BULLETIN OF MARINE SCIENCE, 81(2): 297–308, 2007

GENETIC AND MORPHOMETRIC HETEROGENEITY AMONG RECRUITS OF THE EUROPEAN EEL, ANGUILLA ANGUILLA

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ABSTRACT

The recognition of the vulnerability of marine species to environmental conditions throughout their life-cycle has broadened the scope of investigations on factors influencing their population dynamics and demographics. The European eel *Anguilla anguilla* (Linnaeus, 1758) stock is declining rapidly due to overfishing, pollution, habitat degradation, diseases, and oceanic conditions. We analyzed arrival waves of glass eels collected in the Bay of Biscay and the western Mediterranean area during the period 2001–2003. We observed significant differences in length, weight, and condition between Atlantic and Mediterranean samples, and among arrival waves within sites. All samples were screened for genetic variation using ten allozyme and six microsatellite loci. We observed a pattern of genetic patchiness among arrival waves, namely a highly significant genetic differentiation without a temporal grouping of samples. Although natural selection and gene flow could also play a role, we suggest that the pattern observed results from the high variance in reproductive success in each spawning season. A low effective population size might have contributed to the current decline in the abundance of European eel. A precautionary approach to fisheries should be implemented in order to preserve maximal genetic potential to cope with changing anthropogenic and environment pressures.

Severe declines in populations of marine organisms and catadromous species such as European, American, and Japanese eels have prompted considerable recent research on their ecology and conservation. Recruitment abundance of the European eel *Anguilla anguilla* (Linnaeus, 1758) is outside safe biological limits. The number of new glass eels entering rivers declined in the mid-1980s to 10% of former levels and recent figures show that this has now dropped to 1% (Dekker, 2004). This crash occured over the entire European continent with no single, obvious cause. Whereas some anthropogenic factors such as overfishing, migration barriers (dams and hydroelectric power plants), habitat destruction, diseases (EVEX virus), and parasites (the swimbladder nematode *Anguillicola crassus*) (ICES, 2001) maybe influencing eel reproductive success, climate and ocean current change may also be contributing to declining recruitment of the European eel (Knights, 2003; Friedland et al., 2007).

The complex life cycle of the European eel incorporates two migrations across the North Atlantic at the beginning and end of its life (Tesch, 2003). Larvae (leptocephali) migrate with the Gulf Stream and North Atlantic Drift Current from the Sargasso Sea to European and North African shores (Kettle and Haines, 2006). Upon reaching the continental shelf, larvae metamorphose into glass eels and move into freshwater systems. Arrival of glass eels occurs in pulses or groups known as arrival waves (Boëtius and Boëtius, 1989; Tesch, 2003). After a period of intensive feeding of (on average) 7–8 yrs for males and 11 yrs for females, they metamorphose into silver eels and migrate back to the Sargasso Sea, where they spawn once and die (Tesch, 2003).

A better understanding of crucial aspects of its biology, including genetic diversity, may promote effective measures to protect the species. Early genetic studies using allozyme and mitochondrial DNA markers reported no evidence of spatial substruc-

turing (reviewed in Dannewitz et al., 2005), suggesting the existence of a single panmictic population for the European eel. The panmixia hypothesis was challenged by evidence for an isolation-by-distance (IBD) pattern at both allozyme and microsatellite loci, indicating a spatio-temporal separation of spawning populations with some degree of gene flow (Wirth and Bernatchez, 2001; Maes and Volckaert, 2002). Nevertheless, this pattern proved to be unstable over time when temporal replicates were included in the analysis (Dannewitz et al., 2005). Recently, Pujolar et al. (2006) surveyed a total of 11 intra-annual (arrival waves) samples collected at Den Oever in The Netherlands over three consecutive years (2001–2003). The highly significant heterogeneity in genetic composition among arrival waves suggested a pattern of genetic patchiness attributable to variance in parental reproduction linked to fluctuating oceanic and climatic influences. In a broader geographical scale, which included one annual sample at six sites during three consecutive years (cohorts), Maes et al. (2006) reported an isolation-by-time (IBT) pattern. A highly significant correlation was observed between genetic distance and temporal distance in days between cohorts, irrespective of geographic location. Within cohorts, samples showed no IBT but a genetic patchiness pattern.

