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Characterisation of genetic structure of *Dicentrarchus labrax* larvae in two nurseries of the Gulf of Lions (NW Mediterranean)

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Abstract – Despite extensive knowledge of the genetic structure of sea bass (*Dicentrarchus labrax*) populations, no studies have investigated genetic structure within early life stages, or compared such structure between such stages and (sub)adults. Using nine newly developed microsatellite loci, we investigated patterns of genetic variation and relatedness among juveniles that settled in two NW Mediterranean nursery grounds in close geographical proximity. There was no evidence for differentiation among samples at settlement ($\theta = -0.0010$), and no significant genetic relatedness. Nevertheless, significant departures from Hardy-Weinberg equilibrium were detected in each sample, which could not be attributed exclusively to the presence of null alleles and to Wahlund effect. These results are compared with and discussed in relation to the genetic structure of adults described in the same area 10 years ago.

Key words: Population genetics / European sea bass / Early life stages / Adults / Nursery ground

1 Introduction

The European sea bass, *Dicentrarchus labrax* (L.) (Perciform: Moronidae), is a commercial species distributed in the Eastern Atlantic from Senegal to Norway and throughout the Mediterranean Sea. There is a significant knowledge about genetic structure, evolutionary history, and patterns of gene flow in sea bass, which has accumulated over two decades from a wide array of molecular markers (e.g. Benharrat et al. 1983; Allegrucci et al. 1995, 1997; Garcia de León et al. 1997; Bahri-Sfar et al. 2000; Lemaire et al. 2000; Fritsch et al. 2007 and references therein). Applied issues have also been investigated using genetic markers (e.g. Garcia de León et al. 1998; Bahri-Sfar et al. 2005). Nonetheless, many issues of population genetics remain to be properly investigated in sea bass, in particular for (post-) larval stages, which have never been studied before.

An important general issue within the population genetics of young stages is to understand whether individuals have similar genetic features, sensu lato, as their (sub)adult conspecifics. In particular, for patterns of genetic differentiation among settling juveniles, a phenomenon known as genetic patchiness (Johnson and Black 1982). Genetic patchiness is where the genetic variation observed at a local scale among freshly recruited larvae or early juveniles is greater than that among adults over wider geographical areas. Such differentiation within early life history stages is due to the unpredictability of breeding success coupled with a pelagic larval stage, which together provide a large variance in the contribution of each cohort to the adult population, leading to significant local genetic differentiation due to sampling variance occurring among cohorts of one adult panmictic population (Hedgecock 1994). As for many marine species, the sea bass life cycle may promote this phenomenon because cohorts of post-larval juveniles colonise a wide array of coastal nursery habitats, including coastal lagoons, estuaries, river mouths, and marinas. Genetic patchiness would subsequently arise if a minor proportion of the total sea bass larval pool colonised such coastal habitats, each of them only harbouring a small fraction of the total available genetic diversity.

Genetic patchiness, however, only deals with genetic differentiation among samples, and there are numerous other interesting comparisons to make for the genetics of young recruits versus (sub)adults. These include comparisons of

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within-group structure (i.e. departure or not of Hardy-Weinberg expectations), and investigation of relatedness and/or admixture of subsamples. Then, apart from the stability of observed allele frequencies across life stages that pertain to genetic patchiness, comparison for other genetic features within a single species are scarcely examined.

The NW Mediterranean population of sea bass in the Gulf of Lions provides an opportunity to investigate these issues.

Firstly, using microsatellite loci, Garcia de León et al. (1997) demonstrated that the adult marine individuals were panmictic in this area. However, there is also some evidence that individuals in coastal habitats might be the target of greater local selective pressures than their congeners in the open sea, with different allele frequency distributions among marine and coastal individuals (Lemaire et al. 2000). Nevertheless, to demonstrate such a selective process reliably, it is necessary to ensure that there are identical genotypic distributions in the individuals that enter the nurseries, and that any differentiation in (sub)adults is not simply a result of genetic patchiness in larval stages.

Secondly, the study by Garcia de León et al. (1997) provided a guideline for comparison of genetic structure in adults compared to young stages. For example, despite observed panmixia among Gulf of Lions samples, Garcia de León et al. (1997) established large departures from Hardy-Weinberg expectations. It is interesting to investigate whether this is also true for early life-stages.

