental shelf, larvae metamorphose into glass eels that migrate into continental growth habitats, settle and become pigmented yellow eels. After a period of intensive feeding, they metamorphose into silver eels (on average at age 3–8 for males and age 5–15 for females), and migrate back to the Sargasso Sea, where they reproduce once and eventually die (Van den Thillart et al. 2009).

The stock of the European eel has shown a dramatic decline over the last decades and is considered to be outside safe biological limits (ICES 2008). The European eel was included in 2007 in Appendix II of the Convention on International Trade of Endangered Species (CITES; http://www.cites.org) and was listed in 2008 as critically endangered in the IUCN Red List of Threatened Species (http://www.iucnredlist.org). A management framework for the recovery of the European eel stock was established in 2007 by the Council of the European Union through a dedicated regulation (EU 1100/2007) for eel recovery and sustainable use of the stock requiring the preparation of national eel management plans from any Member States. The available information indicates that the overall stock is at an historical minimum in most of the distribution area and continues to decline, while fishing mortality is still high both in juveniles (glass eels) and adults (yellow and silver eels) (ICES 2008). At present, the European eel shows a historically low recruitment abundance, following a 90–99% decline in comparison with the recruitment observed in the 1970s (Dekker 2000, 2003). Glass eel recruitment has been decimated to 2.0×10^9 individuals per year (Andrello et al. 2010). Most data sets also show a decline in fishing yield and landings of yellow and silver eels from the mid 1960s on (Dekker 2003; ICES 2008). 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 lifecycle. 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 *Anguillicola 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 (Friedland et al. 2007). Large-scale environmental fluctuations such as the North Atlantic Oscillation (NAO) have been associated with a wide range of oceanographic and biological changes, including eel recruitment (Knights 2003).

Recent genetic studies on the European eel support the panmixia hypothesis, namely that all European eel comprise a single randomly mating population, with no evidence of Isolation-by-Distance or Isolation-by-Time (Dannewitz et al. 2005; Palm et al. 2009). However, studies focusing on the genetic composition of glass eels have observed a highly significant genetic heterogeneity without a temporal grouping (genetic patchiness) among intra-annual samples within cohorts (arrival waves) (Pujolar et al. 2006, 2007, 2009a; Maes et al. 2007). 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).

The aim of the present study is to provide an integrated assessment of genetic variability in the European eel in a contemporary context. This has been achieved by estimating the short- and long-term effective population size of the European eel and the possible existence of a genetic bottleneck, expected during or after a severe demographic crash. Genotypes were examined at a total of 22 microsatellite loci derived from Expressed Sequence Tags (ESTs) (Pujolar et al. 2009b), which are single-read sequences produced from partial sequencing of a mRNA pool. Our study also included forward-time simulations in order to assess the power of the genetic softwares used to detect a bottleneck episode (LAMARC, BOTTLENECK, M_P_VAL) under different demographic scenarios varying the magnitude of the decline, initial population size and mating scheme. A better understanding of crucial aspects of the biology of the European eel, including genetic variability and effective population size, will allow examining the relationship between genetic diversity and demographic

processes that determine species distribution and abundance, including spawning and recruitment.

Materials and methods

Sampling collection

Samples of *A. anguilla* were obtained in two separate geographic locations in the Mediterranean Sea, namely Tyrrhenian Sea and Adriatic Sea, including both glass eel and adult life-stages. Tyrrhenian Sea samples were collected in the low course of the Tiber river (41°48′N; 12°25′E) and at the nearby lake of Caprolace (41°21′N; 12°59′E) in the National Park of Circeo. Adriatic Sea samples were collected in the brackish lagoon of Lesina (41°53′N; 15°51′E). Glass eel samples were caught on 16–18 January 2007, while adults were caught during the 2007–2008 fishing season. Otoliths were used to estimate individual age and to assign each fish to a single cohort (year-class). Otoliths were extracted from a total of 1,234 adults, embedded in epoxyn resin, and stained with 3% toluidine blue following grinding of the convex side. 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). A total of 346 adults were further selected for genetic analysis on the basis of good quality of otolith reading, and to include sufficient number of individuals per group to conduct statistical analysis (Table 1). This allowed us to examine a 7-sample temporal series covering the period 1999–2007 for the Tyrrhenian Sea, and, a 5-sample temporal series covering the period 2001–2007 for the Adriatic Sea.

Microsatellite analysis

Minute sections of tissue from ethanol-preserved eel fin-clips were digested in a lysis buffer containing 100 µl TE Buffer, 7 µl 1 M DTT (dithiothreitol) solution pH 5.2 (diluted in 0.08 M NaAC) and 2 µl 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 HWE and was suggested to include null alleles by the program MICRO-CHECKER (Van Oosterhout et al. 2004). PCR products were obtained in a GeneAmp PCR System 2700 Thermocycler (Applied Biosystems) using the QIAGEN Multiplex PCR Kit. PCR reactions consisted of 2 µl template DNA, 5 µl QIAGEN Multiplex PCR Master Mix, 0.2 µl 10 µM forward and reverse primers, and water up to 10 µl. PCR conditions were as follows: 3 min 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.

