



HAL
open science

Candidate gene variation in gilthead sea bream reveals complex spatiotemporal selection patterns between marine and lagoon habitats

B Guinand, C Chauvel, M Lechene, J Tournois, Costas S Tsigenopoulos, Audrey M. Darnaude, David J. Mckenzie, P.-A Gagnaire

► To cite this version:

B Guinand, C Chauvel, M Lechene, J Tournois, Costas S Tsigenopoulos, et al.. Candidate gene variation in gilthead sea bream reveals complex spatiotemporal selection patterns between marine and lagoon habitats. 2016. hal-01370327

HAL Id: hal-01370327

<https://hal.science/hal-01370327v1>

Preprint submitted on 22 Sep 2016

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Candidate gene variation reveals complex spatiotemporal patterns of selection**
2 **between marine and lagoon habitats in the gilthead sea bream**

3
4 B. Guinand^{1,2*}, C. Chauvel^{2,3}, M. Lechene^{2,3}, J. Tournois⁴, C.S. Tsigenopoulos⁵, A.M.
5 Darnaude⁴, D.J. McKenzie⁴, P.-A. Gagnaire^{2,3}

6
7 **Accepted Manuscript: doi 103354/meps1185**

8
9 ¹ : Université de Montpellier, Département Biologie-Ecologie, Place E. Bataillon, 34095
10 Montpellier, France

11 ² : UMR ISEM, Institut des Sciences de l'Evolution de Montpellier, Université de
12 Montpellier, 34095 Montpellier, France

13 ³ : Station Biologique Marine de Sète, 2 rue des Chantiers, 34200 Sète, France

14 ⁴ : UMR MARBEC, Marine Biodiversity, Exploitation and Conservation, Université de
15 Montpellier, Place E. Bataillon, 34095 Montpellier, France

16 ⁵ : Institute of Marine Biology and Genetics, Hellenic Center for Marine Research, PO
17 Box 2214, Gournes Pediados, 71500 Heraklion, Crete, Greece

18
19 *: corresponding author

20
21
22
23 Running head: Complex sea-lagoon selection patterns in sea bream

25 **Abstract:** In marine fishes, the extent to which spatial patterns induced by selection
26 remain stable across generations remains largely unknown. In the gilthead sea bream
27 (*Sparus aurata*), polymorphisms in the growth hormone (*GH*) and prolactin (*Prl*) genes
28 can display high levels of differentiation between marine and lagoon habitats. These
29 genotype-environment associations have been attributed to differential selection
30 following larval settlement, but it remains unclear whether selective mortality during
31 later juvenile stages further shapes genetic differences among habitats. We addressed this
32 question by analysing differentiation patterns at *GH* and *Prl* markers together with a set
33 of twenty-one putatively neutral microsatellite loci. We compared genetic variation of
34 spring juveniles that had just settled in three ecologically different lagoons, against older
35 juveniles sampled from the same sites in autumn, at the onset of winter outmigration. In
36 spring, genetic differentiation among lagoons was greater than expected from neutrality,
37 for both candidate gene markers. Surprisingly, this signal disappeared completely in the
38 older juveniles, with no significant differentiation for either locus a few months later in
39 autumn. We searched for signals of haplotype structure within *GH* and *Prl* genes using
40 next-generation amplicon deep-sequencing. Both genes contained two groups of
41 haplotypes, but high similarities among groups indicated that signatures of selection, if
42 any, had largely been erased by recombination. Our results are consistent with the view
43 that differential selection operates during early juvenile life in sea bream, and highlight
44 the importance of temporal replication in studies of post-settlement selection in marine
45 fish.

46

47 *Key words:* candidate gene, growth hormone, prolactin, genetic differentiation, amplicon sequencing,
48 local selection

49

50 **Introduction**

51 The demography of coastal organisms has a profound influence on the genetic structure
52 of their populations (Hellberg 2006, 2009; Gagnaire et al. 2015). During early life stages,
53 genetic variation can be shaped by a combination of stochastic, dispersive and selective
54 events which affect pelagic larval dispersal but also settlement and colonization of
55 nursery habitats by juveniles (e.g. Larson & Julian 1999; Broquet et al. 2013).
56 Disentangling these various sources of variation throughout the larval and juvenile stages
57 remains extremely challenging.

58 In species with high fecundity, discrete broadcast spawning events followed by
59 very large mortality rates of pelagic larvae (>99%, type-III survivorship curve) can
60 produce successive waves of settlers that differ in their genetic makeup. Because of the
61 marked variance in reproductive success, each group of recruits may comprise a different
62 subset of individuals, representing only a fraction of the parental genetic pool
63 (Hedgecock 1986, 1994). Hence, even if selection occurs during the planktonic stage
64 (Johnson & Black 1984), genetic drift is thought to be the main force shaping genetic
65 structuring among groups of settlers, sometimes promoting increased relatedness among
66 individuals (e.g. Larson & Julian 1999; Planes & Lenfant 2002; Selkoe et al. 2006;
67 Iacchei et al. 2013; Aglieri et al. 2014; review in Hauser & Carvalho 2008). This
68 phenomenon, called ‘sweepstake recruitment’, has been widely documented (Hellberg
69 2006, 2009; Selkoe et al. 2008; Hedgecock & Pudovkin 2011). As the process is random,
70 and depends upon factors such as the strength of the cohorts or variation in local
71 dispersal and connectivity, it generally produces un-patterned spatial (or temporal)
72 genetic differentiation among habitat patches, resulting in ‘chaotic genetic patchiness’
73 detectable over the whole genome (Hedgecock & Pudovkin 2011).

74 Conversely, if the variance in reproductive success is small enough, or if pools of
75 related larvae are sufficiently mixed by currents before settlement, panmixia might be
76 observed among groups of recruits from distinct locations (Domingues et al. 2011;
77 Guinand et al. 2011). In such cases, patterns of locus-specific genetic differentiation may
78 indicate selection operating on juveniles that settled in different nursery habitats (Koehn
79 et al. 1980; Lemaire et al. 2000; Planes & Roman 2004; Guinand et al. 2011). Moreover,
80 patterns of genetic differentiation generated by selection are generally associated with
81 spatial habitat variation (mosaics of different habitats or environmental gradients), and
82 the temporal stability of such patterns provides further support for local selection
83 (Schmidt & Rand 2001; Véliz et al. 2004; Gagnaire et al. 2012).

84 Studies focusing on post-settlement local adaptation in marine organisms usually
85 lack the level of replication of sweepstake recruitment studies (e.g. David et al. 1997;
86 Moberg & Burton 2000). Population studies in marine organisms are often only
87 performed once, with observed genetic patterns then assumed to illustrate species
88 population dynamics (Hedgecock et al. 2007). This may be misleading because within-
89 or among-generational differences in population structure and patterns of connectivity
90 among subpopulations are poorly captured by one single ‘snapshot’ observation (Carson
91 et al. 2010; Bert et al. 2014). The impact on observed genetic structure of demographic
92 and ecological factors such as cohort size, larval mixing, predictability of habitat patches,
93 local currents driving recruitment, or the local strength of selection, most likely varies
94 from one generation to the next (Lottherhos & Markel 2012; Therkildsen et al. 2013).
95 Ideally, a model of fluctuating selection that varies in space and/or time would offer a
96 better representation of reality (Hellberg 2006; Hedgecock & Pudovkin 2011; Moody et
97 al. 2015). This makes the eco-evolutionary dynamics of such metapopulations potentially

98 idiosyncratic, with little scope for the systematic directional changes that are typically
99 expected under habitat-based selection (Hanski 2011).

100 The gilthead sea bream (*Sparus aurata*) is a euryhaline, coastal marine fish in
101 which spatially varying selection between marine and lagoon habitats has been reported
102 in the North Western Mediterranean (Chaoui et al. 2012). After hatching at sea in
103 December-February, a large proportion of the juveniles colonize coastal lagoons in
104 March-April for foraging (Mercier et al. 2011, Isnard et al. 2015), before returning to the
105 open sea in October-November to overwinter when lagoon water temperatures drop
106 (Audoin 1962, Lasserre 1974, Mercier et al., 2012). In the Gulf of Lions, two lagoons
107 located about 30km apart have been studied, which offer very different nursery habitats:
108 (i) the Mauguio lagoon, which is shallow and highly productive, with warm summer
109 temperatures and wide salinity variations, and (ii) the Thau lagoon, which is larger and
110 deeper, and ecologically more similar to the coastal marine environment due to major
111 connections with the Mediterranean (e.g. Mercier et al. 2012, Tournois et al. 2013, and
112 references therein). Using candidate gene markers located in the proximal promoter of
113 the growth hormone (*GH*) and prolactin (*Prl*) genes, Chaoui et al. (2012) found non-
114 neutral allele frequency shifts between young juveniles caught in marine and lagoon
115 habitats, and interpreted these patterns as a footprint of post-settlement selection.
116 Because *cis*-regulatory polymorphisms have been described for both *GH* and *Prl* genes in
117 sea bream (Astola et al. 2003, Almuly et al. 2008; respectively), Chaoui et al. (2012)
118 hypothesized that functional variants affecting gene expression might be selected
119 differently among habitats due to their phenotypic effects on growth and/or
120 osmoregulation (e.g. Streelman & Kocher 2002; Blel et al. 2010; Shimada et al. 2010).