In this paper, newly defined microsatellite loci were used to investigate the genetic structure of two larval cohorts as they entered two NW Mediterranean nurseries located in the Gulf of Lions. Although the number of samples was restricted, it was nonetheless possible to test genetic patchiness in sea bass at the local scale. Genetic patterns in these larval cohorts were then compared to the structure of the adult population discussed by Garcia de León et al. (1997).

2 Materials and methods

Two larval cohorts were sampled with hand nets by snorkelling at the "Port des Quilles" at Sète (post-larvae entering the marina of Sète; Hérault, France; 43°23'33" N, 3°39'51" E) and in Palavas (post-larvae entering the mouth of the Lez river; Hérault, France; 43°31'31" N, 3°56'03" E), two habitats that serve as nursery grounds for sea bass. The two sampling stations are located approximately 20 km apart in the Gulf of Lions; sampling was performed in June 2005 with a one-day interval between the two sites. Individuals from both sites may have belonged to the same event of larval pulse, as sea bass were not observed on previous days at these locations, and had just settled when the sampling was performed. No size difference was apparent among individuals of each sample. The most probable spawning ground in the surveyed area is located offshore of the Aresquier beach, almost at the mid-point of the two sampling sites. Nevertheless, observations of prevailing seasonal shelf currents in this area indicate that these are mainly westward (i.e. from Palavas to Sète) in June (e.g. Petrenko et al. 2005 and references therein). Under such conditions, genetic data provide a further test that larvae could belong to the same larval pulse.

Larvae were transported to the laboratory at the Station Méditerranéenne de l'Environnement Littoral (Sète) where they were maintained for up to eight months for in-vivo ecophysiological studies (not reported here). Samples from all individuals that died during this rearing period were analysed, to minimize any biases in genetic structure due to mortality in captivity. Nevertheless, cannibalism occurred at early stages (≈10%). At the end of this period, the remaining individuals were sacrificed. This resulted in $n = 46$ and $n = 49$ for the Sète and Palavas samples, respectively. Fins or white muscle tissues were collected and stored in 90% ethanol for genetic analyses. Microsatellite loci used in this study are new (Table 1). They have been isolated from AC- and AG-enriched libraries as described in Tsigenopoulos et al. (2003), with the single exception of locus *DLA0064*. This latter was identified in a cDNA sequence of a *c-ski* gene (a family of genes regulating myogenic development; Huang et al. 1999) and mapped in tilapia (Lee et al. 2005). Oligonucleotide primers were optimized for PCR amplification testing over a range of annealing temperatures and $MgCl_2$ concentrations. Furthermore, a multiplex PCR was optimised using the QIAGEN multiplex PCR kit in a 20 μl final volume with 1× Qiagen multiplex PCR master mix, 1× Q-solution, 30 ng of genomic DNA and 0.18 μM of each primer. Amplifications were performed on a PTC-200 (MJ Research) as follows: an initial denaturation at 95 °C for 15 min for HotStarTaq DNA Polymerase activation, 35 cycles at 94 °C for 30 s, annealing at the specific locus temperature (Table 1) for 90 s, and 72 °C for 60 s, followed by a final elongation at 72 °C for 10 min. Genotyping of individuals was performed by allele sizing on an ABI PRISM® 3700 DNA Analyzer (Applied Biosystems), using 5′-labelled reverse primers (Table 1) and the GeneScanTM-500 LIZ® Size Standard (Applied Biosystems) as internal size standard.

MicroChecker v2.2.1 (van Oosterhout et al. 2004) was used to identify scoring errors due to stuttering, PCR amplification bias against large alleles (allelic dropout) and genotyping errors due to null alleles. The number of alleles and the observed (H_{obs}) and unbiased expected (H_{exp}) gene diversities for each population and locus were computed by Genetix v4.05 (http://www.univ-montp2.fr/~genetix/). Using this same software, we investigated deviations from Hardy-Weinberg expectations (HWE) within samples by Weir and Cockerham's f (1984). The null hypothesis of no significant departure from ($f = 0$) was tested by randomly permutating alleles from the original matrix of genotypes using the appropriate procedure. Levels of population differentiation were investigated using Weir and Cockerham's (1984) θ which estimates Wright's (1951) F_{st}. Using Genetix v4.05, we also investigated patterns of linkage disequilibrium in each sample as proposed by Weir (1979). For calculations of genetic relatedness (kinship), the coefficient of relatedness r_{xy} (Queller and Goodnight 1989) was estimated using the Identix computer package (Belkhir et al. 2002), which can detect relatedness in populations using multi-locus genotypic data (Castric et al. 2002). To detect relatedness (kin aggregation of sea bass) within each sample, the original statistics were extended as described by Behrmann-Godel et al. (2006). To test whether larvae within one sample were genetically more related than expected by random distribution (Behrmann-Godel et al. 2006),

