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Population genetics of the American eel (*Anguilla rostrata*): F_{ST} = 0 and North Atlantic Oscillation effects on demographic fluctuations of a panmictic species

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Abstract

We performed population genetic analyses on the American eel (*Anguilla rostrata*) with three main objectives. First, we conducted the most comprehensive analysis of neutral genetic population structure to date to revisit the null hypothesis of panmixia in this species. Second, we used this data to provide the first estimates of contemporary effective population size (N_e) and to document temporal variation in effective number of breeders (N_b) in American eel. Third, we tested for statistical associations between temporal variation in the North Atlantic Oscillation (*NAO*), the effective number of breeders and two indices of recruit abundance. A total of 2142 eels from 32 sampling locations were genotyped with 18 microsatellite loci. All measures of differentiation were essentially zero, and no evidence for significant spatial or temporal genetic differentiation was found. The panmixia hypothesis should thus be accepted for this species. N_b estimates varied by a factor of 23 among 12 cohorts, from 473 to 10 999. The effective population size N_e was estimated at 10 532 (95% CI, 9312–11 752). This study also showed that genetically based demographic indices, namely N_b and allelic richness (*Ar*), can be used as surrogates for the abundance of breeders and recruits, which were both shown to be positively influenced by variation during high (positive) *NAO* phases. Thus, long-term genetic monitoring of American glass eels at several sites along the North American Atlantic coast would represent a powerful and efficient complement to census monitoring to track demographic fluctuations and better understand their causes.

Keywords: American eel, conservation biology, demographic variation, North Atlantic Oscillation, genetic diversity—empirical, fisheries, wildlife management

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Introduction

The extent of population structure within a given species is ultimately determined by the interactions between mutation, migration, genetic drift and selection (Wright 1931). At the proximal level, the balance reached between these forces is determined by ecological factors, such as demography and potential for dispersal as well as the scale and temporal stability of the environment used by a given species to complete its life cycle (Waples & Gaggiotti 2006). Population genetics likely reflects the balance between these counteracting ecological processes, and studies of highly mobile marine fishes have provided evidence for population structure associated with either geographic distance, heterogeneity of oceanographic features or environmental gradients (e.g. Roques *et al.* 2001; Pelc *et al.* 2009). Yet, the extent of observed structuring in marine species is typically very weak even over large geographic distances (e.g. DeWoody & Avise

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2000) and/or temporally and spatially unstable (Hogan *et al.* 2010). Paradoxically, there is growing evidence for the occurrence of meaningful adaptive differences within marine species over relatively short geographic distances, despite the lack of neutral genetic differentiation (Hutchings *et al.* 2007). Interpreting the biological significance of population structure revealed by molecular markers thus remains particularly challenging in marine fishes (Knutsen *et al.* 2011). Yet, knowledge of genetic population structure is crucial for improving the realism of neutral models used to examine adaptive genetic variation as well as for optimizing conservation and management strategies of exploited species.

In addition to understanding population structure, knowledge of the factors influencing demographic fluctuations is also essential for predicting recruitment and for sound management plans of exploited species. Among the many possible causal factors, changes in population abundance of marine species in the Atlantic Ocean have shown significant relationships with climatic and oceanographic variability (Stenseth *et al.* 2003). In particular, the North Atlantic Oscillation (*NAO*), a climatic phenomenon in the North Atlantic resulting from unpredictable temporal fluctuations in the local difference of atmospheric pressure at sea level between the Icelandic low and the Azores high (Hurrell & Deser 2009), has been linked to changes in species assemblages and fluctuations in abundance of several North Atlantic species (e.g. Alheit & Hagen 1997; Attrill & Power 2002; Ottersen *et al.* 2001, 2010).

While it may prove difficult to directly estimate population census size, especially in broadly distributed species, genetic methods can provide valuable information on fluctuations in population abundance (Luikart *et al.* 2010). Molecular markers can be used to estimate the effective population size (N_e), calculated over a time frame of one generation and the effective number of breeders (N_b), which is the effective number of breeders estimated over a unique reproductive event. N_e and N_b can in turn indirectly provide information on fluctuations in census sizes under some circumstances (Antao *et al.* 2011). Moreover, N_e itself is one of the most important parameters in both evolutionary biology (Charlesworth 2009) and conservation biology (Frankham 2005). N_e or N_b estimates using molecular methods are most efficient and precise for relatively small populations but may prove very difficult to obtain for large populations (Waples & Do 2010). However, meaningful estimates of N_e have previously been obtained for exploited marine species (reviewed in Hauser & Carvalho 2008).

Interpreting the biological meaning of weak genetic differentiation has been particularly contentious and yet most relevant in the two Atlantic eels of the genus *Anguilla*: the European (*Anguilla anguilla*) and the American (*A. rostrata*) eel. Based on the species' ecological characteristics, namely random larval dispersal and occurrence of a single spawning area, it has long been implicitly assumed that both Atlantic eels represent truly panmictic species (Tesch 1977). Indeed, previous genetic studies revealed very weak signals of genetic differentiation (F_{ST}) in both species, with species-scale global F_{ST} values measured by microsatellites typically being <0.005 (literature reviewed in the Discussion). Compared with the European eel, relatively modest effort has been devoted to the population genetics of American eel, and thus far the null hypothesis of panmixia has remained unchallenged (Avise *et al.* 1986; Wirth & Bernatchez 2003). Arguably, however, the number of genetic markers used, the number of sampled individuals and sampling coverage were all relatively modest in previous studies on the American eel. Moreover, samples typically comprised specimens of different life stages, which has cast some doubt on the potential of these studies to detect weak but meaningful genetic structure. Also, the temporal stability of genetic variation has never been tested in the American eel.