121 In this study, we first specifically assessed the temporal stability of habitat-based
122 genetic differentiation at the *GH* and *Prl* loci, extending our spatial coverage to another

123 large coastal lagoon, Salses-Leucate, which is a marine-like habitat ecologically similar
124 to Thau lagoon (Bec et al. 2011, Mercier et al. 2012, Tournois et al. 2013). Twenty-one
125 putatively neutral microsatellite loci were analysed together with *GH* and *Prl* markers, in
126 order to partition the relative influence of drift and selection in patterns of genetic
127 variation. We also used next-generation amplicon sequencing to reconstruct haplotypes
128 of the *GH* and *Prl* genes in 24 individuals from Thau and Mauguio lagoons to search for
129 additional haplotype-based signals of selection.

130

131

132 **Materials and Methods**

133

134 *Sampling*

135 The north-western Mediterranean coast of the Gulf of Lions (Fig. 1) is characterized by a
136 series of contiguous lagoons that offer seasonal nurseries for the juveniles of numerous
137 highly prized fish species, including the gilthead sea bream (Quignard et al. 1984). The
138 Salses-Leucate, Thau, and Mauguio (also known as ‘Etang de l’Or’) lagoons are located
139 along a 150 km stretch of coastline, interspersed by various other lagoons. Sea bream
140 juveniles were captured by local fishermen using fyke nets in spring (from late April to
141 May 2011) as they entered the lagoons, and in autumn (from September to November
142 2011) as they started to migrate out to sea, as described in Isnard et al. (2015). Sampling
143 locations were located within each lagoon (i.e. not at the inlets/outlets, where the strong
144 marine influence makes this transition zone more similar to the marine environment).
145 Autumn samples considered in this study were also analysed by Isnard et al. (2015) for
146 their differences in growth rates and condition. Using back calculation from otolith
147 readings, Isnard et al. (2015) showed that autumn individuals have recruited at different

148 periods in the three lagoons (Electronic Suppl. Fig S1). Estimation of the recruitment
149 date in each lagoon closely matched the dates of sampling for our entering individuals in
150 Salses-Leucate and Thau. In Mauguio, however, individuals were sampled after the
151 estimated recruitment date (see Electronic Suppl. Fig S1). Fork length (mean \pm SD) of
152 entering individuals were 31.3 ± 2.5 mm in Salses-Leucate, 34.7 ± 3.7 mm in Thau, and
153 41.3 ± 4.4 mm in Mauguio, with populations means being significantly different from
154 each other (Tukey post-hoc test; all P 's < 0.05). Larger sizes observed at Mauguio should
155 thus reflect longer time for growth between estimated recruitment and fish sampling
156 dates. Hence, it is likely that entering and migrating sea bream individuals belong to the
157 same demographic unit within each lagoon (i.e., individuals sampled in autumn are likely
158 to have recruited in the same lagoon in spring, and do not belong to distinct arrival waves
159 except in Mauguio). Total lengths (mean \pm SD) of autumn (migrating) individuals were
160 found significantly larger in Mauguio (184 ± 11 mm) than in other lagoons (Thau: $172 \pm$
161 11 mm; Salses-Leucate: 167 ± 7 mm) that were not different of each other (Isnard 2012;
162 Isnard et al. 2015). On the day of capture, fish were transported on ice to the laboratory
163 and tissues were sampled and kept in ethanol for DNA analysis ($N = 256$), as
164 summarized in Table 1.

165

166 *Microsatellite genotyping*

167 A small piece of tissue was incubated overnight in a lysis buffer with $5\mu\text{l}$ proteinase K
168 (Qiagen). DNA was isolated using the protocol described in Aljanabi & Martinez (1997),
169 and the concentration of each individual DNA sample was evaluated using NanoDrop
170 8000 (ThermoScientific) and standardised to $20\text{ng}\cdot\mu\text{l}^{-1}$ of genomic DNA. Twenty-three
171 microsatellite loci were analyzed (Electronic Suppl. Table S1). Two of them were
172 candidate *cis*-regulatory microsatellites described for *GH* (Almuly et al. 2005) and *Prl*

173 (Astola et al. 2003) loci, respectively. Protocols for PCR amplification and genotyping at
174 these two loci are reported in Chaoui et al. (2009). The remaining twenty-one
175 microsatellite loci were developed by Franch et al. (2006) and Coscia et al. (2012)
176 (Electronic Suppl. Table S1), and were considered as putatively neutral. These loci were
177 grouped in two multiplexes of ten and eleven loci, respectively. Multiplexed PCR was
178 optimised using the QIAGEN multiplex PCR kit in a 10 μ l final volume with 4.5 μ l
179 Qiagen multiplex PCR master mix, 1 μ l Q-solution, 1 μ l of genomic DNA and 1.5 μ l of
180 each primer. PCR conditions were as follow: initial denaturation 5 min (95°C), 30 cycles
181 at 94°C for 30s, annealing at 58°C for 30s, elongation at 72°C for 60s, then a final
182 elongation of 5 min. Amplifications were performed on a Mastercycler Gradient
183 (Eppendorf) or a Gradient Cycler PTC200 (Bio-Rad) according to the above-mentioned
184 PCR conditions. Genotyping of individuals was performed on an ABI PRISM® 3130XL
185 DNA Analyzer (Life Technologies), using 5'-labelled primers and the GeneScan™-500
186 LIZ® Size Standard (Life Technologies) as internal size standard (1 μ l of multiplex-PCR
187 product; 12 μ l formamide, 0.2 μ l internal size standard). Allele scoring was performed
188 using the GeneMapper software v.4.0 (Life Technologies).

189

190 *Population genetic analyses*

191 Preliminary to genetic analyses, data consistency was checked with Micro-Checker 2.2.3
192 using default settings (van Oosterhout et al. 2004) to evaluate the presence of null alleles,
193 large allele drop-out and scoring errors. Deviations from the Hardy–Weinberg
194 expectations (HWE) within samples were investigated using GENETIX v4.05
195 (<http://mbb.univ-montp2.fr/MBB/subsection/downloads.php?section=2>), by testing the
196 null hypothesis of no significant departure from HWE ($f = 0$) through 5000 random
197 permutations. Among-population differentiation was measured using θ (Weir &

198 Cockerham's 1984) which estimates Wright's (1951) F_{ST} . In order to deal with unequal
199 sample sizes between spring (non-limiting) and autumn migrating samples (Table 1), we
200 tested for consistency of population differentiation estimates by randomly sampling
201 twenty-six individuals in each spring sample. This made the sample sizes of spring and
202 autumn samples similar (Table 1), and allowed us to perform unbiased comparisons. Five
203 hundred subsamples of $N = 26$ were randomly drawn for each spring sample, and these
204 bootstrapped samples were randomly associated in triads containing one sample of each
205 location: MA, TH and SL. Each triad was then associated to the real (i.e. non
206 bootstrapped) autumn samples, and population differentiation indices were estimated as
207 described above.

208 Patterns of linkage disequilibrium in each sample were investigated as proposed
209 by Weir (1979). Corrections for multiple testing were performed according to Narum
210 (2006) when necessary, for both real and bootstrapped data to maintain the significance
211 level at $\alpha = 0.05$. We estimated genetic relatedness among individuals within each
212 sample (i.e. kin aggregation) by estimating the coefficient of relatedness r_{xy} (Queller &
213 Goodnight 1989) using IDENTIX (Belkhir et al. 2002). Briefly, to test whether individuals
214 within each sample were genetically more related than expected by chance, the mean
215 identity index of all sea bream pairs was compared with the null distribution assuming no
216 relatedness. The null distribution was obtained by calculating identity indices for
217 randomly generated samples of similar size (Table 1).

218 Finally, we searched for putatively selected loci using the F_{ST} -outlier detection
219 method by Beaumont & Nichols (1996) implemented in the software LOSITAN (Antao et
220 al. 2008). Outlier loci were searched using three different levels of comparison that were
221 performed among (i) all six samples from Mauguio (MA), Thau (TH) and Salses-Leucate
222 (SL) lagoons, (ii) three entering (E) and (iii) three migrating (M) juvenile samples. In all

223 three tests, locus-specific F_{ST} values were compared with the null distribution of F_{ST}
224 generated with 100,000 simulated loci, using the estimated neutral mean F_{ST} to determine
225 the average differentiation level targeted in simulations.