Table 1. Locus and primers used in this study (F: forward; R: reverse; bp: base pair). The annealing temperature (T_a) is in °C and the $MgCl_2$ concentration in mM. 5'-labelled fluorochromes are indicated. GenBank accession numbers are given in brackets. All microsatellite loci have been specifically developed for *D. labrax* except locus *DLA0064* that was linked to a gene (*c-ski*) mapped in *Oreochromis sp.* (Lee et al. 2005).

Locus [accession No.]	Repeat		Primers 5'–3'	5'-label	Allele size range (bp)	T_a (°C)	$MgCl_2$
DLA0060	$(CA)_{12}(TA)_3AA(CA)_2$	F	GAGAGTTCATCCTGTTCGCTC	FAM	120–128	55	3
[*DQ363883*]		R	TGTAGTAATAATGCGCTCTGCAA				
DLA0061	$(TG)_{14}$	F	AAAGGCCAGTGAAACTCATGT	FAM	150–172	55	1.5
[*DQ363884*]		R	CTCCCTGTCCATCTGTCCTC				
DLA0064	$(GA)_{23}$	F	GTCAGTCACATTCCTGGCTG	FAM	202–246	56	1.5
[*AJ012011*]		R	TTCTATGCTCCTGCGGTTTT				
DLA0066	$(AG)_{22}$	F	GTTGACCGGAGTCCTAGC	ROX	124–168	55	1.5
[*DQ363887*]		R	GGCCATATGTGTCTTGCTT				
DLA0068	$(CA)_7CGCACG(CA)_3$	F	CAACACCTGTTCCTCTGAACC	TAMRA	245–265	55	3
[*DQ363889*]		R	GCATTAGCATTGATTGTCCTG				
DLA0073	$(CT)_{36}$	F	CATGACTTCATGTGCTAATGTCC	TAMRA	141–181	58	1.5
[*DQ363894*]		R	AGTTCAGAGCGGCAACTGT				
DLA0078	$(AG)_{29}$	F	AAGACTGGACCTCTGGAGACC	HEX	217–253	52	3
[*DQ363899*]		R	CACAAGGAACCGAGACAAGA				
DLA0086	$(AC)_{26}$	F	GCTAGAGGATTCATGTCGCTT	FAM	179–205	55	1.5
[*DQ363807*]		R	ACCTGGTGATTGGCAATTCT				
DLA0089	$(GT)_{15}$	F	ACGAGTAATGAGGACCCA	TAMRA	122–136	55	1.5
[*DQ363810*]		R	GTCAAAACAGCCCACCTA				

the mean identity index of all sea bass pairs within one sample was compared with the null distribution of no relatedness. As null distribution, we calculated the distribution of identity indices of randomly generated samples of the same sample size (45 individuals to consider a number close to observed sample sizes). Subsamples were generated by random permutation of genotypes in 1000 randomized subsamples, using each individual larval sample successively, and then the total sample as genotype pools (i.e. successively searching for significant relatedness of individuals of one sample by comparing with relatedness estimates established for random distributions of individuals belonging to this sample, to the other sample, then to the pooled samples). This procedure does not search for rigorous comparison of observed relatedness within a given sample with any theoretical value (e.g. $r_{xy} = 0$); it compares each observed mean relatedness estimate within sample with a random distribution of r_{xy}. We also computed the variance in relatedness in each sample, and then similarly compared this variance to random distributions.