Table 1 Summary of genetic variability estimates across *A. anguilla* samples including number of individuals (*N*), observed (H_o) and expected (H_e) heterozygosities and allelic richness (AR) at 21 microsatellite loci

Sample	N	H_o	H_e	AR
Tyrrhenian				
Caprolace-1999	33	0.497 (0.236)	0.569 (0.255)	6.333 (4.234)
Caprolace-2000	41	0.497 (0.238)	0.553 (0.253)	6.121 (3.559)
Caprolace-2001	29	0.536 (0.226)	0.574 (0.233)	6.102 (3.395)
Tiber-2002	33	0.536 (0.251)	0.561 (0.260)	6.160 (3.907)
Tiber-2003	42	0.520 (0.270)	0.553 (0.265)	6.407 (4.017)
Tiber-2004	34	0.538 (0.233)	0.589 (0.238)	6.536 (3.891)
Tiber-2007	50	0.538 (0.260)	0.566 (0.258)	6.520 (3.933)
Adriatic				
Lesina-2001	30	0.553 (0.235)	0.565 (0.237)	6.185 (3.410)
Lesina-2002	31	0.516 (0.242)	0.558 (0.255)	6.020 (3.455)
Lesina-2003	46	0.497 (0.212)	0.562 (0.259)	6.250 (3.943)
Lesina-2004	27	0.510 (0.266)	0.552 (0.266)	6.396 (4.336)
Lesina-2007	57	0.515 (0.225)	0.574 (0.245)	6.480 (4.025)

Standard deviation in parentheses

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. 1996–2004), and allelic richness (AR) using FSTAT (Goudet 2002). Deviations from Hardy–Weinberg equilibrium, linkage disequilibrium, differences in allele and genotype frequencies among samples, and *F*-statistics were tested using GENEPOP version 3.4 (Raymond and Rousset 1995). Significance levels for multiple comparisons were adjusted using the sequential Bonferroni technique (Rice 1989). Presence of null alleles was tested using the program MICRO-CHECKER version 2.2.3 (Van Oosterhout et al. 2004).

Neutrality of the markers was tested using the fdist program in the selection detection workbench LOSITAN (Antao et al. 2008), which implements a F_{ST} outlier detection approach. This method evaluates the relationship between F_{ST} and expected heterozygosity (H_e), describing the expected distribution of Wright's inbreeding coefficient F_{ST} vs. H_e under an island model of migration with neutral

markers. This distribution is used to identify outlier loci that show excessively high or low F_{ST} values compared to neutral expectations. Such outlier loci are candidates for being subject to selection. Given that low F_{ST}-values might evade detection in LOSITAN due to the non-linear behaviour of $F_{ST} = 1/4\ \mathrm{Nm} + 1$ when F_{ST} approaches zero (Antao et al. 2008), outlier loci were also identified using the lnRH and lnRV test statistics (Kauer et al. 2003; Schlötterer 2003). The principle of both test statistics is that the occurrence of a recent selective sweep is expected to reduce variability at a microsatellite locus linked to the selected site, but other regions of the genome should not be affected. Hence, for both test statistics a selected locus is expected to differ significantly in repeat number (lnRV) and gene diversity (lnRH) from the remainder of the genome. It has been demonstrated that lnRV and lnRH are approximately normally distributed under neutrality (Kauer et al. 2003). Therefore, after standardization (mean = 0; SD = 1) 95% of neutral loci are expected to have values between −1.96 and 1.96 (99% CI between −2.58 and 2.58; 99.9% CI between −3.29 and 3.29). In all cases when a locus was monomorphic in one population, we added a single different allele to the sample in order to avoid the heterozygosity value being zero. Correction for multiple tests was applied by comparing observed number of significant values with the expected number of false-positives at $\alpha = 0.05$ using a chi-squared test.

An estimation of short-term effective population size, which can be affected by recent changes in genetic variation, was evaluated using the temporal method with overlapping generations described in Jorde and Ryman (1995, 1996). An estimate of $N_e(\widehat{N_e})$ was obtained using $\widehat{N_e} = C/2G\bar{F}'$, where C is a correction factor obtained from age-specific survival rates (l_i) and reproductive success (p_i), G is the mean generation length, and $\bar{F}'$ is the grand mean of temporal allele frequency change among all adjacent cohorts and over all loci derived from Pollak (1983) and corrected for sampling variance. Equations to compute F, C, and G are found in Jorde and Ryman (1995). In addition, the 95% confidence interval (CI) for $\bar{F}'$ and $\widehat{N_e}$ was calculated by a non-parametric approach bootstrapping all the individual F values from each combination of locus and pair of consecutive cohorts. Values of l_i (i.e., the probability of a newborn fish to survive to age i) were estimated for each age class from the annual survival rates reported by Andrello et al. (2010). In order to estimate the relative reproductive success p_i at different ages, first we estimated the proportion of breeders (silver eel survival fraction in the year of spawning migration) per age class according to Andrello et al. (2010). Mean number of offspring per male (b_i^m) and female (b_i^f) of age class i were obtained from Andrello et al. (2010), after correcting the values so as to result in a constant population size (i.e., net reproductive rate, $R_0 = \sum l_i b_i = 1$, for each sex). Finally, p_i was estimated using the age-specific estimates of b_i and l_i by giving the sexes equal weight: $p_i = l_i\left(b_i^m + b_i^f\right)/2$.

Estimation of long-term effective population size, which is influenced by demographic processes over a much greater historical scale, was conducted using the Bayesian method in LAMARC 2.1.3. (Kuhner 2006), a Markov chain Monte Carlo coalescent genealogy sampler. Bayesian estimation searches simultaneously among genealogies and among values of the population parameters. Most probable estimates and credibility intervals are generated by recording the parameter values visited by the search and doing one-dimensional curve-smoothing to obtain the posterior probability curve for each parameter. The program estimates: (1) $\theta = 4N_e\mu$, where N_e is the long-term effective population size and μ is the neutral mutation rate per site per generation. N_e was estimated assuming a conservative mutation rate of $\mu = 5 \times 10^{-4}$ that is lower than the rate suggested for fish (10^{-3}; Fraser et al. 2007) but reflects the lower variability of EST-linked microsatellites. Simulations confirmed the adequacy of the rate (see below); (2) the exponential growth rate g. Positive g values are indicative of an expanding population, negative g values indicate a declining population, while g values statistically not different from zero (confidence intervals centred around zero) suggest a stationary population. Parameters were estimated for each locus separately, as well as a joint estimate over all loci. Two replicates were run for each sample, with two million genealogies generated, sampling every 200 genealogies, and with a burn-in of 200,000 iterations. Mutation was modelled using a Brownian-motion approximation to the stepwise model (Beerli and Felsenstein 2001). Priors for θ and g were drawn from uniform distributions ($1^{-10} < \theta < 100$; $-50 < g < 50$). Tracer v1.4 was used to check the convergence of the chains (Rambaut and Drummond 2007). Samples were analyzed individually due to the highly significant genetic heterogeneity found across samples. A sample of 20 individuals per subpopulation is fully adequate, especially if multiple loci are available, which will improve the estimate more than adding individuals (Kuhner 2006).