226

227 *Next-generation sequencing of GH and Prl long-range amplicons*

228 Long-range amplicon sequencing of the two candidate genes was performed in order to
229 investigate haplotype structure. We also tested whether estimates of genetic
230 differentiation reached higher levels at each candidate microsatellite locus, or if nearby
231 gene regions provided even larger estimates, potentially indicating the causative
232 mutations experiencing selection. We hypothesized that under habitat-based selection,
233 genetic differentiation would be higher in older juveniles caught in autumn than in
234 younger ones caught soon after entering coastal lagoons. Therefore, we randomly
235 selected 12 autumn individuals from MA and 12 individuals from TH to evaluate genetic
236 differentiation between the two most extreme habitats (Chaoui et al. 2012). New primers
237 were designed to amplify the full gene sequences of *GH* and *Prl* genes using Long-Range
238 (LR) PCR (see LR- PCR primers Electronic Suppl. Table S1). We targeted a 3685 base
239 pair (bp) region extending from the first (E1) to the last exon (E6) for the *GH* gene, and a
240 4277 bp region spanning the full gene sequence, including both 5' and 3'UTRs
241 (UnTranslated Regions) for the *Prl* gene. LR-PCR was performed using the PCR
242 Extender System (5 Prime). Reaction mix comprised 2 μ l of 10X LR-PCR buffer (15 mM
243 MgCl₂), 1 μ l of each primer (20 μ M), 0.5 μ l of enzyme mix and 2 μ l of DNA (25ng. μ l⁻¹) in
244 a 20 μ l final reaction volume. LR-PCR amplification parameters were as follows: initial
245 denaturation at 94°C for 3 min, 10 cycles of denaturation at 94°C for 20s, annealing at
246 59°C for 30s, elongation at 68°C for 4 min, 25 cycles of denaturation at 94°C for 20s,
247 annealing at 59°C for 30s, elongation at 68°C for 4 min + 2s per cycle, and ended by 10

248 min of final elongation at 68°C. LR-PCR products obtained for 24 autumn juveniles from
249 MA and TH were quantified for each gene using Qubit dsDNA BR Assay Kit
250 (Invitrogen), and pooled in equimolar proportions for each individual. The 24 individual
251 pools were then submitted to tagmentation and individual indexing using the Nextera XT
252 DNA Sample Preparation Kit 24 Samples (Illumina), following the library preparation
253 guide. The 24 individual libraries were pooled using 1ng DNA per individual, and
254 quantified on a Bioanalyzer 2100 (Agilent) prior to sequencing on the Illumina MiSeq
255 platform, with 250 paired-end reads.

256

257 *Reconstruction and analysis of individual haplotypes*

258 Individual raw sequencing data were quality-filtered using TRIMMOMATIC (Bolger et al.
259 2014), to remove adapters and low quality reads. We used *GH* and *Prl* gene sequences
260 retrieved from GenBank to perform a reference assembly for each individual using
261 GENEIOUS 7.1.5 (Kearse et al. 2012). Five iterations were used for alignment, after each
262 of which the reference sequence was corrected using the previous consensus alignment.
263 Heterozygote positions were identified for each individual and encoded using the IUPAC
264 ambiguity code, and individual consensus sequences were aligned for each gene using
265 CLUSTALW (Thompson et al. 1994). Alignments were edited by hand to remove
266 ambiguities within regions of low complexities (mini- and microsatellites). Therefore,
267 variation in repeat-number was not scored and we only focused on SNP variation in
268 subsequent analyses.

269 Individual haplotype reconstruction was performed using the coalescent Bayesian
270 inference method implemented in PHASE v2.1 (Stephens et al. 2001) using default
271 settings. We then evaluated the extent of haplotype structure to search for signatures of
272 partial selective sweeps. To do this, phased individual haplotypes were then used to

273 compute haplotype diversity (H), nucleotide diversity (π), and Tajima's D (Tajima 1989)
274 for the whole gene sequences, as well as in a 100bp sliding window using DNASP
275 (Librado & Rozas 2009). Synonymous and nonsynonymous polymorphisms were
276 searched with the coding regions of each gene. Finally, the similarity relationships
277 among haplotypes were represented with a NEIGHBORNET network for each gene using
278 SPLITSTREE4 (Huson & Bryant 2006).

279

280

281 **Results**

282

283 *Hardy-Weinberg equilibrium and relatedness*

284 No microsatellite marker displayed evidence of null alleles, allelic drop-out or allele
285 scoring problems. No consistent linkage disequilibrium was found among loci in any
286 sample. Thirty-six significant linkage disequilibrium values were detected over 1516
287 tests [$\sim 3.37\%$]; a proportion that would be expected by chance for $\alpha = 0.05$. Six loci
288 displayed significant departures from HWE (deficits in heterozygotes: *Dd16*, *172EP*,
289 *C67b*, *Fd92H*, *GH*, *Prl*), but only two of them (*C67b*, *Prl*) presented significant $f > 0$
290 values in four samples (spring and autumn samples of Manguio and Thau: MA-E, MA-
291 M, TH-E, and TH-M, Table 1). Deficits in heterozygotes were detected in all samples
292 except SL-M, and remained significant in only two samples when the *C67b* and *Prl* loci
293 were removed from the data set (Table 1). This reduced dataset indicated that deviations
294 from HWE were most likely due to a few loci, and were not generated by a genome-wide
295 effect due to the mixing of larvae from discrete reproductive units. No significant
296 relatedness was detected within each individual sample, with all values of r_{xy} being

297 slightly negative (Table 1). This did not mean that related individuals were not present,
298 but simply that their number was too low to influence genetic structure.

299

300 *Microsatellite spatiotemporal variation patterns*

301 *Real datasets* - The level of genetic differentiation estimated using all 23 markers over
302 the whole data set was not significant ($\theta = 0.0020 \pm 0.0160$), nor was genetic
303 differentiation among E and M samples ($\theta = 0.0049 \pm 0.0108$, and $\theta = -0.0035 \pm 0.0094$;
304 respectively). However, these multilocus average values did not reflect the variance
305 among loci (Fig. 2). The *GH* and *Prl* loci showed a significant global genetic
306 differentiation (Fig. 2A; $\theta_{GH} = 0.0192$, $\theta_{Prl} = 0.0188$; P 's < 0.001). Surprisingly, genetic
307 differentiation at *GH* and *Prl* markers was significant among the E samples but not
308 among the M samples (Fig. 2B&C). All pairwise differentiation values calculated
309 between spring E samples were significant for both *GH* and *Prl* loci (*GH*: $\theta_{SL-E/TH-E} =$
310 0.0285 , $\theta_{SL-E/MA-E} = 0.0331$, $\theta_{TH-E/MA-E} = 0.0199$, all P 's < 0.01; *Prl*: $\theta_{SL-E/TH-E} = 0.0110$, θ_{SL-}
311 $_{E/MA-E} = 0.0223$, $\theta_{TH-E/MA-E} = 0.0162$, all P 's < 0.01). Significant genetic differentiation at
312 these loci in spring is moreover related with observed phenotypic differentiation for size
313 recorded among E samples (see “Materials and Methods – *Sampling*”). The only marker
314 showing significant genetic differentiation among autumn M samples was locus *C67b*
315 ($\theta_{C67b} = 0.0181$; $P < 0.01$). Significant genetic differentiation at this locus was mostly
316 explained by the SL-M sample that differed from both TH-M and MA-M ($\theta_{SL-M/TH-M} =$
317 0.0224 , $P = 0.013$; $\theta_{SL-M/MA-M} = 0.0286$, $P < 0.001$), while TH-M and MA-M samples
318 were not differentiated ($\theta_{MA-M/TH-M} = 0.0055$, NS). This result did not match phenotypic
319 differentiation for size, as the size of M individuals was found significantly larger in MA
320 compared to the other lagoons, but similar between SL and TH (see “Materials and
321 Methods – *Sampling*”). Outlier detection tests corroborated the outlying genetic

322 differentiation level of *GH* and *Prl* loci among the three E samples, as well as for locus
323 *C67b* among the three M samples (Electronic Suppl. Fig. S2).

324 Genetic differentiation estimates between E and M samples within each lagoon
325 revealed no significant temporal genetic differentiation in MA (*Prl*: $\theta = 0.0094$, NS; *GH*:
326 $\theta = 0.0143$, NS), but significant allele frequency changes in TH (*Prl*: $\theta = 0.0233$, $P < 0.01$;
327 *GH*: $\theta = 0.0193$, $P < 0.01$) and in SL (*Prl*: $\theta = 0.0254$, $P < 0.01$; *GH*: $\theta = 0.0289$, $P < 0.01$).
328 Moreover, autumn juveniles migrating out of TH and SL were genetically
329 undifferentiated (*Prl*: $\theta = -0.0045$, NS; *GH*: $\theta = 0.0004$, NS). Thus, the loss of genetic
330 differentiation among lagoons in migrating samples was largely due to similar temporal
331 changes in allele frequencies that occurred in TH and SL lagoons between spring and
332 autumn. Fish sampled in TH and SL were showed to likely belong to a demographic unit
333 that undergone a similar life-history throughout the year (see “Materials and Methods –
334 *Sampling*”, and Electronic Suppl. Fig. S1), suggesting that these demographic units
335 inhabiting ecologically similar lagoons undergone similar allele frequency variation
336 between spring and autumn.