The aim of the present study was to examine small-scale temporal differentiation by means of analyzing arrival waves of glass eels obtained throughout the year to test whether genetic differences exist between arrival waves at the same location. Samples were collected during two consecutive years at two sampling sites: (1) the western Mediterranean, which has a different arrival period and putative migration route in comparison with the rest of European glass eels (Boëtius and Boëtius, 1989; Friedland et al., 2007); and (2) the Bay of Biscay, an area of particular interest since natural recruitment is more than ten times as high as elsewhere in Europe, with glass eel fisheries in the Biscay area constituting 87% of all European glass eel catches (Dekker, 2003). We compare our results to data from a previous study carried out in The Netherlands in the period 2001–2003 (Pujolar et al., 2006), corresponding to arrival waves in the North Sea. Our sampling scheme allowed us to quantify and partition the amount of genetic variation found into a spatial component (among geographic sites), an inter-annual temporal component (among cohorts within sites), and an intra-annual temporal component (among arrival waves within cohorts).

Material and Methods

In total, 500 glass eels were collected in the period 2001–2004 at two separate geographic locations: (a) Western Mediterranean samples were obtained at the River Ebro delta (40°40′N; 0°40′E) in Northeast Spain, comprising two different arrival waves from 2003 and two from 2004; (b) samples from the Bay of Biscay were obtained at the River Adour (43°30′N; 1°35′W) and the River Loire (47°17′N; 02°12′W) in West France, comprising a total of 5 different arrival waves (Table 1). All individuals were measured for standard length (L) and body weight (W) (Fig. 1). Ricker's (1975) condition factor [$CI = 1000(W/L^b)$] was calculated for each individual, where L is standard length in mm, W is body weight in mg, and b is the slope from the log length-log weight regression for all samples. Differences in morphometric measures among inter- and intra-annual samples were tested by one-way ANOVA and nested ANOVA.

All individuals were analyzed for allozyme variation using Cellulose Acetate Gel Electrophoresis (CAGE). Tissue extraction, electrophoresis, procedures for visualizing proteins, and buffer systems [Tris Glycine (TG) and Tris Malate (TM)] are described in Maes and Volckaert (2002). Seven enzymatic systems were examined: aspartate aminotransferase (AAT-1*, AAT-2*,

Table 1. Summary of all glass eel (*Anguilla anguilla*) samples including country, sampling site, geographical coordinates, sampling date, number of individuals analyzed (N), mean length in mm (L), weight in mg (W), and condition index (CI). Standard deviations in parentheses.

Country	Site	Location	Date	Code	N	L	W	CI
Spain	River Ebro	40°40′N; 0°40′E	13 January 2003	CAT301	60	67.57 (3.89)	275.00 (56.89)	0.88 (0.12)
			25 February 2003	CAT302	60	65.10 (5.28)	226.63 (104.22)	0.78 (0.15)
			28 January 2004	CAT401	50	64.20 (4.36)	202.80 (44.68)	0.76 (0.12)
			25 March 2004	CAT402	50	61.24 (3.38)	134.00 (37.90)	0.58 (0.14)
France	River Adour	43°30′N; 1°35′W	5 December 2001	FR201	100	71.69 (3.81)	410.62 (71.33)	1.11 (0.12)
			24 February 2002	FR202	100	70.23 (4.15)	282.70 (63.45)	0.81 (0.10)
	River Loire	47°17′N; 02°12′W	24 February 2003	FR301	60	76.13 (3.45)	400.07 (102.97)	0.90 (0.18)
			4 March 2003	FR302	60	77.40 (3.33)	376.70 (56.37)	0.81 (0.08)
			14 April 2003	FR303	60	73.05 (3.97)	396.95 (67.79)	1.01 (0.10)

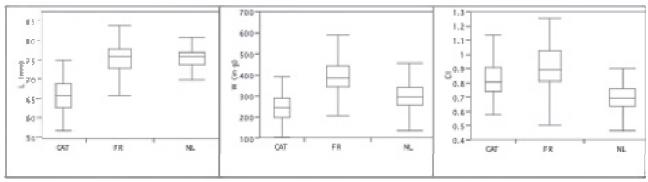

Figure 1. Boxplots representing length (L), weight (W), and condition index (CI) for all 2003 samples from Spain (CAT), France (FR), and The Netherlands (NL; Pujolar et al., 2006).