The effect of a population contraction resulting in a drop of genetic diversity was tested in two different ways. First, we used the software BOTTLENECK (Piry et al. 1999), based on the principle that after a recent reduction of their effective population size, number of alleles (k) decreases faster than heterozygosity (H_e) at polymorphic loci. Thus, in a recently bottlenecked population, the observed gene diversity is higher than the expected equilibrium gene diversity (H_{eq}) which is computed from the observed

number of alleles (k), under the assumption of a constant-size (equilibrium) population (Cornuet and Luikart 1996). We used the Two-Phased (TPM) model (Di Rienzo et al. 1994), which consists of mostly one-step mutations but a small percentage of multi-step changes, and is the recommended model for microsatellite data sets rather than the Infinite Allele (IAM) or Single-step Stepwise (SMM) models (Luikart et al. 1998). The proportion of single-step mutation events was set to 90%, with variance set to 12%. Observed and expected heterozygosities were compared using a one tail Wilcoxon sign-rank test that provides high power with 15–40 individuals and 10–15 polymorphic loci, and is recommended over the "sign test" or the "standardized differences test" (Piry et al. 1999). Second, we calculated M, the mean ratio between number of alleles (k) and range in allele size (r), assuming that during a bottleneck episode k decreases faster than r (M_P_Val; Garza and Williamson 2001). Hence, the value of M decreases when a population is reduced in size. Average M was calculated across loci and compared with the critical value M_{crit} estimated after 10,000 simulations and assuming the population to be at equilibrium. In all simulations, three different values of θ were used (5, 10 and 20). A range of mutation models were examined and conservative values were used for p_s (frequency of one-step mutations) and Δ_g (average size of non one-step mutations), $p_s = 0.90$ and $\Delta_g = 3.5$. As in the case of LAMARC, samples were analyzed individually due to the observed temporal genetic heterogeneity. For BOTTLENECK, Cornuet and Luikart (1996) had enough power to detect a bottleneck when re-examining Taylor et al. (1994) data on the endangered wombat *Lasiorhinus krefftii* using 9 polymorphic loci in a sample of 16–25 individuals. Similarly, re-examination of data from the microsatellite study of the Sardinian human population of Di Rienzo et al. 1994 showed a clear signal of expansion using 10 markers and 46 individuals. For M_P_VAL, a minimum sample size of 25 diploid individuals is recommended (Garza and Williamson 2001); Guinand and Scribner (2003) detected a bottleneck signal using five microsatellite loci and sample sizes around 50 individuals in six lake trout populations.

Forward-time simulations

Genotypic data were generated by SIMUPOP v1.0.4 (Peng and Amos 2008). A finite island model without migration was considered with 100 subpopulations (replicates) of constant size N and equal sex ratio. We considered two different cases, with $N = 5{,}000$ and $N = 10{,}000$, respectively, which correspond to the lower and upper limit in effective population sizes in our real data, and is also concordant with the study of Wirth and Bernatchez (2003). Each generation a Wright-Fisher model (Random Selection, RS, random single-parent parent chooser with replacement) and a sexual mating scheme (Random Mating, RM, random parents chooser with replacement) were simulated to produce a diploid genotype for 22 independent gene loci for each individual.

Because few microsatellite loci follow a strict (one step) Stepwise Mutation Model (SMM), we used a Two-Phased Model of mutation (TPM), as recommended by Luikart et al. (1998), which consisted of 90% one-step mutations and 10% multi-step changes. Simulations were initiated with the allele with the highest frequency in the real data, with no limit in possible allelic states. Average mutation rate was set to $\mu = 5 \times 10^{-4}$, a more conservative value than the one suggested in fish (10^{-3}; Fraser et al. 2007) in accordance with the lower heterozygosity of EST-derived microsatellites. Relative mutation rate across loci was chosen to approximately reflect the expected heterozygosity and number of alleles observed in the real data. Specifically, we estimated the relative rate for each locus by using the equilibrium relationship between mutation rate, heterozygosity and effective population size (Ohta and Kimura 1973):

$$\mu = \frac{1}{8N_e}\left[\left(\frac{1}{1-H_e}\right)^2 - 1\right],$$

and fixing N_e to a common arbitrary value for all loci. The formula is strictly valid for SMM only, but to our knowledge no analogues are available for TPM. The resulting distribution of relative rates was shifted appropriately to reflect the chosen average rate. Preliminary simulations using $\mu = 5 \times 10^{-4}$ showed that heterozygosities and number of alleles were similar to the values found in the empirical dataset. Further simulations using lower mutation rates (i.e., 10^{-4} and 5×10^{-5}) resulted in lower heterozygosities and too few alleles.

We run each simulation for 50,000 generations before collecting data in order to attain an approximate mutation-drift equilibrium. At generation 50,000 a bottleneck episode was simulated in a single generation, and data were collected during the 10 next generations after the bottleneck. We simulated two different magnitudes of the bottleneck (99%, 90%) for each N ($N = 5{,}000$, $N = 10{,}000$) and for each mating scheme (RM, RS), for a total of eight different scenarios.

