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## Genetic population structure of anchovy (*Engraulis encrasicolus*) in North-western Europe and variability in the seasonal distribution of the stocks

Huret Martin <sup>1,\*</sup>, Lebigre Christophe <sup>1</sup>, Iriondo Mikel <sup>2</sup>, Montes Iratxe <sup>2</sup>, Estonba Andone <sup>2</sup>

<sup>1</sup> IFREMER, STH/LBH, B.P. 70, Plouzané, 29280, France

<sup>2</sup> Department of Genetics, Physical Anthropology and Animal Physiology, University of Basque Country (UPV/EHU), Leioa, Spain

\* Corresponding author : Martin Huret, email address : [martin.huret@ifremer.fr](mailto:martin.huret@ifremer.fr)

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### Abstract :

A prerequisite in fisheries management is to identify biological meaningful delineations of stocks, the fundamental exploited units. The European anchovy is one such species in need to better identify stock boundaries. Indeed, despite a spawning aggregation behaviour both in the southern Bay of Biscay and southern North Sea in spring and summer, it has a seemingly continuous distribution in autumn across a broad region ranging from the Bay of Biscay to the northern North Sea including the English Channel, thus with potential mixing of the spawning units. We therefore used genetic markers (single nucleotide polymorphisms) to quantify the degree of gene flow between the currently managed fish stock of the bay of Biscay and northern areas of European anchovy's distribution. Our results confirm the clear distinction between the Bay of Biscay and northernmost populations, with assignment of all English Channel samples to the latter. We also found a clear overall pattern of isolation by distance that resulted primarily from an increasing differentiation with geographic distance in the Northern group magnified by the lack of gene flow with the anchovies of the Bay of Biscay. The small reduction in gene diversity towards northern latitudes may indicate partial isolation of the leading-edge component of the Northern population. Quotient plots relating anchovy's distribution to environmental covariates showed that anchovies sampled in autumn in the English Channel originate from the summer spawning aggregation in the warm, low saline and plankton rich south-eastern North Sea. This change in the seasonal distribution of the northern population mimics, but in opposite direction, the one of the Bay of Biscay where anchovy spread towards the north from the spawning habitat in the south-eastern bay. The encounter, without mixing, of the two populations west of Brittany in autumn suggests strong spawning site fidelity. Finally, we identified for the first time anchovies belonging to the estuarine ecotype in the Loire estuary. Our results therefore support current management units, yet with some uncertainties for the catches in the transition zones between the Bay of Biscay and the English Channel, and within river plumes in the vicinity of estuaries hosting the estuarine ecotype.

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## Highlights

► A clear genetic boundary between the Bay of Biscay and the northern stock. ► Loire estuary (France) host anchovies members of the estuarine ecotype. ► Spatial overlap is possible at stocks' boundaries but without interbreeding. ► A founder effect at the northern leading edge with small decrease in gene diversity. ► Autumn English Channel anchovies originate from the southern North Sea spawning habitat.

**Keywords** : European anchovy, English channel, Single nucleotide polymorphism, Spawning site fidelity, Quotient plot, Ecotype

## INTRODUCTION

60

Fish stocks, the fundamental exploited units in fisheries management, are commonly defined on the basis of morphological and demographic characteristics, fishing patterns, connectivity patterns (adult movement and larval dispersal), and more and more frequently the existence of genetic differentiations (Ovenden et al., 2015; 65 Cadrin et al., 2004). Even if the results of genetic studies need to be cautiously interpreted (Waples and Gaggiotti, 2006), many examples reveal that genetic tools can clearly improve our understanding of connectivity patterns among marine populations (Hedgecock et al., 2007) and eventually refine the delineation of management units, provided existing genetic knowledge is translated operationally 70 (Ovenden et al., 2015; Reiss et al., 2009): stocks should fit the population structure of exploited species. One of the difficulties in defining geographical boundaries between fish populations (Reiss et al., 2009) is to account for variability in populations' spatial distribution over time, such as seasonal migration between reproductive, feeding or overwintering areas. Genetically distinct populations may exist in spite of a seemingly 75 continuous range, and even overlap seasonally, if individuals exhibit fidelity to natal spawning grounds. Yet, quantifying such a process is challenging as often we lack appropriate survey and data on this temporal scale.