AAT-3*, EC 2.6.1.1, TM), alcohol dehydrogenase (ADH*, EC 1.1.1.1, TG), glucose-6-phosphate isomerase (GPI-1*, GPI-2*, EC 5.3.1.9, TG), isocitrate dehydrogenase (IDHP*, EC 1.1.1.42, TM), malate dehydrogenase (MDH-2*, EC 1.1.1.37, TM), mannose-6-phosphate isomerase (MPI*, EC 5.3.1.8, TG), and phosphoglucomutase (PGM*, EC 5.4.2.2, TG). Genetic nomenclature followed the suggestions of Shaklee et al. (1990). Allele assignment was carried out comparing the relative distance with the most common allele (*100). The same individuals analyzed with allozymes were screened for microsatellite variation, except for those samples consisting of 100 individuals, in which a sub-sample of 60 individuals was used. DNA Purification and PCR amplification are described in Pujolar et al. (2005). Genotypes were examined at six dinucleotide repeat microsatellite loci: Aan 01, Aan 03, Aan 05 (Daemen et al., 2001), Aro 063, Aro 095, and Ang 151 (Wirth and Bernatchez, 2001). PCR products were visualized on an automated sequencer (LICOR 4200), using a molecular ladder (Westburg) in order to quantify allele sizes. Fragment data were analyzed using Gene ImagIR ver 4.03 (Scanalytics Inc).

Within sample genetic variation was assessed by level of polymorphism ($P_{0.95}$) and observed (H_o) and expected (H_e) heterozygosity per locus using GENETIX version 4.05 (Belkhir et al., 2005), and, allelic richness (AR) using FSTAT (Goudet, 2002). Deviations from Hardy-Weinberg equilibrium, linkage disequilibrium, and differences in allele and genotype frequencies among samples were tested using GENEPOP version 3.4 (Raymond and Rousset, 1995). Partitioning of genetic differentiation into an inter- and intra-annual component was performed using a hierarchical locus-by-locus AMOVA with 10,000 permutations as implemented in ARLEQUIN v. 2.001 (Schneider et al., 2000). Significance levels for multiple simultaneous comparisons were adjusted using the sequential Bonferroni technique (Rice, 1989). Pairwise multilocus comparisons among samples were calculated by Cavalli-Sforza and Edwards' (1967) chord distance and a Neighbor-Joining (NJ) tree was constructed using PHYLIP 3.5 (Felsenstein, 1996). IBD and IBT were tested using single and partial Mantel tests (Mantel, 1967) implemented in GENETIX and FSTAT 3.5, by correlating coastal distance (between sites) and temporal distance (years and days between recruiting cohorts) vs $F_{ST}/1 - F_{ST}$ as suggested by Rousset et al. (1997) and Hendry and Day (2005). Single population effects and variance in recruitment time (3 mo delayed recruitment in the North Sea, Boëtius and Boëtius, 1989) were tested by sequentially removing populations or correcting recruitment time for the Netherlands (approx. 90 d). Average relatedness of all individuals to each other (r, Queller and Goodnight, 1989) was calculated within and among samples at 6 microsatellite loci using the program IDENTIX (Belkhir et al., 2002). Values among groups were compared by one-way ANOVA. All statistical analyses were performed in STATISTICA version 6.0 (StatSoft Inc).