A random sub-sample of 50 individuals was analyzed for each post-bottleneck scenario by the softwares BOTTLENECK (all 100 replicates for each scenario), M_P_VAL (all 100 replicates) and LAMARC (10 replicates) in order to assess their power to detect bottleneck episodes of different magnitude. Additionally, simulation results were analyzed using 35 individuals from the simulated data for a more realistic comparison for the

empirical data. Efficiency in bottleneck detection remained similar for samples of 35 individuals (data not shown). BOTTLENECK and M_P_VAL results were combined using the Fisher's method combining probabilities of independent tests (Sokal and Rohlf 1995). Pre-bottleneck samples were also analyzed to ensure equilibrium had been reached and no bottleneck was present.

Results

All samples showed moderate to high levels of genetic variation at 22 microsatellite loci, with no significant differences in H_e, H_o or AR (Table 1; Supplementary Table 1). Comparable values were found across geographic regions for heterozygosities (Tyrrhenian: $H_e = 0.55$–0.59; Adriatic: $H_e = 0.55$–0.57) and allelic richness (Tyrrhenian: AR = 6.1–6.5; Adriatic: AR = 6.0–6.5). When testing the temporal stability of genetic variability, no differences were found for the Tyrrhenian samples in the period 1999–2007 (H_e: $P = 0.909$, AR: $P = 0.885$) or for the Adriatic samples in the period 2001–2007 (H_e: $P = 0.926$, AR: $P = 0.799$). All loci were in Hardy–Weinberg Equilibrium (HWE).

Overall genetic differentiation was low ($F_{ST} = 0.0048$) but highly significant ($P < 0.001$). 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.

A neutrality test was conducted prior to the N_e/bottleneck analyses. Using LOSITAN, no loci were suggested to be subject to balancing selection and only one out of 22 loci (AAN24A09) was suggested to be under directional selection. Locus AAN24A09 was identified as outlier (95% criterion), showing significant larger differences between locations than expected for a neutral locus ($P = 0.011$). However, comparison of allele frequencies across samples showed that temporal genetic differentiation does not involve most loci but only a few ones, and that loci showing temporal genetic differentiation are not identical at each location, suggesting that the observed genetic heterogeneity is not due to selection. Similarly, no signatures of selection were suggested using the lnRH and lnRV tests. Out of 1,012 (22 loci × 46 population pairs) comparisons, only one case was significant for the lnRH test and four cases for the lnRV test (all involving different loci), which was lower than the expected number of false-positives (50.6 in total, 1,012 cases at $\alpha = 0.05$) for each test ($P > 0.05$).

Standardized variance of shifts in allele frequency (F) estimated by the temporal method are reported in Table 2 for all possible adjacent cohorts. An estimated $\bar{F}'$(95% CI) = 0.0042 (0.0016–0.0070) was obtained by averaging F' values across cohort pairs. On the basis of the demographic data we calculated the following values of $G = 8.43$ and $C = 354$, which provide an overall point estimate plus 95% CI of short-term effective population size N_e of 5,031 (2,986–12,810). The high value of C obtained is a consequence of the large variance in age-at-maturation in adults of European eel, which ranges from 3 to 20 years old, and varies between males and females (Vøllestad 1992).

Values of long-term effective population size N_e were inferred by a coalescent-based genealogical method (Table 3). Convergence of chains appeared satisfactory following validation with Tracer. Estimates of θ were of the same magnitude across all samples, ranging from 10.89 (Adriatic-2001) to 20.95 (Tyrrhenian-2002). The estimates obtained and their 95% confidence intervals (CI) were consistent around a value of $\theta = 15$. Using a mutation rate of $\mu = 5 \times 10^{-4}$, long-term N_e values ranged between 5,444 and 10,474, predominantly around a value of $N_e = 8{,}500$. Growth rate was also estimated using the same method. For all samples, point estimates were statistically not different from zero (confidence intervals were centred around zero) suggesting a demographically stationary population (Table 3). For both θ and g, similar estimates were suggested across samples and loci, and also when pooling temporal samples.

The signatures of a recent population bottleneck were tested by comparing the decline in allele number versus heterozygosity (BOTTLENECK), and allele number versus allele size range (M_P_Val) (Table 4). The first approach showed no signs of a bottleneck, as the observed gene diversity in all samples was lower than the expected equilibrium gene diversity (H_{eq}), whereas the opposite scenario ($H_{eq} < H_e$) is expected in a recently bottlenecked population. 10 out of 10 tests (one-way Wilcoxon test for each population sample) showed no significant heterozygosity excess (Table 4), and similar results were obtained when pooling all Tyrrhenian samples ($P = 0.999$), all Adriatic samples ($P = 0.999$) and total samples ($P = 0.999$), which suggests no apparent drop in number of alleles. Similarly, the M-ratio bottleneck test showed no signals of demographic bottleneck after failing to detect a drop in allele size range (Table 4). All samples showed M-ratios close to 0.9, and in all comparisons M-ratios were always significantly above the critical value M_{crit} independently of the θ considered ($\theta = 5$, 10 and 20), indicative of no bottleneck. M-ratio tests are reliable only when the results are consistent regardless of the value of θ used (Guinand and Scribner 2003). M-ratios were also significantly higher than M_{crit} when pooling all

Table 2 Estimator of allele frequency change corrected for sampling error (F') calculated between adjacent cohorts of *A. anguilla*