The global abundance of the American eel as well as European eel has been declining during the past 30 years. There are indications that abundance of all stages of eel (glass eel, yellow eel and silver eel) remains at a historical low level. For example, over the last 5 years, glass eel recruitment of the European eel has averaged between <1% (continental North Sea) and 5% (elsewhere in Europe) of the 1960–1979 levels (ICES 2011). Numerous factors are suspected to be responsible for the steep decline of Atlantic eels, among which global oceanic changes could be particularly important (e.g. Castonguay *et al.* 1994; Friedland *et al.* 2007). Indeed, studies on the European eel have documented a negative correlation between glass eel recruitment and the *NAO* (Knights 2003). For the American eel, the *NAO* has been hypothesized to influence local recruitment and year-class strength structure (Sullivan *et al.* 2006). However, there has been no attempt to associate demographic fluctuations of this species with changes in oceanic conditions reflected by the *NAO*.

In this context, this study had three main objectives. First, we conducted the most comprehensive analysis to date of neutral genetic population structure in American eel to revisit the null hypothesis of panmixia in this species. Second, we used this data to provide the first estimates of contemporary effective population size (N_e) and to document temporal variation in the effective number of breeders (N_b) for the American eel. Third, we tested for statistical associations between temporal variation in the *NAO*, effective number of breeders and two indices of recruit abundance.

Materials and methods

Sampling

American glass eels recruitment begins in Florida around December and progresses northward to Newfoundland–Labrador until June–July (Helfman *et al.* 1987). The first waves of glass eels at each location were sampled in 2008 following this latitudinal trend at 17 sites evenly distributed along eastern North America up to the St. Lawrence estuary (Table 1). For each location, 50 individuals were measured and preserved in 95% ethanol. Yellow eels were also collected between May and September 2008 at 15 locations ranging from the upper St. Lawrence River to the Atlantic coast of Canada (Table 2). The emphasis on yellow eel sampling in this region was motivated by the occurrence of strikingly different recruitment trends reported between Atlantic Canada versus the upper St. Lawrence River and Lake Ontario (Cairns *et al.* 2008). Sample size varied from 69 to 100 yellow eels per location (Table 2, Table S2, Supporting information). Fin clips were preserved in 95% ethanol for DNA extraction and genotyping, and heads were kept for otolith extraction.

Table 1 Description of sampling locations (origin) and dates of capture, mean body measurements and sample sizes for glass eels

Lat./long.	Origin	Tributary	Capture	Length (mm)	Mass (mg)	Sample
30.02N–81.33W	Florida	Guana River Dam	28 Jan 2008	53.2 ± 2.9	106 ± 16	50
31.31N–81.47W	Georgia	Mornings-AR	8 Jan 2008	52.9 ± 3.7	106 ± 43	50
32.93N–80.01W	South Carolina	Cooper River	13 Feb 2008	52.9 ± 2.6	111 ± 25	50
34.77N–76.81W	North Carolina	Black Creek	5 Feb 2008	55.2 ± 2.7	n/a	50
37.22N–76.49W	Virginia	Wormley Creek York	28 Mar 2008	57.5 ± 3.4	130 ± 27	50
38.59N–75.29W	Delaware	Millsboro Pond Spillway	5 Mar 2008	59.5 ± 3.6	189 ± 39	50
39.56N–74.58W	New Jersey	Patcong Creak Linwood	4 Apr 2008	59.2 ± 3.6	170 ± 38	50
40.05N–74.98W	Pennsylvania	Crum Creek	5 May 2008	57.7 ± 3.0	157 ± 32	50
41.30N–72.40W	Connecticut	Tayler River	5 May 2008	57.9 ± 3.1	147 ± 28	50
41.68N–70.92W	Massachusetts	Parker River	16 Apr 2008	56.9 ± 2.9	142 ± 27	50
42.93N–70.86W	New Hampshire	Tayler River	23 Apr 2008	61.1 ± 3.1	160 ± 30	50
43.84N–69.65W	Maine	West Harbor Pond	1 Jun 2008	61.5 ± 4.0	135 ± 30	50
44.59N–64.17W	Nova Scotia	Mira River	25 Apr 2008	61.8 ± 3.2	n/a	50
			15 May 2007	n/a	n/a	50
48.78N–67.69W	Québec	Grande-Riviere-Blanche	14 Jun 2008	64.9 ± 3.3	195 ± 27	50
46.43N–63.24W	Prince Edward Island	Rustico Bay	8 Jun 2008	64.9 ± 3.8	191 ± 56	50
47.84N–59.26W	Newfoundland	Codroy River	1 Jul 2008	62.9 ± 2.9	n/a	50

Table 2 Description of sampling locations and date of capture, mean body measurements and sample sizes for yellow eels

Lat./long.	Origin	Tributary	Capture	Length (mm)	Mass (g)	Sample
45.01N–74.79W	Ontario	Moses-Saunders Dam	17 Jul 2008	367.6 ± 75.9	72 ± 58	100
			20 Jun 2007	402.1 ± 77.5	98 ± 68	76
45.31N–73.90W	Québec	Beauharnois	19 Aug 2008	308.2 ± 76.5	41 ± 40	100
45.44N–73.26W	Québec	Chambly	24 Jul 2008	317.1 ± 76.1	43 ± 45	100
48.28N–68.95W	Québec	Rivière Sud-Ouest	1 Jun 2008	224.6 ± 31.4	13 ± 7	82
49.52N–67.28W	Québec	Rivière Petite Trinité	16 Jul 2008	133.5 ± 55.5	4 ± 6	80
48.82N–64.83W	Québec	Rivière St-Jean	1 May 2008	407.2 ± 41.0	90 ± 28	100
47.52N–64.91W	New Brunswick	Tracadie River	3 Jun 2008	499.9 ± 35.5	183 ± 34	80
47.09N–65.22W	New Brunswick	Miramichi Estuary	28 May 2008	403.0 ± 153.9	154 ± 203	401
			4 Sep 2007	494.1 ± 104.1	223 ± 162	01
45.87N–66.15W	New Brunswick	Grand-Lake	7 Sep 2008	423.6 ± 28.1	129 ± 24	80
44.36N–64.46W	Nova Scotia	La Have River	15 Jun 2008	429.8 ± 33.3	123 ± 32	80
45.84N–60.80W	Nova Scotia	Bras d'Or Lake	1 Jun 2008	475.3 ± 58.8	154 ± 66	80
46.43N–61.10W	Nova Scotia	Margaree Harbour	12 Sep 2008	n/a	n/a	69
47.60N–53.26W	Newfoundland	Roberts Bay	5 Sep 2008	n/a	n/a	100
47.85N–59.26W	Newfoundland	Codroy Valley	18 Aug 2008	453.2 ± 22.2	141 ± 22	100
46.43N–63.24W	Prince Edward Island	Rustico Bay	20 Aug 2008	430.5 ± 33.1	132 ± 32	80