3 Results

No linkage disequilibrium was detected among loci in each individual sample or in the total sample. Descriptive genetic statistics of each sample are summarised in Table 2. One hundred and seven alleles were recorded among the 95 individuals sampled in the two samples, with a number of alleles varying from four (*DLA0068*) to eighteen (*DLA0073*) (mean allelic richness: 11.89 alleles per locus). Average allelic richness of each larval sample was slightly lower than the one recorded in the full sample, indicating that some alleles were missing in one sample or the other (10.22 and 10.44 alleles per locus in the Palavas and Sète samples, respectively). Observed gene diversities for the whole sample varied from 0.413 (*DLA0089*) to 0.833 (*DLA0073*) with mean $H_{obs} = 0.573$. Similar values were observed for individual samples. HWE was not observed within each sample and f values were highly significant ($f = 0.215$ and 0.207 in Palavas and Sète samples, respectively; Table 2). This was largely due to large departures from HWE at loci *DLA0064* and *DLA0078* (Table 2), which were the only loci for which MicroChecker identified null alleles as the most probable source of deviation causing such a pattern. The occurrence of null alleles was possibly due to cross-species amplification for locus *DLA0064*, as this locus was originally not developed for sea bass (see Sect. 2). According to MicroChecker, the other loci were not affected by null alleles, stuttering or large allele dropout (details not shown), despite some significant departures from HWE at locus *DLA0066*. When deleting loci *DLA0064* and *DLA0078*, estimates of f are $f = 0.137$, $f = 0.111$, $f = 0.158$ (each $p < 0.001$) for the total, Palavas, and Sète samples, respectively. Although not identified by MicroChecker v2.2.1, we found that deleting locus *DLA0066* led to $f = 0.115$ ($p < 0.01$), $f = 0.085$ ($p < 0.05$), and $f = 0.138$ ($p < 0.01$) for the total, Palavas, and Sète samples, respectively. As demonstrated by jacknife estimates that successively computed f by deleting one individual locus, the remaining loci had only a minor influence on f values (details not shown). The results therefore indicated that significant observed departures of HWE were not only due to potential null alleles or other processes occurring at specific loci identified using MicroChecker but, rather, were constitutive of each individual sample. No significant differentiation was detected when considering nine ($\theta = -0.0010$) or seven loci ($\theta = 0.0005$). No individual locus demonstrated significant genetic differentiation among larval samples (Table 2). Locus *DLA0064* did not differ from other loci for level of population differentiation.

Departure from HWE within each sample could suggest that cohorts were composed of related individuals or that each

Table 2. Descriptive statistics of the sea bass population structure at nine microsatellite loci for the total data set and each larval sample taken separately. Expected (H_{exp}) and observed (H_{obs}) gene diversity (= heterozygosity) are reported, together with standard deviations (± SD) for each mean estimate. Locus specific and multilocus f values are reported together with their level of significance indicating departure from Hardy-Weinberg expectations. θ values represent the estimates of genetic differentiation among larval samples; no significant genetic differentiation was found among samples. f and θ are Weir and Cockerham's (1984) estimators of Wright's (1951) F_{is} and F_{st}, respectively.

Locus	Observed number of alleles			Gene diversity						f			θ
	Total	Palavas	Sète	Total		Palavas		Sète		Total	Palavas	Sète	
				H_{exp}	H_{obs}	H_{exp}	H_{obs}	H_{exp}	H_{obs}				
DLA0060	5	4	4	0.528	0.451	0.529	0.450	0.532	0.452	0.147	0.152	0.151	−0.0106
DLA0061	9	8	8	0.573	0.507	0.469	0.412	0.653	0.589	0.117	0.124	0.098	0.0186
DLA0064	16	15	14	0.901	0.555	0.894	0.471	0.904	0.618	0.398***	0.477***	0.320***	0.0025
DLA0066	17	12	17	0.898	0.696	0.892	0.726	0.908	0.686	0.227**	0.211**	0.248**	−0.0082
DLA0068	4	4	3	0.509	0.425	0.484	0.429	0.518	0.422	0.167	0.116	0.188	0.0265
DLA0073	18	15	17	0.911	0.833	0.899	0.884	0.921	0.880	0.082	0.016	0.133*	−0.0031
DLA0078	16	15	13	0.865	0.492	0.881	0.554	0.847	0.536	0.433***	0.488***	0.372***	−0.0078
DLA0086	13	12	11	0.861	0.791	0.859	0.781	0.868	0.800	0.082	0.092	0.080	−0.0080
DLA0089	9	7	7	0.487	0.413	0.428	0.405	0.543	0.451	0.152*	0.054	0.227**	0.0012
Mean	11.89	10.22	10.44	0.726 (± 0.193)	0.573 (± 0.160)	0.704 (± 0.216)	0.555 (± 0.187)	0.744 (± 0.178)	0.592 (± 0.149)	0.212***	0.215***	0.207***	−0.0010

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

sample represented a mix of more than one differentiated group of unrelated individuals (i.e. Wahlund effect). The coefficient of relatedness r_{xy} in each sample did not reveal any significant difference for both the nine and seven locus data sets, and the mean estimated values did not differ significantly from those observed for the generated null distributions (for the nine locus data set: $r_{xy\,Palavas} = 0.0015$, $p = 0.60$; $r_{xy\,sète} = 0.0056$, $p = 0.59$; $r_{xy\,Pooled} = 0.0024$, $p = 0.48$). Results pertaining to variance in relatedness among samples were not significant either from those observed for the generated null distributions ($p = 0.29$, $p = 0.41$, $p = 0.39$ in Sète, Palavas and in the pooled sample, respectively, for the nine locus data set).