Results

Tests for morphometric variation between Western Mediterranean samples showed significant differences in mean L, W, and CI when comparing all Spain-2003 samples (One-way ANOVA: $P < 0.001$), all Spain-2004 samples ($P < 0.001$), and Spain-2003 (L: 66.33 ± 4.78; W: 250.82 ± 87.06; CI: 0.83 ± 0.14) vs Spain-2004 (L: 62.72 ± 4.16; W: 168.40 ± 53.80; CI: 0.67 ± 0.16) pooled samples (One-way ANOVA: $P < 0.001$;

Fig. 1, Table 1). Comparison of all Bay of Biscay samples showed highly significant differences in L, W, and CI among arrival waves in France-2002 and France-2003 except for W in 2003 (One-way ANOVA: $P = 0.020$). Significant differences were also found when comparing pooled France-2002 (L: 70.96 ± 4.04; W: 346.66 ± 92.98; CI: 0.96 ± 0.18) vs France-2003 (L: 75.53 ± 4.00; W: 391.24 ± 78.21; CI: 0.91 ± 0.15; One-way ANOVA: $P < 0.001$) samples.

Between regions, pooled Bay of Biscay samples (L: 73.12 ± 4.62; W: 367.78 ± 89.02; CI: 0.93 ± 0.17) had higher size and condition (One-way ANOVA: $P < 0.001$) than Western Mediterranean samples (L: 64.69 ± 4.84; W: 213.35 ± 84.37; CI: 0.76 ± 0.17). When using the 2003 samples for comparison, Mediterranean samples were smaller in size than Atlantic samples (One-way ANOVA: $P < 0.001$), especially in W (Mediterranean: 250.82 ± 87.06 mg; Atlantic: 391.24 ± 78.21 mg). While the minimum W observed in Atlantic samples was 173 mg, 16.7% of all Mediterranean samples were between 102–173 mg. Similarly, while the maximum L observed in Mediterranean samples was 75 mm, 72.2% of all Atlantic samples measured between 75–83 mm.

A Nested ANOVA showed significant differences within and among cohorts ($P < 0.001$) in L, W, and CI for the Western Mediterranean, and in L and W for the Bay of Biscay, which showed larger differences in CI among arrival waves within cohorts ($P < 0.001$) than among cohorts ($P = 0.150$). When comparing all 2003 samples, there were more differences in CI among sites ($P < 0.001$) than among arrival waves within sites ($P = 0.052$), and, slightly larger differences in L among sites ($P < 0.001$) than within sites ($P < 0.010$).

Overall tests for Hardy-Weinberg proportions with all allozyme loci and for linkage disequilibrium among all loci showed no significant departures from expected values. At microsatellite loci, only 2 out of 54 tests for Hardy-Weinberg equilibrium revealed significant deviations after Bonferroni correction, which were attributed to a heterozygote deficit at locus Aro 095 in one Western Mediterranean and one Bay of Biscay sample. Dannewitz et al. (2005) suggested the presence of null alleles at locus Aro 095 but proved that null alleles did not affect the results obtained after redoing all analyses with and without this locus. Pooled samples from the Bay of Biscay showed higher genetic variation at allyzomes ($H_e = 0.154 \pm 0.185$, $P_{0.95} = 0.060$, AR = 2.90) than pooled samples from the Western Mediterranean ($H_e = 0.140 \pm 0.175$, $P_{0.95} = 0.050$, AR = 2.45), although differences were not statistically significant (Table 2). At microsatellites, pooled samples from the Bay of Biscay showed higher heterozygosities ($H_e = 0.720 \pm 0.288$) than pooled Western Mediterranean samples ($H_e = 0.713 \pm 312$), while AR for Bay of Biscay samples (11.4–12.5) was higher than that for Mediterranean samples (10.6–11.3), although not significantly so (Table 2).

Overall genetic differentiation among samples at microsatellite loci was low but highly significant ($F_{ST} = 0.003$, $P < 0.001$). Genetic differentiation partitioned significantly among samples within sites ($F_{SC} = 0.0042$, $P = 0.003$), while no significant differentiation was found among sites ($F_{CT} = -0.008$, $P = 0.774$). At allozymes we observed a lower differentiation ($F_{ST} = 0.001$) which was marginally non-significant ($P = 0.053$). All tests for either IBD or IBT showed a lack of correlation between genetic and geographic distance (allozymes: $r = 0.240$; $P = 0.140$; microsatellites: $r = 0.113$; $P = 0.491$) and between genetic and temporal distance (allozymes: $r = 0.018$; $P = 0.915$; microsatellites: $r = 0.004$; $P = 0.978$). Figures 2 and 3 show the constructed NJ trees based on allozyme and microsatellite data, respectively, which include five additional samples from the Netherlands (Pujolar et al., 2006) for comparison. No evidence

Table 2. Genetic diversity estimates for all samples including expected heterozygosities (H_e), polymorphism ($P_{0.95}$), and allelic richness (AR). Standard deviation (SD) in parentheses. Sample abbreviations as in Table 1.