Age determination

From the 2146 samples, age using otoliths (Tremblay 2009) was determined on 946 yellow eels (2008). Other samples were either glass eels (850) or yellow eels (346) from previous sampling (2007) for which otoliths were not conserved or were lost or degraded. Thus, nearly 75% of all yellow eels could be subdivided into annual cohorts. Sagittal otoliths were extracted, stored in glass vials in a 95% ethanol : glycerine solution (1: 1 ratio), and cleaned with successive baths of bleach, water and 95% ethanol. Once dried, otoliths were embedded in a mix of epoxy resin and hardener (4 : 1 ratio) inside gelatine capsules for 24 h, ground to the core on the sagittal plane and polished with alumina powder on a polishing disc. Sections were etched, decalcified in 5% *EDTA* for annuli enhancing, stained in 0.01% toluidine blue solution and digitally photographed (e.g. Tremblay 2009). The first annulus after the dark central nucleus was considered as the elver check (metamorphosis from leptocephalus larva to glass eel) and subsequent annuli as winter checks (e.g. ICES 2011). Eels were considered by convention to be of age 0+ in their year of arrival in continental waters, and their 'cohort year' was thus defined as so. Each otolith was aged twice by two eel experts to confirm aging. A total of 946 yellow eel otoliths were readable, representing 17 different cohorts, each comprising 1–127 individuals.

DNA extraction, PCR and genotyping

Genomic *DNA* was extracted from about 50 μg of tissue using a salt-extraction protocol (Aljanabi & Martinez 1997). A total of 26 microsatellite loci were tested and optimized on a subset of samples (Table S1, Supporting information) using the same multiplex protocol developed and used for the European eel by Als *et al.* (2011). Single and multiloci amplifications were carried out using the Promega amplification kit following the manufacturer's protocol and performed in simplex and multiplex combinations. The 15 μL reaction volume comprised 500 ng genomic *DNA*, 5 U of GoTaq Flexi *DNA* Polymerase (Promega, Madison, WI, USA), 10 μM of each forward and reverse primer, 3 μL of 5× *PCR* buffer, 10 mM *dNTPs*, 25 mM $MgCl_2$ and brought to volume with Milli-Q water. *PCR* amplification parameters included an initial denaturation at 95 °C for 5 min followed by 30 cycles of denaturation, annealing and extension fixed at respectively 94 °C for 30 s, 57 °C for 90 s, and 72 °C for 60 s and a final extension at 72 °C for 30 min. *PCR* products were then diluted (1 : 100) and mixed with formamide and LIZ molecular scale (1 : 10 : 1). The mixture was denatured at 95 °C for 2 min and quickly chilled for 4 min on ice before migration on an ABI3100 sequencer (Applied Biosystems, Foster City, CA, USA). *PCR* fragment size was calibrated using GeneScan software (ABI) and visualized and scored with GENOTYPER 3.7 (ABI). All genotypes were scored twice by two independent experienced people; genotype scoring disagreements were resolved by rescreening the sample, and *PCR* reruns to obtain a consistent genotype database. Binning and allele scoring were automatically processed with ALLELOGRAM software (Morin *et al.* 2009). Among the 26 microsatellite markers that were initially optimized, 21 were genotyped after amplification and scoring checking.

We used the MICRO-CHECKER software (Van Oosterhout *et al.* 2004) to detect large allele dropouts, null alleles and scoring incongruities based on the whole-genotype matrix. Statistical significance ($\alpha = 0.05$) was adjusted for multiple comparisons using Bonferroni correction. We finally retained 18 of the 21 microsatellite markers that conformed to Hardy–Weinberg equilibrium proportions for all subsequent analyses (Table S3, Supporting information). The statistical power of the data set was then evaluated with the POWSIM software (Ryman & Palm 2006). Our microsatellite genotype database is available on DRYAD (doi: 10.5061/dryad.39jb0).

Population genetics analyses

We computed standard genetic parameters, including the observed numbers of alleles (n_a), allele richness (*Ar*, standardized to $n = 36$), observed (H_o) and expected (H_e) heterozygosities, F_{IS}, and global and pairwise F_{ST} values using the ARLEQUIN 3.5 software (Excoffier & Lischer 2010). We performed different *AMOVA* to assess the component imputable to genetic variance among life stages, sampling sites and cohorts. Isolation by distance (*IBD*) was tested within single cohorts of glass eels (because the largest geographic range was covered for this life stage) using a Mantel test and linear regression between pairwise genetic distance as measured by $F_{ST}/(1 - F_{ST})$ (Rousset 2000) and pairwise coastal geographic distance. Isolation by time (*IBT*) was tested among yellow eel cohorts by linear regression between pairwise genetic distance and temporal distance in years. Statistical analyses were performed with SYSTAT 13 (Cranes Software International Ltd., Chicago, IL, USA).