Locus	Caprolace1999 vs. 2000	Caprolace 2000 vs. 2001	Tiber 2002 vs. 2003	Tiber 2003 vs. 2004	Lesina 2001 vs. 2002	Lesina 2002 vs. 2003	Lesina 2003 vs. 2004
03I14	0.002	−0.007	−0.007	0.007	−0.010	0.002	0.005
06E24	0.009	0.012	−0.003	0.003	−0.004	−0.002	0.003
22D01	−0.010	−0.016	−0.015	−0.004	−0.020	−0.009	0.006
22M07	0.005	0.024	0.002	0.022	−0.023	0.010	−0.004
24A09	0.018	0.020	0.012	0.002	0.030	0.014	0.006
24L24	−0.011	−0.018	0.028	0.015	0.025	0.013	−0.007
26N13	0.001	0.013	0.021	0.001	0.020	−0.005	0.010
33D15	0.009	−0.001	0.006	0.013	0.025	0.010	−0.003
35N10	−0.015	−0.025	0.002	0.008	0.069	0.018	−0.027
41E24	0.006	−0.010	0.006	−0.004	−0.005	0.006	0.007
41F01	−0.018	0.005	0.010	−0.002	−0.015	0.009	−0.014
42O08	−0.010	0.001	0.033	0.015	−0.005	0.007	0.030
44B14	−0.004	−0.007	0.000	0.038	0.008	0.000	0.041
44B22	0.025	−0.008	0.008	0.005	0.009	0.006	−0.002
44E05	−0.010	−0.023	−0.007	0.024	−0.016	0.017	0.001
CT070	−0.009	0.018	0.002	0.001	0.010	0.018	0.016
CT202	−0.003	0.035	−0.002	−0.024	−0.033	−0.005	−0.008
CT347	0.038	0.000	−0.017	0.009	−0.004	0.005	0.051
CT390	−0.011	0.007	0.004	−0.009	0.005	0.034	0.015
CT446	−0.012	0.010	−0.012	−0.025	−0.027	0.045	0.019
CT812	0.002	0.014	−0.009	−0.011	0.049	0.037	0.010
CT859	−0.018	−0.005	0.001	0.004	0.004	0.009	−0.015
Mean	−0.001	0.002	0.002	0.004	0.004	0.011	0.006
SD	0.014	0.016	0.013	0.014	0.025	0.014	0.018

SD standard error

Table 3 Long-term estimates of θ, N_e and growth rate g (including 95% confidence interval) using LAMARC (Kuhner 2006)

Population	θ	95% CI	N_e	95% CI	g	95% CI
Tyrrhenian						
Caprolace-1999	19.02	14.57/28.66	9,509	7,285/14,330	0.12	−0.01/0.31
Caprolace-2000	17.52	13.95/25.56	8,759	6,977/12,781	0.02	−0.02/0.24
Caprolace-2001	16.68	11.73/22.97	8,342	5,864/11,485	0.14	−0.02/0.54
Tiber-2002	20.95	14.08/26.04	10,474	7,038/13,021	0.21	−0.04/0.41
Tiber-2003	14.49	11.79/18.02	7,247	5,893/9,012	0.07	−0.01/0.24
Tiber-2004	17.23	13.50/24.05	8,613	6,752/12,026	0.04	−0.03/0.11
Tiber-2007	19.75	14.47/27.79	9,877	7,238/13,893	0.10	−0.02/0.35
Adriatic						
Lesina-2001	10.89	8.50/13.32	5,444	4,250/6,660	0.03	−0.03/0.17
Lesina-2002	14.94	11.88/20.07	7,468	5,941/10,305	0.01	−0.03/0.09
Lesina-2003	17.94	16.34/19.91	8,970	8,169/9,953	0.01	−0.03/0.10
Lesina-2004	18.82	12.34/29.88	9,412	6,170/14,940	0.04	−0.02/0.32
Lesina-2007	16.68	14.73/21.02	8,342	7,366/10,508	0.02	−0.01/0.19

Tyrrhenian samples ($M = 0.93$, $M_{crit} = 0.75$–0.76), all Adriatic samples ($M = 0.89$, $M_{crit} = 0.76$), and total samples ($M = 0.96$, $M_{crit} = 0.78$–0.79).

With regard to forward-time simulations, a preliminary analysis was conducted to ensure pre-bottleneck simulated samples were at equilibrium and showed no signatures of a

Table 4 Results of the bottleneck analyses

Population	BOTTLENECK	M_P_VAL						
	P (Wilcoxon)	*M*	$M_{crit}/\theta = 5$	*P*	$M_{crit}/\theta = 10$	*P*	$M_{crit}/\theta = 20$	P
Tyrrhenian								
Caprolace-1999	1.000	0.898	0.735	0.996	0.712	1.000	0.688	1.000
Caprolace-2000	1.000	0.915	0.744	0.997	0.723	1.000	0.702	1.000
Caprolace-2001	1.000	0.904	0.732	0.996	0.704	1.000	0.674	1.000
Tiber-2002	0.995	0.907	0.738	0.998	0.712	0.999	0.689	1.000
Tiber-2003	1.000	0.903	0.742	0.994	0.724	0.996	0.705	1.000
Tiber-2004	1.000	0.855	0.737	0.892	0.714	0.895	0.689	1.000
Tiber-2007	1.000	0.902	0.750	0.992	0.732	1.000	0.716	1.000
Adriatic								
Lesina-2001	1.000	0.826	0.733	0.719	0.706	0.930	0.681	0.992
Lesina-2002	1.000	0.840	0.737	0.824	0.711	0.971	0.682	0.998
Lesina-2003	1.000	0.856	0.747	0.869	0.726	0.973	0.712	0.997
Lesina-2004	0.999	0.881	0.729	0.982	0.702	1.000	0.673	1.000
Lesina-2007	1.000	0.909	0.750	0.994	0.736	1.000	0.724	1.000

BOTTLENECK (Piry et al. 1999): *P* value of the one-tail Wilcoxon test for heterozygote excess. M_P_VAL (Garza and Williamson 2001): *M* is the observed average ratio between number of alleles (*k*) and range in allele size (*r*) across loci; M_{crit} is the critical value of *M* estimated after 10,000 simulations and assuming the population to be at equilibrium. For each sample, we performed *M* estimates using $\theta = 5$, $\theta = 10$ and $\theta = 20$

bottleneck. Using LAMARC, all populations were demographically stationary at generation 50,000, with growth *g* values not statistically different from zero. Recovered values of θ were around $\theta = 10$ in the case of populations with $N = 5{,}000$, and around $\theta = 20$ in the case of populations with $N = 10{,}000$, which are congruent with the expected values assuming a 5×10^{-4} mutation rate. Similarly, BOTTLENECK and M_P_Val showed populations to be of constant size at generation 50,0000, with no apparent drop in allele number or allele size range.