		Allozymes			Microsatellites		
Sample	N	H_e	$P_{(0.95)}$	AR	H_e	$P_{(0.95)}$	AR
CAT301	60	0.145 (0.155)	0.060	2.428	0.742 (0.280)	1.000	11.284
CAT302	60	0.148 (0.173)	0.050	2.554	0.690 (0.294)	1.000	11.050
CAT401	50	0.135 (0.174)	0.050	2.588	0.721 (0.251)	1.000	10.640
CAT402	50	0.129 (0.149)	0.060	2.296	0.693 (0.309)	1.000	10.762
FR201	100	0.156 (0.163)	0.060	2.931	0.733 (0.266)	1.000	11.816
FR202	100	0.170 (0.154)	0.070	3.224	0.733 (0.258)	1.000	12.518
FR301	60	0.144 (0.164)	0.060	2.528	0.708 (0.280)	1.000	11.642
FR302	60	0.156 (0.182)	0.050	2.955	0.729 (0.241)	1.000	11.391
FR303	60	0.133 (0.161)	0.050	2.480	0.723 (0.270)	1.000	11.548

was found for any grouping, and samples clustered together without a geographic or temporal pattern.

All samples showed low mean values of relatedness, ranging from −0.074 to 0.029 in the Western Mediterranean, and from −0.046 to 0.027 in the Bay of Biscay (Table 3). Most individuals showed r values between −0.4 and 0.2, which suggests that arrival waves are composed of a majority of unrelated individuals. Despite the low mean relatedness, all samples included about 5% of highly related individuals with mean r values > 0.5.

Discussion

A Pattern of Genetic Patchiness Among Arrival Waves.—The fundamental result from this study is the highly significant genetic differentiation among samples from different arrival waves despite the low observed F_{ST} values. Hierarchical F_{ST} and Neighbor-Joining tree analyses revealed a pattern of genetic patchiness, in which samples did not cluster together according to cohort origin or arrival time within the year. Genetic heterogeneity is likely to result from a large variance in the contribution of individuals to each spawning event determined by genetic drift. Hedgecock (1994) hypothesized that reproduction of marine organisms is mediated

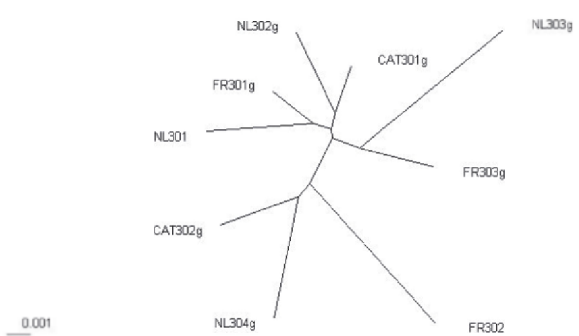

Figure 2. Neighbor-Joining tree based on Cavalli-Sforza and Edwards (1969) chord distance using allozyme loci among all 2003 European eel samples including Spain (CAT), France (FR), and The Netherlands (NL; Pujolar et al. (2006). Sample abbreviations as in Table 1.