Effective number of breeders (N_b) and effective population size (N_e)

We estimated the effective number of breeders (N_b) for each of 12 annual cohorts, including 10 yellow eel cohorts defined by otolith age determination and two glass eel cohorts (sample size ranging from 32 to 145), to document temporal fluctuations in N_b. The linkage disequilibrium (*LD*) method (Hill 1981), implemented in the N_e

ESTIMATOR software (Queensland Government 2004), was used. We also used the LDN_e software (Waples & Do 2008), which generally generated larger confidence intervals, often including infinity (results not shown). As we were mainly interested in variation among annual cohorts—and no population structuring was detected (see Results)—it was not relevant to apply either temporal estimation or methods considering migration between structured populations. For the sake of comparisons with other studies, the total effective population size per generation (N_e) was estimated by $N_e = g \times \tilde{N}_b$, where g = generation length and $\tilde{N}_b$ is the harmonic mean of N_b across years (Waples 2005). The mean generation length (g) of the American eel in the northern range was previously approximated to be 17 years (e.g. Tremblay *et al.* 2006). However, since the maximum of abundance is found in southern United States where mean silver eel age may drop to 3–4 years, it is reasonable to estimate a mean overall generation time of 8 years.

Year class strength index

We estimated a year class strength index (*YCSI*) based on an annual yellow eel monitoring of the Sud-Ouest River from 1999 to 2008. The survey has taken place over the entire upstream migration period, from June to September (Verrreault and collaborators, unpublished data from Ministère des Ressources Naturelles et de la Faune). Using collection grid based on length classes was used to guide sampling throughout the season. Subsamples of five fish per length classes were otolith-aged and used to establish an age-length key (Ricker 1975). The age distribution of fish for a specified length class was combined to estimate the total number of fish in each cohort. The relative YCSI was then estimated stepwise beginning with (i) calculation of the age distribution proportion of each cohort distribution proportion for the entire period. The various year classes in different years were expressed as (ii) frequencies of the mean age distribution; the relative abundance of each cohort (YCSI) was the mean of these proportions (iii) and age groups from 0 to 10 years old in this specific river system.

$$(1)\ \text{Cohort recruitment ratio} = \frac{\#\ \text{of individuals in a specific age class}}{\text{All captures from 1999 to 2008}}$$

$$(2)\ \text{Year recruitment ratio} = \frac{\#\ \text{of individuals at age of a specific year}}{\text{Total of individuals captured in that year}}$$

$$(3)\ \text{YCSI} = \frac{\text{Year recruitment ratio}}{\text{Cohort recruitment ratio}}$$

As not all individuals in the river could be sacrificed for ageing, only a subsample was used. Consequently, while it should be interpreted cautiously, it is the closest available estimate of year-class abundance to be compared with recruitment estimation index estimated from allelic richness (*Ar*).

North Atlantic Oscillation association with fluctuations in demographic indices

We tested for statistical associations among several combinations of the *NAO* time series and temporal fluctuations of three variables used as proxies of relative abundance. Variation in N_b was used as a proxy for relative variation in the number of breeders. Allelic richness, measured for each cohort, was used as a proxy for the relative abundance of recruits because it has previously been proposed to correlate with offspring recruitment (McCusker & Bentzen 2010). *YCSI* was used as a second proxy of recruit abundance.

We first tested for pairwise correlations between N_b, *Ar* and *YCSI*, and time series of these three parameters were then compared with the monthly normalized *NAO* (https://climatedataguide.ucar.edu/sites/default/files/cas_data_files/asphilli/nao_pc_monthly_4.txt). The 'corresponding year' between time series represented the year when glass eels reached the continent for the *Ar*, N_b and *YCSI* time series. To test environmental influence on previous life stages, from 0- to 2-year lags were tested. To assess the statistical significance of climate influence on eel abundance, multivariate models were run where the explanatory variables considered were the *NAO* time series. Stepwise regressions of the three relative abundance variables (N_b, *Ar* and *YCSI*) were fitted to the explanatory variables to determine which ones were significant. The Akaike information criterion (*AICc*) was used to select models. Cross-validation R^2 was computed to determine the prediction strength of the selected model and semi-partial R^2 were computed to assess the relative importance of each selected variable. Analyses were performed using SAS 9.2 software.

Results

Microsatellite variability and test of population structure

All 18 microsatellites retained for their quality were highly polymorphic in all samples, with H_o and H_e ranging from 0.455 to 0.923 and 0.488 to 0.965, respectively. None of these markers showed significant F_{IS} value ($P > 0.05$) across samples. The number of alleles (n_a) per locus for all samples ranged from 15 to 118, and the mean *Ar* ranged from 4.53 to 28.57 (Table S3, Supporting information).

Table 3 Summary of five different AMOVAS to test for the amount and significance of molecular variance imputable to differences between life stages, among sites within either glass eel or yellow eel stages, among annual cohorts within yellow eels and among cohorts including both yellow and glass eels ($n = 100$ per year to balance with average sample size for yellow eel cohorts). No attempt was made to estimate genetic variance among sites within cohorts since sample sizes were too small and highly variable

Dataset	Sum of squares	Variance components	F_{ST}	*P*-value
Among life stages ($n = 2$) Glass eels and yellow eels ($n = 2142$)	4.598	−0.00131	−0.00019	0.99609
Among sites ($n = 17$) Glass eels ($n = 872$)	109.477	0.00104	0.00014	0.91887
Among sites ($n = 15$) Yellow eels ($n = 1270$)	98.193	0.00239	0.00036	0.59042
Among cohorts ($n = 10$) Yellow eels ($n = 886$, cohorts: 1995–2004)	71.169	0.00389	0.00055	0.17009
Among cohorts ($n = 12$) Yellow eels ($n = 886$, cohorts: 1995–2004) Glass eels ($n = 100$, cohorts: 2005–2006)	86.962	0.00421	0.00059	0.15249

The minimal level of genetic differentiation detectable (at $P < 0.05$) as estimated by POWSIM was very small ($F_{ST} = 0.00034$). In comparison, the global genetic differentiation estimated among all samples yielded an extremely weak F_{ST} value of 0.00009, which was not significantly different from zero ($P = 0.998$, 1000 permutations). All AMOVAS revealed null or extremely weak and nonsignificant components of genetic variance attributable to the different levels of sample groupings (Table 3). Thus, there was no significant genetic differentiation between life stages (glass vs. yellow eels), either among sampling sites—both within glass eels and within yellow eels—or among yellow eel cohorts or among yellow eel cohorts combined with the two glass eel cohorts. Furthermore, there was no significant difference between samples within any of these different levels of groupings (data not shown). Thus, all measures of differentiation were essentially null. There was no evidence either of IBD among glass eel samples (Mantel test; $Z = 2.682$, $R = -0.1075$, $P = 0.821$) or of IBT among yellow eel cohorts (Mantel test: $Z = 0.2176$, $R = 0.2028$, $P = 0.906$).