337

338 *Bootstrapped datasets* - Because spring and autumn sample sizes differed, we performed
339 bootstrap resampling to investigate the consistency of patterns of genetic differentiation
340 reported in the previous subsection. Sample sizes of E samples were standardized to $N =$
341 26 individuals to match the sample size of M samples (Table 1). The level of overall
342 genetic differentiation (i.e. among the 6 samples) estimated using all 23 markers was
343 found significant in only 7 (1.4%) out of 500 replicates. Triads of bootstrapped E
344 samples were also compared and significant multilocus genetic differentiation was
345 detected in only 13 cases (2.6%) out of 500 bootstrap resamplings. Such patterns are
346 expected by chance alone, and therefore multilocus genetic homogeneity detected with

347 the real dataset among all 6 samples or among the E samples were likely not affected by
348 unequal sampling sizes between spring and autumn juveniles.

349 When looking specifically at patterns of genetic differentiation at the *GH* and *Prl*
350 loci in bootstrapped samples, results reported significant overall genetic differentiation in
351 448 (89.6%) and 465 (93.0%) cases out of 500 bootstrap resamplings for *Prl* and *GH*,
352 respectively. These numbers reached 463 (92.6%) and 484 (96.8%) for *Prl* and *GH*,
353 respectively, when considering only the triads of bootstrapped E samples. Differentiation
354 patterns observed at the *GH* and *Prl* loci were thus not dependent on sample size. By
355 contrast, we found a lack of genetic differentiation among E samples in 7.4% and 3.2%
356 of resampled data sets for *Prl* and *GH*, (respectively. Hence, the lack of observed genetic
357 differentiation among the real M samples was likely not affected by sample sizes (Fig. 2).

358 Similarly, when standardizing E samples to $N = 26$, locus *C67b* displayed
359 significant genetic differentiation in only 21 (4.2%) cases out of 500. This suggested that
360 the absence of genetic differentiation among real E samples was true for this locus.

361 Overall, our bootstrap procedure supported that the observed genetic
362 differentiation patterns at the *GH*, *Prl* and *C67b* loci were robust to unequal sample sizes
363 with error rates generally <0.05 . We hence considered that estimates of pairwise genetic
364 differentiation and outlier detection test reported in the previous subsection for the real
365 data set were relevant.

366

367 *Haplotype structure in the sea bream for GH and Prl genes*

368 Microsatellite *GH* and *Prl* markers did not display genetic differentiation in autumn
369 samples, so we additionally investigated whether genetic differentiation could be higher
370 at other genomic locations within or close to each candidate gene. Among a total of
371 14,407,916 filtered paired-end reads, 5,067,129 and 5,753,776 were successfully aligned

372 to the *GH* and *Prl* gene reference sequences, respectively, providing extremely profound
373 sequencing depth for each gene in each individual (Electronic Suppl. Table S2). The *GH*
374 and *Prl* gene sequences analysed in this study have lengths of 3,633bp and 4,079bp,
375 respectively (Table 2). Haplotype diversity (H) was close to one at each locus, indicating
376 that almost every individual had two unique haplotypes (Table 2). Nucleotide diversity
377 was almost twice as high for *Prl* ($\pi = 0.0072$) as for *GH* ($\pi = 0.0039$). Albeit non-
378 significant, Tajima's D showed a slight excess of intermediate frequency sites at locus
379 *Prl*, and an excess of rare variants at locus *GH* (positive and negative estimates of D ,
380 respectively; Table 2). Nucleotide diversity (π) profiles calculated in overlapping 100pb
381 windows revealed very heterogeneous diversity along each gene (Fig. 3). These profiles
382 largely reflected the intron/exon structure within each gene, with exonic regions showing
383 reduced diversity. Only a few non-synonymous mutations segregating at low frequencies
384 were found in each gene (not reported), making them unlikely to be related to the
385 functional variants under spatially varying selection. The highest diversity levels were in
386 the 5'UTR, as well as in introns 1 and 3 of the *Prl* gene, and in introns 1 and 3 of the *GH*
387 gene. Little linkage disequilibrium was found among the most variable regions within
388 each gene, indicative of low haplotype structure. However, we could distinguish for each
389 gene two haplotype groups that were evenly distributed among TH-M and MA-M
390 juveniles (Fig. 4).

391

392

393 **Discussion**

394 The aim of this study was to re-investigate patterns of genetic differentiation among sea
395 bream populations collected in coastal lagoons of the Gulf of Lions, using a larger array
396 of microsatellite markers than in previous studies in this species (De Innocentiis et al.

397 2004; Alarcon et al. 2004; Chaoui et al. 2009; but see Coscia et al. 2012). A particular
398 focus was made on microsatellite markers located close to two candidate genes, *GH* and
399 *Prl*, that are functionally related to growth and osmoregulatory capacities in fish
400 (Streelman and Kocher 2002; Almuly et al. 2008; Quéré et al. 2010), and have been
401 previously showed to be involved in habitat-based genetic differentiation in sea bream
402 (Chaoui et al. 2012). This latter study suggested that *GH* and *Prl* genotypes with varying
403 selective advantage among lagoons impact mortality in juvenile sea breams that spent
404 their first year of life in ecologically contrasted lagoons (i.e. with different salinity,
405 temperature, food availability conditions). Here, we re-evaluated this result by analysing
406 genetic differentiation patterns at *GH* and *Prl* genes together with a new set of neutral
407 loci in another cohort of sea bream sampled at two different periods of their first year of
408 life.

409 The set of twenty three microsatellite markers used in this study did not show any
410 significant multilocus genetic differentiation among lagoons in the newly recruited
411 juveniles, confirming that the sea bream population in the Gulf of Lions broadly behaves
412 as a panmictic unit as reported in Chaoui et al. (2009) with fewer markers. Nevertheless,
413 sea bream larvae from discrete reproductive events have been reported to colonise the
414 three lagoons at different dates, separated by up to three weeks (Isnard et al. 2015;
415 Electronic Suppl. Fig. S1). Sweepstake recruitment may therefore occur, although it did
416 not translate into significant multilocus genetic differentiation in our data set. The
417 absence of significant kinship structure was also suggestive of a large local population
418 size, as previously reported for the sea bass (*Dicentrarchus labrax*) in the Gulf of Lions
419 (Lemaire et al. 2000; Guinand et al. 2008).

420 The candidate *GH* and *Prl* markers were found genetically differentiated in our
421 study. Bootstrap resampling showed that genetic differentiation patterns observed at

422 these loci were robust to variation in sampling size among samples. If observed genetic
423 differentiation was due to neutral demographic factors, other loci would have displayed
424 non-null differentiation values. These results thus confirm that habitat-based selection
425 operates at the *GH* and *Prl* genes, as previously reported in Chaoui et al. (2012). Besides,
426 the only other marker showing significant genetic differentiation and a possible footprint
427 of selection was locus *C67b*. In this case, genetic differentiation was observed among M
428 samples, and was mainly explained by the distinctiveness of the SL-M sample. This
429 locus was associated with the ubiquitin ligase *Nedd4-2* (neural precursor cell-expressed,
430 developmentally down-regulated protein 4-2) gene, a gene involved in regulation of the
431 epithelial sodium channel in several organs, including kidney in mouse (Gormley et al.
432 2003; Bens et al. 2006). It may therefore be associated with osmoregulation in sea bream,
433 but his function has never been investigated in fish. This unexpected result for locus
434 *C67b* should be re-evaluated in an independent study and it will not be considered further
435 here.

436 Considering candidate *GH* and *Prl* markers, only E samples were found
437 genetically differentiated. Together with observed differences in body size among E
438 individuals from each lagoon, these results suggest that early selection may have
439 occurred at or just after settlement in response to specific selective pressures in each
440 lagoon. A sequential temporal genetic response to a sea-lagoon selective gradient was
441 already suggested for young sea bass recruits in the MA lagoon, with a possible signature
442 of early selection appearing very soon after colonisation, while further selective
443 differences were only observed after some months (Guinand et al. 2015) (see also Planes
444 & Roman 2004 for the white sea bream, *Diplodus sargus*). In addition Chaoui et al.
445 (2012) showed that a mechanism based on genotype-dependent habitat choice with no

446 selective mortality was very unlikely to produce the observed genetic structure at the *GH*
447 and *Prl* loci.

448 Conversely, no genetic differentiation at candidate microsatellite gene markers
449 was detected among M samples. This result was confirmed by long-range amplicon
450 sequencing which could not detect any signal of haplotype structure. These results
451 challenge the traditional view that genetic differentiation at candidate gene marker
452 accumulates with the time spent within the habitats where selection is at play, as
453 previously recorded in the flounder (*Platichthys flesus*; Laroche et al. 2002) or the
454 common sole (*Solea vulgaris*; Guinand et al. 2011). Temporal genetic differentiation has
455 been tested previously in sea bream, although using a distinct sampling scheme (Chaoui
456 et al. 2012). Interestingly, a trend toward a reduction in genetic differentiation was
457 already observed at locus *Prl* in the MA lagoon. Thus, the genetic homogenization
458 observed in this study between spring and autumn juveniles is not contradictory with
459 previous observations based on a different year cohort. However, it affected both *GH* and
460 *Prl* markers, and resulted in a loss of genetic differentiation at candidate gene markers in
461 autumn.