Forward-time simulations showed that despite LAMARC estimating the long-term effective population size, the software was able to detect a recent bottleneck (Table 5). When a 99% drop was simulated, all replicates analyzed showed a significant negative *g* value, indicative of a drop in effective population size. In addition, we observed a double-fold drop in the recovered values of θ following the simulated bottleneck in both RS and RM mating schemes ($N = 10{,}000$: from $\theta = 22$–24 to around θ= 10; $N = 5{,}000$: from around $\theta = 15$ to around $\theta = 5$). With a 90% drop, the bottleneck was detected when populations fell from $N = 5{,}000$ to $N = 500$ in both RS and RM mating schemes, but not when populations fell from 10,000 to 1,000 individuals, in which the pre- and post-bottleneck recovered values of θ were around $\theta = 20$.

Finally, forward-time simulations showed that a bottleneck was also noticeable with BOTTLENECK and M_P_VAL (Table 6). A 99% decline was visible with both softwares independently, but the power to pick up the bottleneck signal increased considerably when combining the results of both softwares, with a 100% probability to detect a bottleneck under RS mating scheme, and an 84–89% probability under RM. Combining BOTTLENECK and M_P_VAL, a 90% decline was evident under RS, with an 87% probability for initial $N = 5{,}000$ and a 67% probability for initial $N = 10{,}000$, but not under RM.

Discussion

Our study suggests that the firmly established demographic decline experienced by the European eel has not entailed a genetic bottleneck of the same magnitude, as evidenced by the observation of a moderate to high level of genetic diversity, with a continuous allele distribution and many rare alleles; two bottleneck-detection methods (BOTTLENECK, M_P_VAL) showing no signatures of a bottleneck episode; and a coalescent approach indicating a stationary population with growth values not statistically different from zero, which is confirmed by finding comparable values of short-term ($N_e = 3{,}000$–12,000) and long-term ($N_e = 5{,}000$–10,000) effective population size.

Forward-time simulations using different scenarios in terms of magnitude of the decline, initial population size and mating scheme confirmed that even very large exploited marine fish populations can undergo genetic bottleneck episodes and experience a loss of genetic variability. Forward-time simulations showed that a bottleneck

Table 5 Forward-time simulations

Decline	Change in N	Sample	θ	95% CI	g	95% CI
Random selection						
99%	10,000–>100	Before	22.14	20.72/22.84	−0.001	−0.009/0.038
		After	9.32	8.82/10.06	−0.051*	−0.066/−0.019
	5,000–>50	Before	14.23	13.66/14.93	−0.022	−0.033/0.015
		After	5.34	4.75/5.98	−0.085*	−0.115/−0.047
90%	10,000–>1,000	Before	21.52	20.52/24.38	−0.047	−0.093/0.129
		After	18.80	17.91/20.29	−0.021	−0.025/0.003
	5,000–>500	Before	20.88	20.06/22.18	−0.010	−0.018/0.015
		After	10.89	10.12/11.96	−0.039*	−0.042/−0.007
Random mating						
99%	10,000–>100	Before	24.74	23.49/26.25	0.003	−0.007/0.041
		After	9.30	8.68/10.22	−0.049*	−0.072/−0.018
	5,000–>50	Before	14.59	11.83/14.93	−0.013	−0.023/0.021
		After	6.20	5.35/7.02	−0.066*	−0.111/−0.023
90%	10,000–>1,000	Before	18.57	17.60/19.38	−0.010	−0.016/0.016
		After	19.85	18.79/21.14	−0.013	−0.021/0.006
	5,000–>500	Before	19.93	18.88/20.95	−0.002	−0.018/0.073
		After	5.04	4.70/6.34	−0.079*	−0.124/−0.030

Estimates of θ and growth rate g using LAMARC (Kuhner 2006). Simulations included two mating schemes (Random selection, Random mating), two initial population sizes ($N = 10{,}000$, $N = 5{,}000$) and two magnitudes of the bottleneck (99, 90% decline). Pre- and post-bottleneck samples for comparison were collected at generation 50,000 (Before) and generation 50,005 (After), five generations after the simulated bottleneck. Values reported correspond to mean values from 10 replicates with 95% confidence interval

* Significant growth rate g

Table 6 Forward-time simulations

Decline	Change in N	BOTTLENECK	M_P_Val	Combined
Random selection				
99%	10,000–>100	83	97	100
	5,000–>50	87	97	100
90%	10,000–>1,000	53	31	67
	5,000–>500	65	74	87
Random mating				
99%	10,000–>100	69	73	89
	5,000–>50	67	47	84
90%	10,000–>1,000	12	18	29
	5,000–>500	9	15	24

Probability to detect a bottleneck episode (out of 100 replicates) using the softwares BOTTLENECK (Piry et al. 1999), M_P_Val (Garza and Williamson 2001) and the combination of both using the Fisher's method combining probabilities of independent tests (Sokal and Rohlf 1995). Simulations included two mating schemes (Random selection, Random mating), two initial population sizes ($N = 10{,}000$, $N = 5{,}000$) and two magnitudes of the bottleneck (99, 90% decline)

episode is detectable with bottleneck-detection methods (BOTTLENECK, M_P_VAL), indicating that the failure to pick up the signal of a genetic bottleneck in our case study of the European eel is not due to lack of power. While the simulated data showed the predicted bottleneck signatures (loss of rare alleles and reduction in allele size range), our real data presented (1) rare alleles at 205 out of 220 locus/sample combinations, with a mean of 3.26 rare alleles per loci, and (2) an almost continuous allele size range with a non-significant M-ratio ($M = 0.826$–0.915), which explains the failure of the BOTTLENECK and M-ratio test, respectively.