Effective number of breeders (N_b) and effective population size (N_e)

Estimates of effective number of breeders are presented in Table 4. For yellow eels, no attempt was made to estimate N_b for cohorts with <30 individuals. N_b estimates varied by a factor of 23 among cohorts from 473 in 2005 to 10 999 for the 1999 cohort, for an overall mean of 2682. The effective population size N_e was estimated at 10 532 (95% CI, 9312–11 752). We then looked for associations between the number of breeders N_b and the recruitment index (*YCSI*). While there was no correlation between both parameters estimated the same year (data not shown), we observed a positive correlation between *YCSI* and N_b after applying a 2-year lag ($R^2 = 0.340$, $P = 0.047$; Fig. 1A). As suggested by Waples & Gaggiotti (2006), we also tested the correlation using the 95% lower bound values of N_b confidence intervals, which was even more pronounced ($R^2 = 0.461$, $P = 0.015$; Fig. 1B). No correlation was found between other factors.

Table 4 Summary of sample size (n), allelic richness (Ar), effective number of breeders per cohort (N_b), confidence interval (95% CI) in yellow eel samples and the year class strength index (*YCSI*) in the Sud-Ouest River, Québec. The column 'Year' correspond to the year of arrival in estuary of sample aged

			N_b			*YCSI*	
Year	n	Ar	Estimate	95% CI		Year	*YCSI*
1997	32	16.7	667	410	1729	1995	0.7899
1998	35	17.4	1078	627	3667	1996	1.1727
1999	60	17.2	1332	811	3616	1997	0.9107
2000	59	18.1	3161	1707	19 802	1998	0.9251
2001	94	17.9	10 999	3574	∞	1999	1.3716
2002	145	18.1	2869	2087	4556	2000	1.0342
2003	138	17.4	2776	1850	5488	2001	1.2595
2004	82	18.0	2554	1512	8046	2002	1.1152
2005	127	17.5	3387	2337	6088	2003	1.2081
2006	63	17.4	2264	1021	∞	2004	0.9051
2007	50*	18.6	472	356	698	2005	0.8832
2008	150†	17.9	633	436	1138	2006	0.2273

*N_b was only estimated on glass eels samples, which were available only for the Mira River, Nova Scotia.
†N_b was estimated from a mixture of glass eels from Québec, Nova Scotia and Newfoundland to represent a geographic coverage similar to that of the yellow eel cohorts.

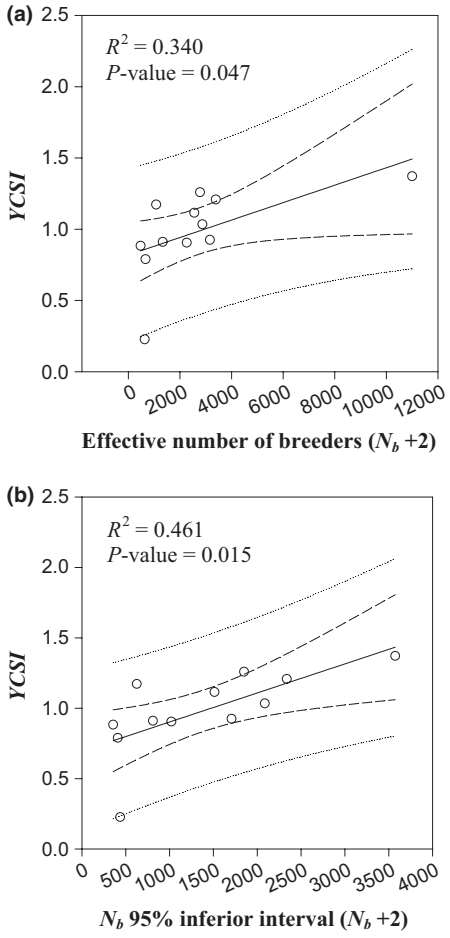

Fig. 1 (A) *Top panel:* Linear correlation between the effective number of breeders per cohort (N_b +2 years) and the year class strength index (*YCSI*) estimated on the Sud-Ouest River, Québec. (B) *Bottom panel*: Linear correlation between N_b 95% inferior intervals (as suggested in Waples 2006a,b) and *YCSI* estimated in the Sud-Ouest River. The central line represents the model estimate, the inner dashed line and the outer dotted lines delimit the 95% and 99% confidence intervals, respectively.

North Atlantic Oscillation association with fluctuations in demographic indices

Highly significant and positive correlations with *NAO* (except in one case) were observed for each of the three eel demographic indices (Table 5). For N_b, the best model explained up to 87% of the temporal fluctuations, and three *NAO* series were retained in the model, which all showed a positive association with N_b: the overall monthly averaged *NAO* lagged 2 years, the February *NAO* lagged 1 year, and the November *NAO* lagged 2 years. The best model for *Ar* explained 82% of the temporal fluctuations for this proxy of relative recruit abundance. The model showed a positive correlation with two *NAO* time periods lagged by 1 year (April and June) and a negative correlation with an *NAO* time period lagged by 1 year (July). The best model for *YCSI* was marginally significant (P = 0.0569). Model showed a positive correlation with an NAO time period lagged by 1 year (May).