462 Hypotheses explaining the genetic homogeneity among M samples of sea bream
463 first involve the potential mixing of individuals from the different habitats, prior to
464 migration of individuals to the sea. Otolith microchemistry analyses have revealed that
465 individuals from MA, TH and SL can be distinguished by specific chemical signatures
466 associated with each nursery habitat (Mercier et al. 2011, 2012; Tournois et al. 2013).
467 These signatures are relatively stable across multiple annual cohorts despite variations in
468 meteorological conditions across the years. This suggests that there are very low levels of
469 migration among lagoons at the juvenile stage during the first year, even at these
470 relatively short distances. While lagoons such as TH are connected by canals to several

471 adjacent lagoons that are ecologically similar to MA, the mixing of juveniles among the
472 different habitats is unlikely.

473 A second hypothesis would be that similar environmental conditions later in the
474 year make allele frequencies at candidate genes to converge to similar equilibrium
475 frequencies. In the present study, allele frequency changes between the E and M
476 juveniles of the 2011 sea bream cohort mainly occurred in TH and SL for both genes.
477 Since these marine-like nurseries have almost identical fish species composition (Pérez-
478 Ruzafa et al. 2011), and similar physico-chemical characteristics (e.g., Bec et al. 2011),
479 temporal changes in allele frequency observed between TH and SL may suggest
480 convergence to an equilibrium frequencies while fish were genetically distinct at the two
481 candidate gene markers in spring. TH and SL individuals were also different for the
482 average size in spring while they were not anymore in autumn, indicating that both
483 genotypic and phenotypic convergence occurred in TH and SL. Isnard et al. (2015)
484 reported that sea bream inhabiting these two lagoons (*i*) have similar characteristics in
485 body condition in autumn compared to MA, and (*ii*) that their growth rates were similar
486 during their life-history, also compared to MA. However, these observations do not
487 explain why similar allele frequencies are also observed in the MA sample in autumn,
488 while this lagoon is ecologically distinct from SL and TH (e.g., Bec et al. 2011; Pérez-
489 Ruzafa et al. 2011) and is also phenotypically differentiated (Isnard et al. 2015). Results
490 observed at candidate genes for the MA lagoon are also puzzling, as no genetic
491 differentiation was observed between the E and M individuals at the *Prl* and *GH* loci in
492 this study, while substantial genetic differentiation was observed at each locus among
493 young and older sea bream in Chaoui et al. (2012). Therefore, the relative roles of
494 migration and post-settlement selection in the autumnal dedifferentiation remain difficult
495 to disentangle in sea bream.

496 Our results hence suggest that patterns of genetic differentiation estimated for
497 markers associated to candidate genes reflect spring selection in the 2011 cohort of sea
498 bream. However, the temporal analysis of genetic differentiation patterns at each
499 candidate gene in each lagoon suggests a lack of stability for the patterns established in
500 spring. This may be due to highly dynamic and heterogeneous environmental conditions
501 of coastal lagoons (Pérez-Ruzafa et al. 2005, 2007; Tournois et al. 2013) that may
502 generate within-year variations in the strength and direction of selection. This
503 emphasizes the need for increased spatiotemporal resolution, to monitor the potentially
504 complex responses to selection during early development of marine organisms (Broquet
505 et al. 2013; Hernandez-Garcia et al. 2015).

506 As a supplement to the study of microsatellite markers located in the proximal
507 promoter region of the *GH* and *Prl* genes, the study of molecular variation by amplicon
508 resequencing allowed the investigation of the footprint produced by spatially varying
509 selection within these genes. A detailed map of genetic variation was obtained along *GH*
510 and *Prl* genes using 250 bp paired-end resequencing. This approach was efficient for
511 reconstructing individual haplotypes defined by combinations of neighbouring SNPs, but
512 could not determine the exact number of repeats associated with micro- and mini-satellite
513 repeats. However, we detected an average number of 35 repeats for the 17 bp motif
514 (GACCTGTCTGTCTCTCT) of the regulatory mini-satellite located in the first intron of
515 the *GH* gene (Almuly et al. 2008). Interestingly, intron 1 was the most variable region
516 within the *GH* gene (due to variation within and among repeats), and the proximal
517 promoter region involved in the modulation of *Prl* gene expression (Astola et al. 2003)
518 was also among the most variable regions of the *Prl* gene. These results are therefore
519 consistent with a form of balancing selection maintaining high levels of polymorphism in
520 regulatory regions whereas, initially, the allele frequency shifts observed at candidate

521 microsatellite loci have been attributed to direct selection acting on *cis*-regulatory
522 polymorphisms (see Chaoui et al 2012). Although our analysis revealed haplotype groups
523 for each gene, close similarities among groups indicated that any possible signature of
524 selection had largely been erased by recombination. This is consistent with the prediction
525 that the chromosomal signature left by local (i.e. habitat-based) selection in panmixia
526 typically affect narrow genomic regions, especially if spatially varying selection has been
527 maintaining polymorphism for a long time (Gagnaire et al. 2015).

528

529

530

531 *Acknowledgements:* This research was funded by IFREMER (Project SpartaGen of the
532 GDR MarCo), by the CNRS (Project EC2CO METASPAR), and the TOTAL Foundation
533 (project LAGUNEX). The authors are grateful to local fishermen for fish collections, to
534 E. Isnard and F. Ferraton for fish dissection, to L. Velez for helping with the figures, and
535 to M.-T. Augé for assistance during lab work. Our studies on population genetics of sea
536 bream were first initiated by F. Bonhomme.

537

538 *Archiving of data:* Data will be deposited on a public database upon final acceptance of
539 the current submission.

540

541 **References**

- 542 Aglieri G, Papetti C, Zane L, Milisenda G, Boero F, Piraino S (2014) First evidence of
543 inbreeding, relatedness and chaotic genetic patchiness in the holoplanktonic jellyfish *Pelagia*
544 *noctiluca* (Scyphozoa, Cnidaria). PLoS ONE 9: e99647
- 545 Alarcón JA, Magoulas A, Georgakopoulos T, Zouros E, Alvarez MC (2004) Genetic comparison
546 of wild and cultivated European populations of the gilthead sea bream (*Sparus aurata*),
547 Aquaculture 230: 65-80.
- 548 Almuly R, Poleg-Danin Y, Gorshkov S, Gorshkova G, Rapoport B, Soller M, Kashi Y,
549 Funkenstein B (2005) Characterization of the 5' flanking region of the growth hormone gene of
550 the marine teleost, gilthead sea bream *Sparus aurata*: analysis of a polymorphic microsatellite
551 in the proximal promoter. Fish Sci 71: 479–490
- 552 Almuly R, Skopal T, Funkenstein B (2008) Regulatory regions in the promoter and first intron of
553 *Sparus aurata* growth hormone gene: repression of gene activity by a polymorphic
554 minisatellite. Comp Biochem Physiol D: Genomics Proteomics 3: 43–50
- 555 Antao T, Lopes A, Lopes RJ, Beja-Pereira A, Luikart G (2008) LOSITAN: a workbench to detect
556 molecular adaptation based on a F_{st} -outlier method. BMC Bioinformatics 9: 323
- 557 Astola A, Ortiz M, Caldach-Giner JA, Perez-Sanchez J, Valdivia MM (2003) Isolation of *Sparus*
558 *auratus* prolactin gene and activity of the *cis*-acting regulatory elements. Gen Comp
559 Endocrinol 134: 57–61
- 560 Audouin J (1962) La daurade de l'étang de Thau *Chrysophrys aurata* (Linné). Revue des
561 Travaux l'Institut des Pêches Maritimes 26
- 562 Beaumont MA, Nichols RA (1996) Evaluating loci for use in the genetic analysis of population
563 structure. Proc R Soc Biol Sci 263: 1619–1626
- 564 Bec B, Collos Y, Souchu P, Vaquer A, Lautier J, Fiandrino A, Benau L, V. Orsoni V, Laugier T
565 (2011) Distribution of picophytoplankton and nanophytoplankton along an anthropogenic
566 eutrophication gradient in French Mediterranean coastal lagoons. Aquat Microb Ecol 63:
567 29–45
- 568 Belkhir K, Castric V, Bonhomme F (2002) IDENTIX, a software to test for relatedness in a
569 population using permutation methods. Mol Ecol Notes 2: 611–614
- 570 Bens M, Chassin C, Vandewalle A (2006) Regulation of NaCl transport in the renal collecting
571 duct: lessons from cultured cells. Pflugers Arch - Eur J Physiol 453: 133-146
- 572 Bert TM, Arnold WS, Wilbur AE, Seyoum S, McMillen-Jackson AL, Stephenson SP, Weisberg
573 RH, Yarbrow LA 2014. Florida Gulf bay scallop (*Argopecten irradians concentricus*) population
574 genetic structure: form, variation, and influential factors. J Shellfish Res 33: 199-236

575 Blel H, Panfili J, Guinand B, Berrebi P, Said K, Durand JD (2010) Selection footprint at the first
576 intron of the *Prl* gene in natural populations of the flathead mullet (*Mugil cephalus*, L. 1758). *J*
577 *Exp Mar Biol Ecol* 210: 60-67

578 Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence
579 data. *Bioinformatics* btu170

580 Broquet T, Viard F, Yearsley JM (2013) Genetic drift and collective dispersal can result in
581 chaotic genetic patchiness. *Evolution* 67: 1760-1775

582 Chaoui L, Kara MH, Quignard JP, Faure E, Bonhomme F (2009) Strong genetic differentiation of
583 the gilthead sea bream *Sparus aurata* (L., 1758) between the two western banks of the
584 Mediterranean. *C R Biol* 332: 329–335

585 Chaoui L, Gagnaire PA, Guinand B, Quignard JP, Tsigenopoulos C, Kara MH, Bonhomme F
586 (2012) Microsatellite length variation in candidate genes correlates with habitat in the gilthead
587 sea bream *Sparus aurata*. *Mol Ecol* 21: 5497–5511.