4 Discussion

Despite relatively extensive knowledge on the genetics of sea bass, the genetic structure of populations at young stages has not been described in *D. labrax*, as all previous studies focused upon older juveniles or adults. Even though a rearing period and some mortalities occurred before genetic analyses were performed upon the sample populations, the current study is the first description of the genetic structure of individuals at the settlement stage in NW Mediterranean Sea. Garcia de León et al. (1997) reported that the adult population in the Gulf of Lions was effectively panmictic. Our results indicate that young individuals deriving from this panmictic population are not differentiated when they enter local nurseries ($\theta = -0.0010$; Table 2). That is, no spatial genetic patchiness (SGP) was observed among sea bass individuals at early life history stages, even though such SGP has been described in many other marine fish species (e.g. Selkoe et al. 2006; Burford and Larson 2007). However, those studies reported SGP at larger scales than investigated here. SGP at this local scale has only been reported in invertebrates (e.g. Moberg and Burton 2000). There is no such local differentiation in adult sea bass in the Gulf of Lions, an absence of SGP at the scale of this study is not particularly surprising. The absence of SGP also indicates that the larval fish may derive from the same spawning grounds. A spawning ground could be located between the two sample sites but, if this ground provided the larvae, then the local westward current, which prevails during the settlement period, cannot play any role in colonisation of the eastward Palavas site. It is, of course, possible that the larval pulse derived from another spawning ground. Further studies could extend these preliminary results to a larger spatial scale.

The sea bass population has a large census size and is homogeneous in the region (Garcia de León et al. 1997). Larval pulses may carry offspring from large spawning groups. In that situation, different larval pulses would display homogeneous allele frequencies and it would be unlikely that related individuals would be sampled as observed in our study. However, large departures of HWE of each sample have already been reported by Garcia de León et al. (1997) for adult populations of sea bass in the Gulf of Lions, and those results are consistent with the current study upon post-larval stages (Table 2). Despite the use of different microsatellite loci in each study, such large departures are poorly consistent with large spawning groups/larval pulses. In such situations, genetic data rather revealed no or few significant departures from HWE proportions (e.g. Saillant et al. 2006; see below for further justification). Furthermore, the patterns described in adults *ca.* 10 years ago are also observed in young stages at recruitment.

A detailed comparison of our results with those presented in Garcia de León et al. (1997) is then warranted. As in their study, our results were initially confounded by null alleles apparently occurring at two (or three) loci. We used MicroChecker (van Oosterhout et al. 2004) to account more thoroughly for these loci. Nonetheless, the loci identified as influenced by null alleles cannot by themselves explain the deficits in heterozygotes in the total sample, or in each individual sample, because the f-values remained significant when these loci were deleted. If the rejection by the software of the loci with null alleles was valid, the deficit of heterozygotes might be a consequence of a number of factors: the Wahlund effect (as previously suggested by Garcia de León et al. 1997), biased patterns of inheritance; inbreeding sensu lato (see below), assortative mating, and/or selection against heterozygotes. Garcia de León et al. (1997) found that the Wahlund effect alone was not sufficient to explain departures from HWE in their study. These authors argued that the Wahlund effect is caused by breeding subunits that are individually at HWE but are differentiated amongst themselves. In such a case, the estimated f values reported in Table 2 could provide a measure of genetic differentiation among subunits. Considering that the measure of genetic differentiation (θ) among the Sète and Palavas samples is smaller than the estimated f values (Table 2), it is unlikely that each sample could be composed of subunits which created a far greater differentiation than that observed amongst samples, an argument that has already been made by Garcia de León et al. (1997) and explaining why studies reporting no significant genetic differentiation should report no HWE departures (e.g. Saillant et al. 2003, 2006). Even though different sea bass microsatellite loci were used, biased transmission was rejected as a possible explanation because patterns of Mendelian inheritance were respected (Garcia de León et al. 1995). Given the similarities between that study and the present results, it can probably be assumed that such patterns are also respected for the loci we used, and it is certainly very unlikely that biased transmission affect several independent loci at the same time. As also discussed in Garcia de León et al. (1997), assortative mating cannot be totally rejected, but this remains hypothetical because it is difficult to conceive of it affecting different unlinked loci in a similar manner (that is, all f-values were positive). Theoretically, selection could act on loci linked to microsatellite markers (genetic hitch-hiking). This would introduce heterogeneity among estimated f-values. Nevertheless, as in the Garcia de León et al. (1997) study, this hypothesis is poorly supported by the present dataset because two outlier loci (*DLA0064* and *DLA0078*) were excluded by MicroChecker, and the deletion of one further locus (*DLA0066*) did not influence the significance of the results obtained with the remaining six loci ($f = 0.115$, $f = 0.085$, and $f = 0.138$, all $p < 0.05$, for the total, Palavas, and Sète samples, respectively). It is also difficult to conceive that two independent studies using different sets of anonymous microsatellite loci (except locus *DLA0064*) would, in both cases, provide distribution skewed towards positive f-values.