Discussion

Confirmed panmixia in the American eel

The first objective of this study was to conduct the most comprehensive analysis to date of the neutral genetic population structure in the American eel to revisit the null hypothesis of panmixia in this species. A total of 2142 eels from 32 locations were genotyped at 18 microsatellite loci that were selected for their high scoring quality. This set of markers also provided high power for detecting very weak level of significant differentiation. All measures of differentiation were essentially null, and there was no evidence for spatial or temporal genetic differentiation based on analyses between or within life stages and cohorts. We conclude that the occurrence of spatial or temporal population genetics structure is very unlikely in American eel and thus propose that the panmixia hypothesis should be accepted for this species.

Our study thus confirms what has been tacitly assumed or suggested based on previous population genetics studies but could not be positively confirmed for various reasons. Williams *et al.* (1973) and Koehn & Williams (1978) documented clinal variation at a few metabolic genes using allozymes. While they proposed that these patterns likely reflected single-generation footprints of spatially varying selection, this interpretation remained ambiguous because their data could not rigorously refute the alternative hypothesis that the observed clines (found at one stable locus out of three variable loci of five loci tested) could instead reflect weak population structure with IBD (Vasemagi 2006). However, by comparing changes in allele frequencies at outlier SNP markers between leptocephali collected in the Sargasso Sea with glass eels from several locations, Gagnaire *et al.* (2012) recently confirmed the hypothesis of Williams *et al.* (1973) by demonstrating the effect of local selection acting on several genes. Previously, Avise *et al.* (1986) had concluded that the species is composed of a single spawning population based on the analysis of a single, maternally inherited mitochondrial DNA locus. Finally, based on the analysis of seven microsatellite markers in eels from eight locations, Avise *et al.* (1988) documented an overall weak but significant genetic differentiation between sampling sites (F_{ST} = 0.0022, $P < 0.01$). Nevertheless, Wirth & Bernatchez (2003) proposed that panmixia was more likely than population structuring because no pattern of

Table 5 Statistical analysis of the relationships between the North Atlantic Oscillation (*NAO*) index and three indices of relative abundance and recruitment for American eel: effective number of breeders (N_b), allelic richness (*Ar*) and year class strength index (*YCSI*). Explanatory variables considered were the *NAO* for every month and different time lags: current year (0), previous year (1) and 2 years before (2). Means of *NAO* for the current year, the previous year and 2 years before were also considered. Stepwise regressions of the four relative abundance variables on the explanatory variables were fitted to select important explanatory variables. The *AICC* was used as the variable selection criteria. Cross-validation R^2 values were computed to determine the prediction strength of the selected model and semi-partial R^2 values were computed to assess the relative importance of each selected variable

Abundance indice	Time series (*NAO*)	Lag NAO (year)	*AICC*	DF	*F*-value	*t*-value	Pr > *F*	R^2
N_b	Model			3	18.26		0.0006	0.8726
	Intercept		206.9834	1		6.20	<0.0001	
	Year average	2	204.9895	1		5.83	0.0004	
	February	1	197.0762	1		4.44	0.0022	
	November	2	196.9286	1		2.38	0.0444	
Ar	Model			3	12.27		0.0023	0.8215
	Intercept		0.0565	1		237.46	<0.0001	
	April	1	−0.2438	1		4.06	0.0036	
	June	1	−1.6180	1		3.37	0.0097	
	July	1	−5.9562	1		−4.63	0.0017	
YCSI	Model			1	4.94		0.0569	0.3818
	Intercept		−8.4331	1		10.13	<0.0001	
	May	1	−8.9575	1		2.22	0.0569	

IBD was observed, which contrasted to what they had reported for the European eel (Wirth & Bernatchez 2001). One aspect of structuring that was not investigated in this study is the pattern of genetic variation among seasonal waves of glass eels arriving at a given sampling site, which has been shown to exceed spatial variation in the European eel (e.g. Pujolar *et al.* 2007). However, such temporal variation in the genetic composition of arriving glass eels does not reflect a stable population genetic structure. Instead, this could result from variance in reproductive success (chaotic genetic patchiness) within cohorts among seasonally separated spawning groups, perhaps originating from fluctuating oceanic and climatic forces (Maes *et al.* 2006).

Much more effort has been devoted to the population genetics of the European eel, and departures from panmixia have been highly debated. Studies interpreted results as weakly genetically differentiated, partially isolated spawning groups (e.g. Wirth & Bernatchez 2001; Maes & Volckaert 2002) or temporal variation within a single gene pool (Dannewitz *et al.* 2005; Pujolar *et al.* 2006). Recently, more studies have documented either a total lack of genetic structure among leptocephali collected in the Sargasso Sea (Als *et al.* 2011) or continental samples (Andrello *et al.* 2011), thus providing very strong, if not definitive, support for panmixia in the European eel.

Given that both American and European eels appear to be truly panmictic, a next step would be to identify the factors responsible for generating the pronounced regional differences in recruitment that have been documented in both species (Vélez-Espino & Koops 2010), which seems paradoxical in the context of panmixia (Cairns *et al.* 2008). Eels are also characterized by pronounced regional (often latitudinal) variations in life history as well as physiological and ecological traits (e.g. Wang & Tzeng 2000; Jessop 2010; Laflamme *et al.* 2012). Arguably, such variations could hypothetically be totally environmentally plastic (e.g. Oliveira & McCleave 2000). However, laboratory-controlled and transplant experiments revealed differences in growth and fatty acid content as well as age and size at the onset of sexual maturation between American eels from different geographic origins, suggesting the existence of quantitative genetic differences for these traits (Côté *et al.* 2009; Verreault *et al.* 2010; Pratt & Threader 2011). The role of 'genetic factors' in explaining differences in growth and behaviour between eels colonizing different habitats has also been proposed based on physiological experiments on European glass eels (Edeline *et al.* 2007). Recently, Gagnaire *et al.* (2012) confirmed the occurrence of local allelic differences at coding genes generated by spatially varying selection between American glass eels from different sampling sites characterized by different sea surface temperatures (SST) at the time when they enter continental waters. This could offer a plausible hypothesis for solving the apparent paradox between observations of important regional variation in life-history traits, demography, and ecology on the one hand and evidence for the existence of a single, randomly mating population in each species on the other. Testing this general hypothesis of a link between genetic variations caused by spatially varying selection and variations in

phenotypes should be a major avenue of research in eel biology in years to come.