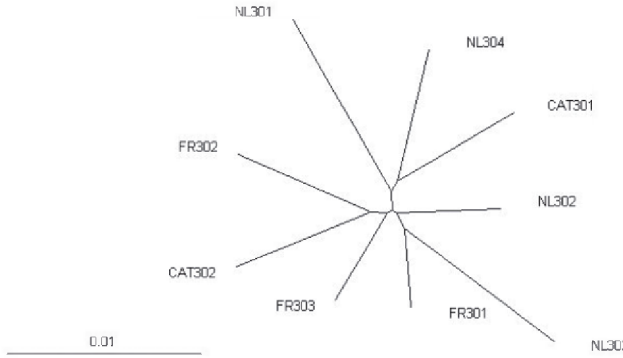

Figure 3. Neighbor-Joining tree based on Cavalli-Sforza and Edwards (1969) chord distance using microsatellite loci among all 2003 European eel samples including Spain (CAT), France (FR), and The Netherlands (NL; Pujolar et al., 2006). Sample abbreviations as in Table 1.

by spatio-temporal fluctuating oceanographic processes affecting spawning, fertilization, larval survival, and recruitment. Under the sweepstakes reproductive success hypothesis, 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. How many and which individuals is determined by chance events. The genetic consequences of a large variance in parental contribution would be the induction of an unpatterned temporal genetic heterogeneity among recruits (genetic patchiness). Several studies have detected genetic patchiness among recruits on a small spatial scale, 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).

Hedgecock (1994) proposed that genetic patchiness reflects the family structure of the larval pools. In European eel, our data suggest a significant genetic heterogeneity both among cohorts and among samples within cohorts, which might be the result of random genetic drift. Genetic relatedness was calculated in all samples to give an indication of the family structure of the arrival waves. A general pattern was observed in the 2002 and 2003 cohorts in which samples consisted of individuals with high values of relatedness mixed with a large group of unrelated individuals. This suggests that arrival waves are composed in part by relatives. As expected in the case of the European eel, larval mixing can occur during the long leptocephali migration from the Sargasso Sea to the European continental shelf and also during the glass eel phase in continental waters, which could explain the low mean relatedness observed for most of the samples.

Table 3. Mean genetic relatedness (r) within each sample including maximum (max r) and minimum (min r) relatedness for all samples. Standard deviations in parentheses. Sample abbreviations as in Table 1.

Relatedness	CAT301	CAT302	CAT401	CAT402	FR201	FR202	FR301	FR302	FR303
Mean r	−0.074	0.007	0.007	0.029	0.014	−0.019	0.027	−0.046	−0.007
	(0.261)	(0.258)	(0.256)	(0.302)	(0.253)	(0.259)	(0.232)	(0.257)	(0.236)
Max r	1.000	1.000	0.897	1.000	0.880	0.844	1.000	1.000	1.000
Min r	−0.885	−0.516	−0.557	−0.895	−0.644	−0.540	−0.451	−0.604	−0.518

Alternatively, genetic heterogeneity among recruits might be due to the different selective histories of the larval pools or gene flow between genetically differentiated populations (Larson and Julian, 1999). Under selection, genetic composition mostly shows clines in allele frequencies that parallel environmental gradients (Koehn et al., 1980; Oakeshott et al., 1982, 1983; Bernardi et al., 1993). Thus, the temporal genetic patchiness observed in our study, with recruit composition varying over time, seems to suggest a lack of consistent selection. The gene flow hypothesis implies the existence of discrete genetic populations, which contradicts the subtle population structure suggested by genetic data (Daemen et al., 2001; Wirth and Bernatchez, 2001; Maes and Volckaert, 2002; Dannewitz et al., 2005; Maes et al., 2006). Although natural selection and gene flow could also play a role in the observed genetic pattern, we suggest that large variance in reproductive success is a contributing factor to the recruit differentiation.

In our study, no significant correlation was observed between genetic distance and temporal distance (measured as difference in days between arrival waves), suggesting that differentiation among arrival waves was not attributable to an IBT pattern. In contrast with the lack of a seasonal IBT pattern in our data, Maes et al. (2006) detected a clear IBT pattern between spawning cohorts differing by 2–3 yrs. IBT on a broader scale and genetic patchiness on a small scale are not contradictory but a consequence of the long migration loop in the European eel and a large variance in adult reproductive success most likely due to stochastic effects.