While LAMARC estimates the long-term effective population size, defined as the harmonic mean N_e over the past $4N_e$ generations (Anderson and Garza 2009), and not the short-term or contemporary effective population size that can be estimated using temporal methods, our simulations show that a severe demographic decline can also be detected with coalescent methods (significant negative g estimates). Furthermore, the drop in N_e in post-bottleneck simulated samples indicates that long-term effective population is also affected by strong demographic declines even if recent. In contrast with the simulated data, our real data showed a static population with non-significant g estimates, suggesting no genetic decline.

Importantly, we obtained similar estimates of short- and long-term N_e using two independent methods based on different approaches and assumptions, the former evaluated using the temporal method with overlapping generations that incorporates data on the demography of the species,

the latter using a coalescent genealogy sampler. Our finding of an N_e of about 3,000–12,000 individuals is consistent with the previous study of Wirth and Bernatchez (2003) that suggested a long-term effective population size of 5,000–10,000 individuals using seven neutral microsatellite loci. Nevertheless, the study of Wirth and Bernatchez (2003) detected an ancient population crash using a different genealogy sampler (MSVAR, Beaumont 1999) that occurred about 20,000 years ago, which was not detected by LAMARC. The differences across studies might be due to the number and nature of the microsatellite markers used, with the study of Wirth and Bernatchez (2003) using genomic microsatellites with heterozygosities exceeding by almost twofold the ones found using EST-derived microsatellites. On the other hand, the differences can be model dependent or due to sampling. In particular, MSVAR has been reported to detect signals of population decline when admixed populations are analyzed (Chikhi et al. 2010), which is the case of the study of Wirth and Bernatchez (2003). Moreover, a preliminary analysis using MSVAR of simulated non-bottlenecked populations sampled after a run of 50,000 generations at constant size also indicated an ancient bottleneck, which suggests that the use of MSVAR was not appropriate for our empirical data.

No apparent fisheries-induced genetic bottleneck in the European eel

Our study shows no signatures of a genetic bottleneck in the heavily exploited European eel, suggesting that the observed demographic decline did not entail a genetic decline of the same magnitude.

Recent genetic studies have challenged the notion that the genetic diversity of exploited fish populations is unaffected by commercial fisheries due to their large stock biomasses, showing instead that collapsed fish stocks may lose neutral genetic diversity. In the exploited New Zealand snapper *Pagrus auratus*, comparison of historical samples from the period 1950–1998 showed a significant drop in number of alleles (from 12.5 to 9.5) and heterozygosities (from 0.76 to 0.73) in a population from Tasman Bay associated with recent signs of overfishing (Hauser et al. 2002). Nevertheless, no evidence of a change in genetic diversity was observed in a population from the more-heavily exploited Hauraki Gulf that only showed random fluctuations in number of alleles and heterozygosities. Despite both populations experiencing a similar reduction of biomass by 85%, population abundance was reduced to about 3.3 million individuals in Tasman Bay, while population size never fell below 37 million individuals in Hauraki Gulf, which could explain why only the former population experienced a genetic effect. Similarly, a decline in number of alleles (from 46 to 37 alleles) was found in North Sea cod *Gadus morhua* during the period 1954–1970 that was paired with a decline in stock biomass due to fishing pressure (Hutchinson et al. 2003). Nevertheless, a similar temporal study showed no drop in genetic diversity in the collapsed Newfoundland cod after experiencing a 99% reduction in biomass in the early 1990s in comparison with its historical maximum in the 1960s (1.6 million tons), which suggests that final population sizes were still large enough that genetic effects caused by declines in population size were unlikely (Ruzzante et al. 2001). Although the decline in the North Sea was not as sharp, it was noted that the minimum size of the population was likely in the order of one magnitude lower than that of the Newfoundland cod, which might explain the discrepancy between studies (Hutchinson et al. 2003).

Hence, it is likely that the genetic pool is only impaired when population size gets past a certain threshold. A population of $N_e = 500$ is considered to be large enough to maintain genetic diversity for key life-history traits, hence genetic diversity would appear depleted only if $N_e < 500$ (Frankham 1995), although it has been argued that a target of $N_e =$ 1,000–5,000 may be a more accurate minimum level for long-term conservation (Lande 1988; Lynch and Lande 1998). It should be taken into account that since the severity of genetic erosion is a function of 1/2 N_e, a serious loss of genetic diversity will only occur when N_e becomes quite small (i.e., $N_e < 50$; Franklin 1980). Furthermore, there might not be strict linear relationships between census size N and N_e over varying N (Palstra and Ruzzante 2008; Luikart et al. 2010). By using 22 EST-linked microsatellite loci we estimated a genetic effective population size roughly between 3,000 an 12,000 individuals. In the case of the European eel, the threshold value of $N_e = 500$ is about 10 times lower than estimated from our data and around the target advocated by Lande (1988). Our results showing a static population with no apparent signatures of genetic bottleneck and moderate to high levels of genetic diversity suggest that the effective population size is large enough to preclude critical losses of neutral genetic diversity, and that a full recovery of the stock is still possible provided that the declining trend is inverted. Furthermore, in constructing models based on eel population data in Camargue lagoons, Bevacqua et al. (2009) noted density-dependent effects on glass eel recruitment limiting effective elver settlement; the shape of the recruitment/settlement relationship reaches a plateau at a carrying capacity of around 1,000 individuals/ha, above which additional input of glass eels has no significant benefit. A similar association was also observed in the Lough Neagh system in Northern Ireland (ICES 2009). This suggests that past (pre-decline) high abundances in recruitment might have not always translated into higher elver settlements, and that despite less glass eels arriving

now in comparison with the 1980s, the number of elvers settling may be the same due to the limited carrying capacity of the system.