Effective number of breeders, effective population size and N_e/N ratio

The second main objective of this study was to document the temporal fluctuations in the effective number of breeders (N_b). Except for two cohorts, confidence intervals around our N_b estimates were relatively narrow compared with similar studies in marine fishes (Hauser & Carvalho 2008). In general, N_e (or N_b) estimators are not expected to perform well in large populations because of the small signal-to-noise ratio. However, estimates can be improved using a large number of individuals and many highly variable markers and by integrating the information over many time periods (e.g. Palstra & Ruzzante 2008; Overgaard *et al.* 2010). Here, the mean sample size of about 100, the number of highly variable microsatellite markers of about 20, and the analysis of 12 cohorts provided relatively accurate N_b estimates ranging on the order of several hundred to a few thousand. Another factor that may also improve N_b or N_e estimates in eel is panmixia, which rules out the strong biases that migration among populations may cause in structured species (Wang & Withlock 2003; Palstra & Ruzzante 2008).

Although this was not a central focus of the present work, N_b estimates allowed us to generate an estimate of contemporary effective population size (N_e) based on the harmonic mean of N_b values. This returned a N_e estimate of 10 532 (95% CI, 9312–11 752). This value is twice as large as the 5000 or so reported for American eel by both Avise *et al.* (1988) and Wirth & Bernatchez (2003) based on the analysis of mitochondrial *DNA* and seven microsatellites, respectively. However, these latter estimates may not be readily comparable because they were quantified from methods reflecting historical rather than contemporary estimates. Using 22 *EST*-derived microsatellite loci, Pujolar *et al.* (2011) recently reported comparable short-term (N_e = 3000–12 000) and long-term (N_e = 5000–10 000) effective population sizes for the European eel. While being very small compared with the species census size (see below), the N_e estimate of 10 532 ranks among the highest values reported for marine fishes, which are generally <2000 (Hauser & Carvalho 2008). Despite uncertainties surrounding any N_e estimate, this indicates that the effective population size for the whole panmictic species must be much smaller than its current census size. Although no rigorous estimates are available, we propose that the total numbers of eels reproducing each year could be on the order of 50–100 million considering local estimates of seven million breeders in the southern Gulf of St. Lawrence (Cairns *et al.* 2008) and 2–3 million breeders in the Potomac River, Maryland (Fenske *et al.* 2011), which represent only small fractions of the total habitat. This suggests that the N_e/N ratio for the American eel could be on the order of 10^{-3} to 10^{-4}. This is similar to the N_e/N ratio of 10^{-4} proposed for the European eel (Pujolar *et al.* 2011) and is also within the range of values reported for most marine exploited fishes (10^{-2}–10^{-6}, mean = 10^{-4}) (Hauser & Carvalho 2008).

North Atlantic Oscillation and variation in number of breeders and recruits

Temporal variations in environmental conditions encountered during migration and reproduction may also cause strong interannual variation in survival and recruitment in marine fishes (Ottersen *et al.* 2010). Moreover, climatic and oceanic processes influence survival during early development, which depends on biological production at the oceanic scale (e.g. Hurrell *et al.* 2003). In the Atlantic Ocean, the *NAO* has been linked to fluctuations in abundance of many marine species (e.g. Alheit & Hagen 1997; Ottersen *et al.* 2001; Attrill & Power 2002; Stenseth *et al.* 2003). We observed a pronounced temporal change in the effective number of breeders: it varied by a factor of 23 among years, from 473 to 10 999 per cohort. Assuming an N_b/N_a (N_a = annual number of breeders) ratio of 10^{-4}, this would suggest that the number of breeders that contributed to reproduction in any given year varied approximately between 4.73×10^6 and 1.09×10^8. We also observed that the *YCSI* varied among years and showed a significant positive correlation with N_b lagged by 2 years. These observations suggest that good climate and environmental conditions during maturation to silver eels and oceanic adult migration could hypothetically produce larger cohorts, perhaps with larger individuals, which may exhibit higher survival. We also found highly significant and generally positive correlations between temporal variation in the *NAO* and N_b as well as *Ar* and nearly significant with *YCSI*. This suggests that *NAO* may influence the abundance of American eel by impacting both the number of adult eels contributing to reproduction as well as the number of recruits. It has long been suspected that oceanic conditions may have a profound impact on eel abundance (Castonguay *et al.* 1994), and the *NAO* has previously been hypothesized to influence local recruitment in American eel (Sullivan *et al.* 2006). Moreover, Knights (2003) reviewed a significant negative correlation between the NAOI and the number of glass eels immigrating to Den Oever in the period 1960–1990. Nevertheless, after an all-time low in 1991 for abundance and length, both the NAOI and length recovered

to average values, while abundance dropped to a new all-time low in 2001 (Dekker 2004). Finally, Pujolar *et al.* (2006) have found a negative correlation between NAO and genetic variability in European eels. However, to our knowledge, this study is the first to report a strong correlation between temporal variation during a positive cycle of the *NAO* and annual fluctuations of adult abundance and recruitment of the American eel.

As for population genetics studies, more efforts have been devoted towards testing for an association between the *NAO* and demographic fluctuations in the European than in the American eel. Knights (2003) was the first to document negative correlations between recruitment (based on the Den Oever glass eel recruitment index (*DOI*) in the Netherlands) and the *NAO* lagged by 1 year. Given that the transatlantic larval migration of the European eel is about 2 years (Kettle & Haines 2006; Bonhommeau *et al.* 2010), a 1-year lag between *DOI* and the *NAO* would suggest that factors encountered during larval migration rather than during early feeding in the Sargasso Sea would be at play (Friedland & Knights 2007).