The European anchovy (*Engraulis encrasicolus*, hereafter anchovy) is one of such species in need for a clarification of its management units. In the Bay of Biscay (ICES 80 subarea VIII, 43°N-48°N, 11°W-0°W, see Fig.1) it is managed as a single stock and is under TAC regulation following annual assessment (ICES, 2017). Conversely, northern anchovy populations (North Sea, Irish Sea, Celtic Sea and English Channel) are not assessed and not regulated, as those population's abundances and fishing

have not been considered large enough to require management efforts (ICES, 2017).

85 Indeed, in subareas IV (North Sea) and VI (western Scotland) reported landings have never exceeded a few tons. The English Channel has long hosted an anchovy population (Cunningham, 1890). In subarea VII (encompassing Celtic Sea and English Channel), landings (mostly from French and British fleets) have historically been scarce until 1996, with a maximum of 25 tons (ICES, 2017). However, the

90 northern populations have increased substantially since the mid-90's (Beare et al., 2004a; Beare et al., 2004b) probably due to warming that increased the recruitment potential of local populations (Alheit et al., 2012; Petitgas et al., 2012). Consequently, landings of the French and British fleets increased to up to 1,754 tons in subarea VII in 2015. There has also been evidence that the Bay of Biscay anchovy stock

95 sometimes expand further north of the subarea VIII (ICES, 2017). Part of the French landings occur in statistical rectangles 25E4 and 25E5 of subarea VII (see Fig.1), just north of the Bay of Biscay subarea VIII, a region called 'Iroise Sea' in front of Brittany. Therefore the assessment group traditionally considered that this portion of the catch in rectangles offshore the tip of Brittany consisted of individuals from the

100 Bay of Biscay stock. However, during the fishery closure (2005-2009) some catches were reported in the subarea VII, then out of the stock delineation and regulation measures (ICES, 2010a). If anchovy abundance and fishing pressure are to continuously increase in the English Channel, we need to understand accurately the population structure and connectivity of anchovy in this area in order to set up an

105 appropriate management of this/these stock(s).

Since the late 90's, there has been a continuous effort to characterise population structure of anchovy in European waters based on evolving genetic technologies, from allozymes (Sanz et al., 2008), mitochondrial DNA (Magoulas et al., 2006; Silva

et al., 2014), microsatellites (Zarraonaindia et al., 2009; Silva et al., 2014) and more  
110 recently Single Nucleotide Polymorphisms (SNPs; Montes et al., 2016; Zarraonaindia  
et al., 2012). The Bay of Biscay, hosting the largest population in European Atlantic  
waters population (Uriarte et al., 1996) with a collapse between 2005 and 2009, was  
logically the focus for most genetic studies. Only recently have some samples from  
northern Europe been analysed (Montes et al., 2016; Silva et al., 2014;  
115 Zarraonaindia et al., 2012). These studies indicated that North Sea and English  
Channel samples were genetically homogenous, exhibiting significant genetic  
differences with the Bay of Biscay samples (Zarraonaindia et al., 2012; Silva et al.,  
2014). Moreover, Bay of Biscay samples appeared to be genetically more similar to  
the western Mediterranean samples than to the North Sea-English Channel samples  
120 (Zarraonaindia et al., 2012). Within the Bay of Biscay, (Montes et al., 2016) used a  
large genetic dataset (456 SNPs) and clearly showed the occurrence of two  
ecotypes: an 'offshore' or 'marine' ecotype spread over the continental shelf, and a  
'coastal' ecotype constrained to estuaries and/or their plumes such as the Gironde,  
Abra (Montes et al., 2016) or Adour (Le Moan et al., 2016). Hereafter we will prefer  
125 the designations 'marine' vs. 'estuarine' since both ecotypes can be found along the  
coast, thus offshore habitat is too restrictive for the former ecotype, whereas coastal  
is not restrictive enough for the latter one. In spite of their clear isolation, 'estuarine'  
samples were genetically more similar with one another (including samples from the  
Netherlands and the North-western Mediterranean) than to the neighbouring and  
130 sometimes spatially overlapping 'marine' anchovies (Catanese et al., 2017; Le Moan  
et al., 2016; Montes et al., 2016). Such genetic differences between 'marine' and  
'estuarine' ecotypes are consistent with phenotypic differences, 'estuarine' ecotype  
being described as whitish-skinned and with a lower growth. Reduced gene flow

between the two ecotypes would result from divergence between two previously  
135 isolated gene pools adapted to contrasting habitats and now in secondary contact,  
with reproductive isolation reinforced by hindered gamete compatibility (Montes et al.,  
2016).