588 Carson HS, López-Duarte PC, Rasmussen L, Wang D, Levin LA (2010) Reproductive timing
589 alters population connectivity in marine metapopulations. *Curr Biol* 20: 1926-1931

590 Coscia I, Vogiatzi E, Kotoulas G, Tsigenopoulos CS, Mariani S (2012) Exploring neutral and
591 adaptive processes in expanding populations of gilthead sea bream, *Sparus aurata* L., in the
592 North-East Atlantic. *Heredity* 108: 537-546

593 De Innocentiis S, Lesti A, Livi S, Rossi AR, Crosetti D, Sola L (2004) Microsatellite markers
594 reveal population structure in gilthead sea bream *Sparus auratus* from the Atlantic Ocean and
595 Mediterranean Sea. *Fish Sci* 70: 852-859

596 David P, Perdieu MA, Pernot AF, Jarne P (1997) Fine-grained spatial and temporal population
597 genetic structure in the marine bivalve *Spisula ovalis* L. *Evolution* 51: 1318–1322

598 Domingues CP, Creer S, Taylor MI, Queiroga H, Carvalho GR (2011) Temporal genetic
599 homogeneity among shore crab (*Carcinus maenas*) larval events supplied to an estuarine
600 system on the Portuguese northwest coast. *Heredity* 106: 832–840

601 Franch R, Louro B, Tsalavouta M, Chatziplis, Tsigenopoulos CS, Sarropoulou E, Antonello J,
602 Magoulas A, Mylonas CC, Babbucci M, Patarnello T, Power DM, Kotoulas G, Bargelloni L
603 (2006) A genetic linkage map of the hermaphrodite teleost fish *Sparus aurata* L. *Genetics* 174:
604 851-861

605 Gagnaire P-A, Normandeau E, Côté C, Hansen MM, Bernatchez L (2012) The genetic
606 consequences of spatially varying selection in the panmictic American eel (*Anguilla rostrata*).
607 *Genetics* 190: 725-736

608 Gagnaire P-A, Broquet T, Aurelle D, Viard F, Souissi A, Bonhomme F, Arnaud-Haond S, Bierne
609 N (2015) Using neutral, selected, and hitchhiker loci to assess connectivity of marine
610 populations in the genomic era. *Evol Applic* 8: 769-786

611 Gormley K, Dong Y, Sagnella GA (2003) Regulation of the epithelial sodium channel by
612 accessory proteins. *Biochem J* 371: 1-14

613 Guinand B, Dujardin E, Dufour V, Tsigenopoulos C (2008) Characterisation of genetic structure
614 of *Dicentrarchus labrax* larvae in two nurseries of the Gulf of Lions (NW Mediterranean).
615 *Aquat Living Resour* 21: 81–87

616 Guinand B, Durieux EDH, Dupuy C, Cerqueira F, Bégout ML (2011) Phenotypic and genetic
617 differentiation in young-of-the-year common sole (*Solea solea*) at differentially contaminated
618 nursery grounds. *Mar Environm Res* 71: 195-206

619 Guinand B, Quéré N, Desmarais E, Lagnel J, Tsigenopoulos CS, Bonhomme F. (2015) From the
620 laboratory to the wild: salinity-based genetic differentiation of the European sea bass
621 (*Dicentrarchus labrax*) using gene-associated and gene-independent microsatellite markers.
622 *Mar Biol* 162: 515-538

623 Hanski IA (2011) Eco-evolutionary spatial dynamics in the Glanville fritillary butterfly. *Proc*
624 *Natl Acad Sci USA* 108: 14397-14404

625 Hauser L, Carvalho GR (2008) Paradigm shifts in marine fisheries genetics: ugly hypotheses
626 slain by beautiful facts. *Fish Fish* 9: 333–362

627 Hedgecock D (1986) Is gene flow from pelagic larval dispersal important in the adaptation and
628 evolution of marine invertebrates. *Bull Mar Sci* 39: 550-564

629 Hedgecock D (1994) Does variance in reproductive success limit effective population size of
630 marine organisms? In: AR Beaumont (Ed.) *Genetics and evolution of aquatic organisms*.
631 Chapman & Hall, London, p 122-134

632 Hedgecock D, Launey S, Pudovkin AI, Naciri Y, Lapègue S, Bonhomme F (2007) Small
633 effective number of parents (N_b) inferred for a naturally spawned cohort of juvenile European
634 flat oysters *Ostrea edulis*. *Mar Biol* 150: 1173–1182

635 Hedgecock D, Pudovkin AI (2011) Sweepstakes reproductive success in highly fecund marine
636 fish and shellfish: a review and commentary. *Bull Mar Sci* 87: 971-1002

637 Hellberg ME (2006) Genetic approaches to understanding marine metapopulation dynamics. In:
638 JP Kritzer, Sale PF (eds) *Marine metapopulations*. Elsevier, Amsterdam, p 431–455

639 Hellberg ME (2009) Gene flow and isolation among populations of marine animals. *Ann Rev*
640 *Ecol Evol Syst* 40: 310–491

641 Hernández-García R, Muñoz I, López-Capel A, Marcos C, Pérez-Ruzafa Á (2015) The influence
642 of environmental variability of a coastal lagoon ecosystem on genetic diversity and structure of
643 white seabream [*Diplodus sargus* (Linnaeus 1758)] populations. *Mar Ecol* 36: 1144-1154

644 Huson DH, Bryant D (2006) Applications of phylogenetic networks in evolutionary studies. *Mol*
645 *Biol Evol* 23: 254-267

646 Iacchei M, Ben-Horin T, Selkoe KA, Bird CE, García-Rodríguez FJ, Toonen RJ (2013)
647 Combined analyses of kinship and F_{ST} suggest potential drivers of chaotic genetic patchiness in
648 high gene-flow populations. *Mol Ecol* 22: 3476-3494

649 Isnard E (2012) Comparison of condition and life history traits of gilthead seabream *Sparus*
650 *aurata* L. juveniles after their first summer in various lagoons on the Gulf of Lions
651 (Mediterranean Sea). MSc. Thesis. Univ. Montpellier 2. 41p

652 Isnard E, Tournois J, McKenzie DJ, Ferraton F, Bodin N, Aliaume C, Darnaude AM (2015)
653 Getting a good start in life? A comparative analysis of the quality of lagoons as juvenile
654 habitats for the gilthead seabream *Sparus aurata* in the Gulf of Lions. *Estuar Coasts* 38: 1937-
655 1950

656 Johnson MS, Black R (1984) Patterns beneath the chaos: the effect of recruitment on genetic
657 patchiness in an intertidal limpet. *Evolution* 38: 1371-1383

658 Kearse M, Moir R, Wilson A, Stone-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A,
659 Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic:
660 an integrated and extendable desktop software platform for the organization and analysis of
661 sequence data. *Bioinformatics* 28: 1647-1649

662 Koehn RK, Newell RI, Immermann F (1980) Maintenance of an aminopeptidase allele frequency
663 cline by natural selection. *Proc Natl Acad Sci USA* 77: 5385-5389

664 Laroche JQuiniou L, Juhel G, Auffret M, Moraga D (2002) Genetic and physiological responses
665 of flounder (*Platichthys flesus*) populations to chemical contamination in estuaries. *Environm*
666 *Toxicol Chem* 21: 2705-2712.