The issue of relatedness (kinship) or inbreeding sensu lato, as defined in Garcia de León et al. (1997) deserves more attention. A common interpretation of relatedness results in sea bass is that each larval sample was apparently not composed of more related individuals than those generated in random distributions as mean coefficients of and variance in relatedness did not differ. Results might possibly be improved by using more loci (Blouin 2003), as shown in successive results obtained in red snapper (*Lutjanus campechanus*; Saillant et al. 2003, 2006). Studies using less or similar number of loci have nonetheless reported significant relatedness in other fish species (Behrmann-Godel et al. 2006; Selkoe et al. 2006). In this study, however, null distributions and observed relatedness derived from one genetically unique sample ($\theta = -0.0010$). Selkoe et al. (2006) reported a significant relationship among pairwise θ values among samples and the ability to detect significant relatedness. Another common problem with relatedness is to dissociate inbreeding sensu stricto (i.e. gene correlation within individuals) and coancestry (i.e. gene correlation between individuals of the same breeding group) (Cockerham 1969, 1973). Those parameters together define relatedness and population structure (e.g. Chesser 1991). We more implicitly look at coancestry in this paper when referring to relatedness by considering that samples might be derived from different families (Chesser 1991). Using simulations, Guinand et al. (2006) reported that detecting changes in distributions of samples composed of individuals with any inbreeding level sensu stricto, and whatever the coancestry level considered within each sample, was difficult for loci with an average of 10 alleles and low θ-value (0.01), if contrast of those quantities among samples is low. When increasing inbreeding/coancestry, data sets generated in simulations by Guinand et al. (2006) demonstrated distributions of f-values skewed to positive values (B. Guinand, unpubl. results). Even if present, results by Selkoe et al. (2006) and results from simulations indicated that detecting significant differences in mean relatedness and successfully comparing variance in relatedness within- or among samples is extremely difficult.

In turn, if we assume that the positive f-values found in the present study and in Garcia de León et al. (1997) are due to undetectable non-zero levels of coancestry/inbreeding sensu stricto in the sea bass population, this would imply that this population may have low local effective population size despite large census size. Garcia de León et al. (1997) provided a broad estimate of effective population size for sea bass that was potentially as low as $N_e = 10\,000$–$60\,000$ in the area encompassing the Gulf of Lions and the Gulf of Valence (Spain). It would, therefore, be important to investigate those processes that might reduce effective population size in sea bass (e.g. a bottleneck, see Turner et al. 2002 for other factors), and how they can impact upon relatedness (see Bierne et al. 2000). In marine organisms, a bottleneck should not be conceived as a global population crash but, rather, as a component of the life-cycle which occurs during recruitment. This hypothesis agrees well with the general population dynamics of marine organisms, whose numbers can vary greatly as a function of many factors (e.g. Cushing 1990; Leggett and Deblois 1994). Furthermore, in red drum (*Sciaenops ocellatus*) – one estuarine-dependent fish species sharing common life-history features with sea bass – Turner et al. (2002) demonstrated that variance in productivity of spawning and/or nurseries habitats necessary for successful recruitment was probably very large and played a significant role for determining genetic diversity. Such elements should provide the impetus for a more complete genetic study in sea bass to further understand key demographic factors that shape observed genetic structure in larvae and adults.

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