Higher census than effective population size

Our findings also confirm the discrepancies between census numbers and effective genetic population size that have been reported for a wide range of marine taxa (Hauser and Carvalho 2008; Palstra and Ruzzante 2008). For marine fish, effective population size might be 2–6 orders of magnitude smaller than census size, with the most extreme differences observed in snapper *Pagrus auratus* (Hauser et al. 2002) and plaice *Pleuronectes platessa* (Hoarau et al. 2005). In the case of the European eel, a census size of about 3×10^9 individuals has been estimated (Dekker 2000), which means that the effective population size of 3,000–12,000 individuals in our study might be five orders of magnitude lower than the census size.

One of the possible explanations for a low effective population size/census size ratio might be the complex spawning and recruitment dynamics in marine species (Hauser and Carvalho 2008). This holds particularly true for the European eel, which has a protracted reproductive season with spawning occurring all throughout the year. By distributing reproductive efforts across seasons, the number of breeders per spawning event could be low despite the high effective population size. Furthermore, it has been hypothesized that the most likely and most important factor reducing N_e in marine species is large variance in reproduction success of adults mediated by spatio-temporal fluctuations in climate and oceanographic processes affecting spawning, fertilization, larval survival, and recruitment (Hendrick 2005; Hauser and Carvalho 2008; Palstra and Ruzzante 2008). Under the sweepstakes reproductive success hypothesis (Hedgecock 1994), many individuals fail to contribute to recruitment and a small fraction of individuals replaces the entire population by a sweepstakes-chance matching of reproductive activity with oceanic conditions. Although individuals in a given species may number in the thousands, if the sweepstakes effect occurs, N_e and the N_e/N ratio might be quite low (Hendrick 2005). Very specific circumstances are required to generate high variance in reproductive success, but it seems plausible in the case of the European eel. The species has to cover 5,000 km in the spawning migration to the Sargasso Sea and get together in an area of c. 1,000 km wide and 3,000 km long, although it has been proposed that distinct temperature fronts may be used as clues to locate spawning grounds (McCleave 1993). In this sense, the observation of genetic patchiness (unpatterned genetic heterogeneity) among intra-annual glass eel samples (arrival waves) using genetic markers (Pujolar et al. 2006, 2007, 2009a; Maes et al. 2007) was attributed to a large variance in the contribution of parents to each cohort determined by genetic drift. Although natural selection and gene flow could also play a role in the pattern of genetic patchiness found, the authors suggested that recruitment differentiation is a consequence of each spawning event involving a restricted number of spawners. While an ideal population ($N_e = N$) assumes random reproductive success, a scenario in which only a subset of adults contribute to spawning would reduce N_e to numbers lower than N.

What caused the eel decline?

It has been argued that the spawning stock biomass of the European eel could be depressed as a consequence of the long decline in yield occurring for decades, and that insufficient spawning stock biomass might have caused the recruitment collapse currently observed (Dekker 2003). However, our genetic data suggest that the number of migrating adults is still large enough so that effective population size is not affected and remains stable over time.

We propose that reduced reproduction success due to poor quality of the spawners rather than low number of spawners is a key factor in explaining the European eel crash. Due to the detrimental effects of continental factors (overfishing, pollution, parasites) on fitness and fecundity, the spawning stock may fail to produce enough good quality eggs, which translates into a decline in the recruitment of the stock. First, fisheries-mediated selection against largest eels could have caused a decline in fecundity and fitness of migrating females that might translate in a lower number of eggs released in the Sargasso Sea during spawning. Second, evidence has been presented that the European eel is prone to bioaccumulating contaminants such as PCBs, pesticides and heavy metals (Robinet and Feunteun 2002; Belpaire et al. 2009). A recent study has shown that gonadal levels of organic pollutants such as dioxin-like PCBs have a detrimental effect on survival and development of eel embryos, arguing that the current level of dioxin-like contaminants seriously impair the reproduction of the European eel (Palstra et al. 2006). Reduced fitness may be exacerbated by oceanic processes inducing changes in primary production in the Sargasso Sea and/or pathway of transport across the Atlantic leading to a higher larval mortality (Knights 2003; Friedland et al. 2007; Bonhommeau et al. 2008).

Conclusions

All statistical methods utilized for inferring demographic history failed to detect the occurrence of a population

bottleneck. Those methods have limitations, including the fact that mutation models are by no means perfect and the mutation rate estimate might be inaccurate, and that simulated mating schemes might be too simplistic. Yet, considering the fact that we find comparable values of short- and long-term effective population size between 3,000 and 12,000 individuals, and that simulations support the empirical data, our main conclusion regarding the absence of a genetic bottleneck of the same magnitude as the demographic decline appears justified. The notion that genetic variability has not been critically lost means that there is still time to safeguard the species if measures are urgently implemented and the decline does not progress further. Although our data suggest a population large enough to maintain genetic variability for important life history traits, conservation efforts should not be relaxed. Reduction in eel exploitation as foreseen by EU regulation 1100/2007 would allow spawning stock enhancement. Ecosystem factors contributing to anthropogenic stress on eels such as habitat alterations, barriers to upstream and downstream migration or deterioration of water quality should be addressed in eel management plans. Finally, quality of spawners should also be evaluated and incorporated into the assessment of the status of the stock and in defining management policies.

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