667 Larson RJ, Julian RM (1999) Spatial and temporal genetic patchiness in marine populations and
668 their implications for fisheries management. *CalCOFI Rep* 40: 94-99

669 Lasserre G (1974) Stock-number, growth, production and migration of giltheads *Sparus auratus*
670 (L. 1758) of group 0+ from étang de thau. *Cah Biol Mar* 15: 89-111

671 Lemaire C, Allegrucci G, Naciri M, Bahri-Sfar L, Kara H, Bonhomme F (2000) Do discrepancies
672 between microsatellite and allozyme variation reveal differential selection between sea and
673 lagoon in the sea bass (*Dicentrarchus labrax*)? *Mol Ecol* 9: 457-467

674 Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA
675 polymorphism data. *Bioinformatics* 25: 1451-1452

676 Lotterhos KE, Markel RW (2012) Oceanographic drivers of offspring abundance may increase
677 or decrease reproductive variance in a temperate marine fish. *Mol Ecol* 21: 5009-5026

678 Mercier L, Panfili J, Paillon C, N'diaye A, Mouillot D, Darnaude AM (2011) Otolith reading and
679 multi-model inference for improved estimation of age and growth in the gilthead seabream
680 *Sparus aurata* (L.). *Estuar Coast Shelf Sci* 92: 534-545

681 Mercier L, Mouillot D, Bruguier O, Vigliola L, Darnaude AM (2012) Multi-element otolith
682 fingerprints unravel sea-lagoon lifetime migrations of gilthead sea bream *Sparus aurata*. Mar
683 Ecol Progr Ser 444: 175–194

684 Moberg PE, Burton RS (2000) Genetic heterogeneity among adult and recruit red sea urchins,
685 *Strongylocentrotus franciscanus*. Mar Biol 136: 773–784.

686 Moody KN, Hunter SN, Childress MJ, Blob RW, Schoenfuss HL, Blum MJ, Ptacek MB (2015)
687 Local adaptation despite high gene flow in the waterfall-climbing Hawaiian goby, *Sicyopterus*
688 *stimpsoni*. Mol Ecol 24: 545-563

689 Narum SR (2006) Beyond Bonferroni: less conservative analyses for conservation genetics.
690 Conserv Genet 7: 783–787

691 Pérez-Ruzafa A, Fernández AI, Marcos C, Gilabert J, Quispe JI, García-Charton JA (2005)
692 Spatial and temporal variations of hydrological conditions, nutrients and chlorophyll in a
693 Mediterranean coastal lagoon (Mar Menor, Spain). Hydrobiologia 550: 11–27

694 Pérez-Ruzafa A, Marcos C, Pérez-Ruzafa IM, Barcala E, Hegazi MI, Quispe J (2007) Detecting
695 changes resulting from human pressure in a naturally quick-changing and heterogeneous
696 environment: spatial and temporal scales of variability in coastal lagoons. Estuar Coast Shelf
697 Sci 75: 175-188

698 Pérez-Ruzafa A, Marcos C, Pérez-Ruzafa I, Pérez-Marcos M (2011) Coastal lagoons:
699 “transitional ecosystems” between transitional and coastal waters. J Coast Conserv 15: 369-392

700 Planes S, Lenfant P (2002) Temporal change in the genetic structure between and within cohorts
701 of a marine fish, *Diplodus sargus*, induced by a large variance in individual reproductive
702 success. Mol Ecol 11: 1515-1524

703 Planes S, Roman P (2004) Evidence of genetic selection for growth in new recruits of a marine
704 fish. Mol Ecol 13: 2049-2060

705 Queller D, Goodnight K (1989) Estimating relatedness using genetic markers. Evolution 43: 258-
706 275

707 Quéré N, Guinand B, Kuhl H, Reinhardt R, Bonhomme F, Desmarais E. 2010. Genomic
708 sequences and genetic differentiation at associated tandem repeat markers in growth hormone,
709 somatolactin and insulin-like growth factor-1 genes of the sea bass, *Dicentrarchus labrax*.
710 Aquat Living Resour 23: 285-296.

711 Quignard JP, Man-Wai R, Vianet R (1984) Les poissons de l’Etang de Mauguio (Hérault,
712 France) inventaire, structure du peuplement, croissance et polymorphisme des tailles. Vie
713 Milieu 34: 173–183

714 Schmidt PS, Rand DM (2001) Adaptive maintenance of genetic polymorphism in an intertidal
715 barnacle: habitat- and life-stage-specific survivorship of *Mpi* genotypes. Evolution 55: 1336-
716 1344

717 Selkoe KA, Gaines SD, Caselle JE, Warner RR (2006) Current shifts and kin aggregation explain
718 genetic patchiness in fish recruits. *Ecology* 87: 3082–3094

719 Selkoe KA, Hentzler CM, Gaines SD (2008) Seascape genetics and the spatial ecology of marine
720 populations. *Fish Fish* 9: 363-377

721 Stephens M, Smith NJ, Donnelly P (2001) A new statistical method for haplotype reconstruction
722 from population data. *Am J Hum Genet* 68: 978-989

723 Streebman JT, Kocher TD (2002) Microsatellite variation associated with prolactin expression
724 and growth of salt-challenged tilapia. *Physiol Genom* 9:1-4

725 Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA
726 polymorphism. *Genetics* 123: 585-595

727 Therkildsen NO, Hemmer-Hansen J, Als TD, Swain DP, Morgan MJ, Trippel EA, Palumbi SR,
728 Meldrup D, Nielsen EE (2013) Microevolution in time and space: SNP analysis of historical
729 DNA reveals dynamic signatures of selection in Atlantic cod. *Mol Ecol* 22: 2424-2440

730 Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of
731 progressive multiple sequence alignment through sequence weighting, position-specific gap
732 penalties and weight matrix choice. *Nucl Acids Res* 22: 4673-4680

733 Tournois J, Ferraton F, Velez L, McKenzie DJ, Aliaume C, Mercier L, Darnaude AM (2013)
734 Temporal stability of otolith elemental fingerprints discriminates among lagoon nursery
735 habitats. *Estuar Coast Shelf Sci* 131: 182-193

736 van Oosterhout C, Hutchinson WF, Willis DPM, Shipley PF (2004) Micro-checker: software for
737 identifying and correcting genotyping errors in microsatellite data. *Mol Ecol Notes* 4: 535–538

738 Véliz D, Bourget E, Bernatchez L (2004) Regional variation in the spatial scale of selection at
739 *MPI** and *GPI** in the acorn barnacle *Semibalanus balanoides* (Crustacea). *J Evol Biol* 17:
740 953-966

741 Weir BS (1979) Inference about linkage disequilibrium. *Biometrics* 31: 235–254

742 Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure.
743 *Evolution* 38: 1358–1370

744 Wright S (1951) The genetical structure of populations. *Ann Eugen* 15: 323–354

745

746 **Legends of tables:**

747

748 **Table 1:** *Sparus aurata*. Spring and autumn 2011 samples used in this study, from south-
 749 west to north-east: SL: Salses-Leucate, T: Thau, and M: Mauguio (Fig. 1). Spring
 750 samples are composed of newly recruited ‘entering’ (E) juveniles, while autumn samples
 751 are made of late juveniles ‘migrating’ (M) out of the lagoons to the open sea. f and f_{corr}
 752 represents, respectively, estimates of deviations from Hardy–Weinberg expectations for
 753 the original data set (23 loci) and after removing the *C67b* and *Prl* loci from the analysis
 754 (see Results section). Levels of significance are reported. For each sample, the
 755 coefficients of relatedness r_{xy} (Queller and Goodnight 1989) estimated using the Identix
 756 computer package (Belkhir et al. 2002) is also reported, together with their associated p -
 757 values. Corrections for multiple tests were applied when necessary. N : sample size.

758

759

		N	f	P	f_{corr}	P	r_{xy}	p
Spring	SL-E	56	0.0279	<0.05	0.0115	-	-	0.354
	TH-E	55	0.1038	<0.001	0.0861	<0.001	-	0.394
	MA-E	54	0.0710	<0.001	0.0472	<0.01	-	0.140
Autumn	SL-M	36	0.0238	-	0.0075	-	-	0.723
	TH-M	27	0.0487	<0.01	0.0234	-	-	0.902
	MA-M	26	0.0673	<0.001	0.0219	-	-	0.845

- : not significant

760

761 **Table 2:** *Sparus aurata*. Genetic diversity indices for *Prl* and *GH* genes after haplotype
762 reconstruction. Tajima's *D* values are both non-significant (ns).

763

Gene	Length (bp)	No. of individuals	No. of haplotypes	Haplotype diversity (<i>H</i>)	Nucleotide diversity (π)	Tajima's <i>D</i>
<i>Prl</i>	4079	24	46	0.997	0.0072	0.0707 (ns)
<i>GH</i>	3633	24	47	0.999	0.0039	-0.5711 (ns)

764

765 **Legends of figures**

766

767 **Fig. 1:** *Sparus aurata*. Map of the study area showing the three studied lagoons
768 (Mauguio, Thau and Salses-Leucate) in bold.