Morphometric Heterogeneity.—Our study shows significant heterogeneity in size and condition between Western Mediterranean and North Atlantic samples, with the sampled Mediterranean larvae generally smaller than the sampled Atlantic larvae. Mediterranean samples were also smaller in comparison with arrival waves collected in the estuary of the Burrishoole river system in western Ireland during 1987–1988 (Poole et al., 2004), in which all monthly mean L values (70–74 cm) were higher than the maximum mean L (67.57 ± 3.89 cm) found in the Mediterranean. Boëtius and Boëtius (1989) proposed that a different migration route might account for the smaller size of Mediterranean individuals. Despite the circulation patterns and oceanic currents being complex and poorly understood, it is well-documented that leptocephali larvae are transported along the Gulf Stream and the North Atlantic Current for a journey of at least 6–9 mo to the eastern Atlantic coast (Lecomte-Finiger, 1994; Arai et al., 2000), with the main bulk of larvae arriving in the Bay of Biscay area (Dekker, 2003). As the Gulf Stream turns into the North Atlantic Current, a second branch termed the Azores Current, first flows southeast towards the Mid-Atlantic ridge, and then eastwards until it nears the African coast (Mann, 1967). Here it meanders eastward towards the Gulf of Cadiz, where some of its water is entrained in the outflow of Mediterranean water at Gibraltar (Johnson and Stevens, 2000). A shorter transatlantic journey for Mediterranean individuals was also indicated by the modelling study of Kettle and Haines (2006).

By comparison, samples obtained in Den Oever (The Netherlands) in the same period as this study (Pujolar et al., 2006), were of similar length to those from the Bay of Biscay but significantly smaller in terms of weight and condition. Such differences might be attributable to the longer distance to be travelled by non-feeding glass eels from the edge of the continental shelf to The Netherlands. Furthermore, glass eels in the colder waters of the North Sea must wait offshore until temperatures rise to approximately 6 °C before ascending the rivers (Boëtius and Boëtius, 1989). In The

Netherlands this period has been estimated to last about 3 mo (Desaunay and Guerault, 1997), which might explain the lower condition observed, as larvae must survive on their stored energy reserves for that time.

Highly significant differences in L, W, and CI were also observed within sites, both among arrival waves within years and among years. A significant loss in size and condition was observed during the arrival period in the Bay of Biscay (2002) and the western Mediterranean (2003 and 2004), which is concordant with the significant decreases throughout the season in L, W, and CI observed by Poole et al. (2004). Desaunay and Guerault (1997) proposed that seasonal changes throughout the year were probably directly linked to feeding conditions, with larvae migrating across the ocean during plankton production peaks reaching a higher size and condition.

In summary, we detected a significant size and genetic heterogeneity among arrival waves of European eel. The observed genetic patchiness (highly significant genetic differentiation among arrival waves without a temporal pattern) is most likely due to stochastic effects, although natural selection and gene flow could also play a role. A direct implication of our results is that if only a subset of the adults contribute to spawning, the effective population size in European eel might be considerably lower than the census size. Unfortunately, the calculation of the effective population size remains complex in the European eel, as adults mature at variable ages (from 6–40 yrs), making a reliable calculation of effective population size highly speculative. A proper calculation would require the analysis of historical (long-term time series) samples and a larger number of samples and markers. Together with anthropogenic (overfishing, pollution, diseases) and oceanic factors, a low effective population size might have contributed to the current decline in the abundance of European eel. A precautionary approach to fisheries should be implemented (as currently being advised, FAO, 2001; ICES, 2001) in order to avoid the loss of genetic diversity and preserve maximal genetic potential to cope with changing anthropogenic and environment pressures.

Acknowledgments

We thank the personnel at the Parc Natural del Delte de l'Ebre (Spain), CEMAGREF Bordeaux (France) and Royaal BV (The Netherlands) for assistance with sampling. Research was funded by the EU contract EELREP (Q5RS-2001-01836). J. M. Pujolar received a postdoctoral fellowship from the Ministerio de Educación, Cultura y Deporte (Spain). G. E. Maes received a Ph.D. fellowship from the I.W.T. (Institute for the Promotion of Innovation by Science and Technology in Flanders) and is now a post-doctoral researcher funded by the FWO (Scientific Research Fund Flanders).

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