More recently, Durif *et al.* (2010) investigated the association between the *NAO* and eel abundance based on the analysis of the Skagerrak beach seine survey of yellow-silver eels in Norway. They found that the *NAO* was negatively correlated with yellow eel abundance, with a lag of 11 years, and proposed that this was due to increased current transport that would shorten migration time and bring larvae too early to northern continental waters. The most detailed study on the impact of the *NAO* on European eel demography was carried out by Kettle *et al.* (2008), who analysed data comprising 26 times series of glass eel indices and 15 time series of *FAO* eel landings. They found a significant negative correlation between glass eel indices from most locations and the *NAO* lagged 1–2 years, as in previous studies. However, their results also revealed a strong correlation between adult (silver and yellow) eel landings and the *NAO*. This correlation was negative for sampling sites from southern Europe but positive for northern Europe. Positive *NAO* are associated with high rainfall in northern Europe whereas negative *NAO* indices are associated with high rainfall in southern Europe (Stenseth *et al.* 2003). Kettle *et al.* (2008) proposed that the numbers of outward migrating adult eels were associated with rainfall patterns caused by the *NAO*, with more rain triggering the outward migration of more eels. Hence, Kettle *et al.* (2008) suggested that migrations of the youngest and oldest eels may be linked to the *NAO*. Finally, it is important to mention other oceanographic indices that were not analysed here, like SST and primary productivity (PP), which have also been reported to correlate with demographic and genetic parameters (Bonhommeau *et al.* 2008a,b). SST and PP are influenced by more than one factor and may be not independent from the NAO phenomenon which in our sense makes them more complicated to interpret.

Our results also revealed that both the abundance of adults and recruits of American eels are linked to the *NAO* as we observed highly significant and positive correlations (except for one *NAO*) for N_b and *Ar* demographic indices and close to be significant to *YCSI*. The strongest correlation was observed for N_b, and the three *NAO* indices retained in the best model showed a positive association, the strongest being with the monthly average with a 2-year lag. As N_b reflects the abundance of breeders and was estimated from the cohort of glass eels arriving in continental waters in the year following reproduction and that it likely takes 5–6 months for adults to migrate from their continental habitats to the Sargasso Sea, the 2-year lag reflects environmental conditions encountered by silver eels prior to or during the onset of their outward migration to the ocean. As positive *NAO* events generally increase temperature and precipitation in eastern North America (Stenseth *et al.* 2003), this suggests that the abundance of out-migrating silver eels that will reproduce in a given year is positively associated with the amount of rainfall during the year that the reproductive migration is undertaken; this was also proposed by Kettle *et al.* (2008) for the European eel. The November monthly index was also positively associated with N_b with a 2-year lag, whereas the February monthly index was positively associated with a 1-year lag. This suggests that conditions encountered during the oceanic spawning migration to the Sargasso Sea (approximately between November and February) could also potentially impact on the number of adult breeders.

We also observed positive correlations between several monthly *NAO* indices and the two indices of recruit abundance (*Ar* and *YCSI*) that were all lagged by 1 year. This suggests that recruit abundance is influenced by environmental conditions encountered mainly during the year preceding arrival in continental waters. Thus, knowing that the American eel spawns in the Sargasso Sea mainly during February-April (McCleave *et al.* 1987) and that the first waves of glass eels arrive as early as the following December on the Florida coast and not until the subsequent June in the St. Lawrence estuary in Québec (Laflamme *et al.* 2012), this means that American eel larvae and glass eels take approximately 8–16 months to reach rivers, depending on the location. Although the exact significance of the correlation with each of the monthly *NAO* indices involved cannot be rigorously interpreted, the fact that they span from February lagged −1 to April of the year corresponding to recruitment suggests that conditions encountered during the spawning period, the oceanic

migration, and the arrival in continental waters could all influence recruitment. What is clear, however, is that high *NAO* indices have a positive effect on recruit abundance of American eel whereas they negatively impact that of European eel. There is evidence of a northward shift of the Gulf Stream during positive phases of the NAO (Hurrell & Deser 2009). One hypothesis is that this northward shift could increase larval and glass eel transport and hence abundance in the northern part of the range, as was proposed by Castonguay *et al.* (1994). In contrast, faster and northward transport has been proposed to cause a loss of recruits that cannot reach the European coast at the right time (Durif *et al.* 2010). Moreover, since 1980, the *NAO* has been mainly positive, so our data set only reflects interannual variations in abundance during this period. We thus cannot rigorously form conclusions on the possible effects of negative *NAO* events on American eel abundance on a longer time scale until more data with negative NAO indices become available.

Conclusions

In summary, spatial or temporal population genetics structure is very unlikely in American eel, and thus, the panmixia hypothesis should be accepted for this species. Second, this study illustrates that estimates of the effective number of breeders with relatively narrow confidence intervals can be obtained for a species having a large census size, such as the American eel, when large sample sizes and many highly variable markers are used, and that N_e/N ratio is small, as reported in other marine exploited species. Third, we also showed that genetically based demographic indices, namely N_b and Ar, can be used as meaningful proxies for the abundance of breeders and recruits. Thus, our results suggest that interannual variations in abundance of both breeders and recruits are positively influenced by atmospheric and oceanographic variability during positive *NAO* phases. Long-term genetic monitoring of glass eels at several sites along the North American Atlantic coast would provide a powerful and efficient complement to census monitoring for tracking demographic changes and better understanding their causes.

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Data accessibility

Genotypes, age, sampling location and SAS modelling script in DRYAD entry doi: 10.5061/dryad.39jb0.

North Atlantic Oscillation Index available at: https://climatedataguide.ucar.edu/sites/default/files/cas_data_files/asphilli/nao_pc_monthly_4.txt.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Summary of tested microsatellite markers: locus name, primer sequences (forward and reverse), allele size range (*bp*), citation, and GenBank accession no.

Table S2 Number of yellow eels for each annual cohort used for estimating N_b and temporal genetic variation from each sampling location as estimated by otolith age determination.

Table S3 Levels of genetic diversity measured at 18 microsatellites retained for population genetic analyses.