769

770 **Fig. 2:** *Sparus aurata*. Observed genetic differentiation as measured by F_{ST} at each of the
771 twenty-three loci considered in this study. Loci that showed significant genetic
772 differentiation are indicated by filled circles, while absence of genetic differentiation is
773 indicated by open squares (bars represent one standard deviation). The order of loci along
774 the horizontal axis is the same in each panel; this order corresponds to the one adopted in
775 Supplementary Table S1. Only labels of markers showing significant genetic
776 differentiation are indicated in the figure. Left panel: genetic differentiation calculated
777 among all six samples. Central panel: genetic differentiation among the three samples of
778 young recruits entering the lagoons in spring. Right panel: genetic differentiation among
779 the three samples of individuals migrating out of the lagoons in autumn (Table 1).

780

781 **Fig. 3:** *Sparus aurata*. Profiles of nucleotide diversity averaged in 100 base pair sliding
782 windows along *GH* and *Prl* genes in the Mauguio and Thau lagoons. E: exons; I: introns.

783

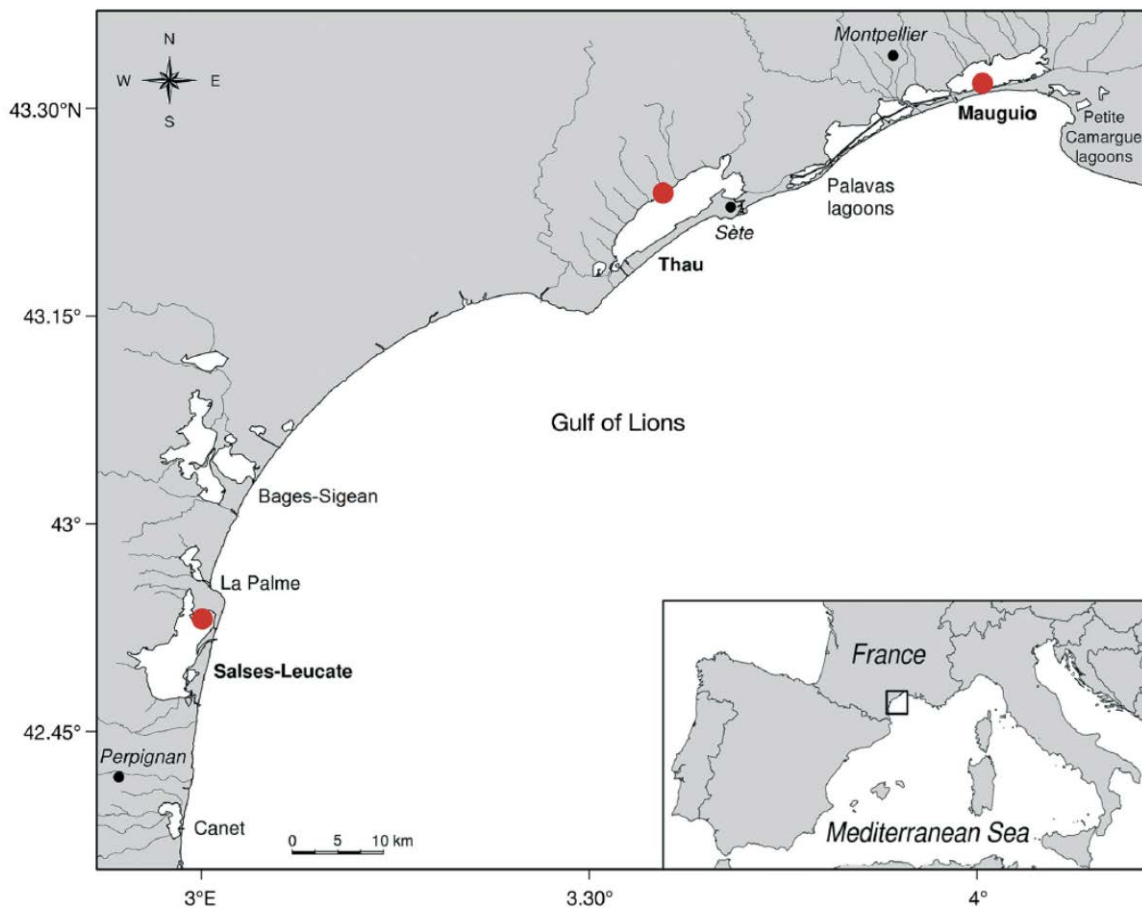
784 **Fig 4:** *Sparus aurata*. NEIGHBORNET networks representing similarities among
785 haplotypes for *GH* and *Prl* genes. Shaded areas indicate groups of related haplotypes (i.e.
786 located further apart in the network) in each gene. Haplotypes found in the Mauguio and
787 Thau migrating juveniles are represented by green and blue dots, respectively.

788

789

790 Fig. 1

791

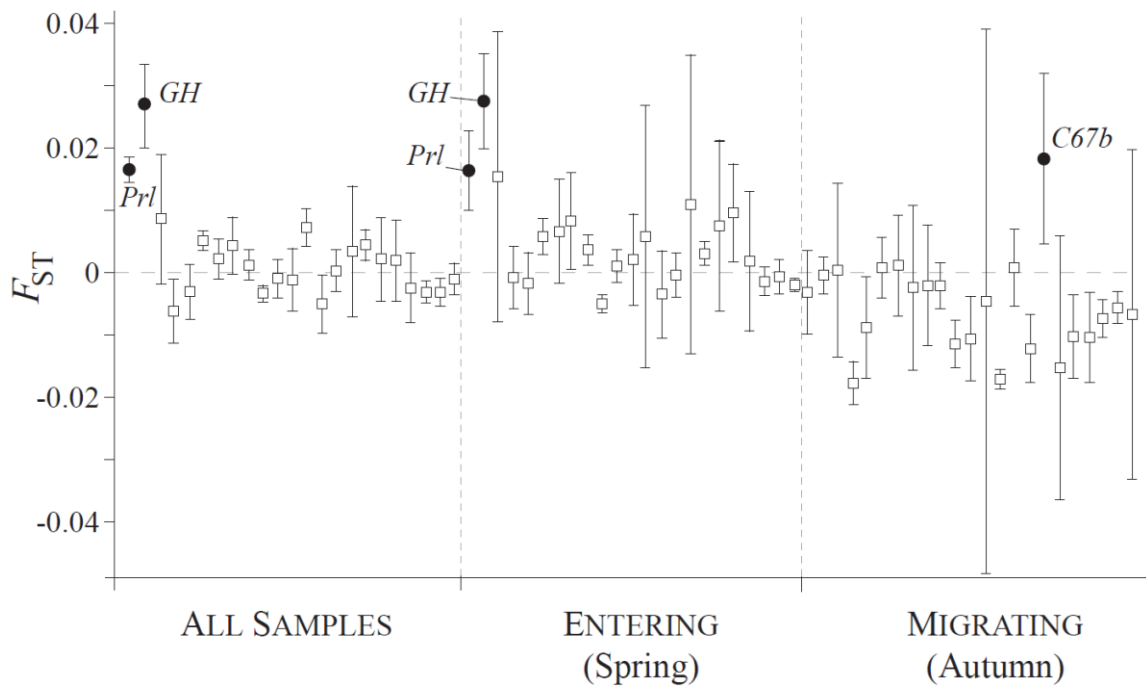


792

793

794 Fig. 2

795

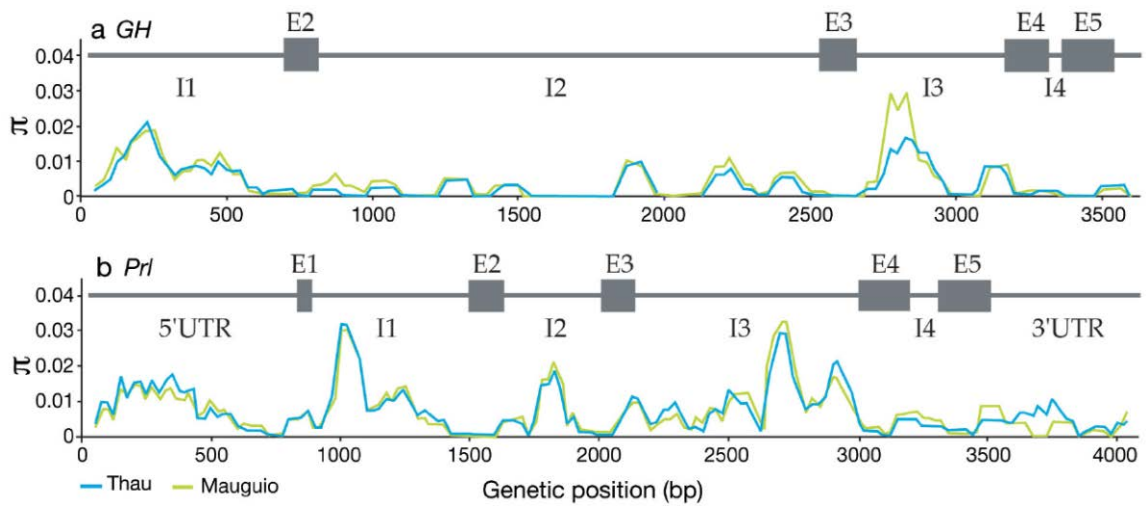


796

797

798 Fig.

799



800

801