In spite of their remarkable achievements, the inference that can be drawn from  
these studies regarding the actual population structure of western European  
140 anchovies is limited by the lack of sampling in some of the areas and seasons.  
Indeed, the genetic structure between anchovies sampled in the English Channel  
and the Bay of Biscay was drawn either from a single sample of the English Channel  
from an approximate location (south of Cornwall, UK) that consisted in a pool of  
multiple years (2002-2007) in Zarraonaindia et al. (2012), or from a single sample  
145 collected at the boundary with the North Sea in Silva et al. (2014). Montes et al.  
(2016) managed to collect additional samples in the Irish Sea to confirm the overall  
result of Zarraonaindia et al. (2012) on the distinction between the northernmost  
population and the one of the Bay of Biscay. Yet, the English Channel constitutes a  
clear gap in which data have long been missing while this area makes the junction  
150 between northernmost anchovies and those inhabiting the Bay of Biscay.  
Furthermore, the part of the Bay of Biscay north of the Loire estuary has never been  
sampled for genetic studies while such data collected around Brittany (see Fig.1) are  
needed to determine whether there is a clear boundary between the Bay of Biscay  
and northern populations or whether there is continuous and ongoing gene flow.  
155 Anchovy is known to move within the Bay of Biscay, its life cycle showing a marked  
change in spatial occupation with both ontogeny and on a seasonal scale between  
spawning (April to August) and overwintering habitats (Boyra et al., 2013; ICES,  
2010b). Similar seasonal movements may occur in 'northern' anchovies. Indeed, no

egg presence has ever been reported in the English Channel, suggesting its absence  
160 from the area from spring to summer if we rely on the Bay of Biscay spawning  
season, or in summer based on North Sea spawning season (June to August, Alheit  
et al., 2012). In addition landings in the Celtic Sea – English Channel area mostly  
occur during the second semester by French fleets just north of the Bay of Biscay,  
and in winter south of Cornwall (ICES, 2017). Absence of spawning and seasonality  
165 in the landings overall suggests that anchovies come to overwinter from one or  
several spawning areas located in the south-eastern North Sea, the Irish Sea or the  
Bay of Biscay. More than a century ago, Cunningham (1895) and Radeke (1916)  
assumed a migration from the spawning grounds occupied between May and August  
along the Dutch Coast to explain its presence in the western English Channel in  
170 autumn-winter, whereas no observation was made in that area in spring-summer.  
Surprisingly, the connectivity pattern of anchovy between the Bay of Biscay and the  
North Sea through the English Channel was not studied further until recently, to  
identify the processes that led to the recent increase of abundance in the northern  
European seas (Petitgas et al., 2012; Zarraonaindia et al., 2012). Finally, the  
175 existence of genetically distinct ecotypes ('marine' vs 'estuarine') has relied on the  
investigation of a few sites located in IJsselmeer and the estuaries of the Adour and  
Gironde (Bay of Biscay). Thus, it is important to determine whether anchovies living  
in or in the vicinity of other western European estuaries also belong to the 'estuarine'  
ecotype. Given the large distribution of western European anchovies and the recent  
180 increase in abundance in its northernmost part, the potential migration patterns within  
this range of distribution, as well as the existence of different ecotypes, there is a  
clear need to clarify the existing links between 'marine', 'estuarine', 'Biscay', and  
'Northern' anchovies and determine the degree to which they form genetically

homogeneous units that should be managed independently in spite of their apparent  
185 continuous range.

To address this issue we conducted a large scale sampling effort to collect individuals  
of western European anchovies from the Bay of Biscay to the North Sea, at different  
seasons when possible, with a special effort in the English Channel. We genotyped  
them with the genetic markers (SNPs) developed by Montes et al. (2016), enabling  
190 us to combine our recent data with those of the northernmost range of this species  
(Montes et al., 2016). More specifically, we quantified the degree to which anchovies  
in the English Channel were different from those of the Bay of Biscay and the Irish  
Sea and North Sea, and compared such genetic differentiation to that existing  
between 'estuarine' and 'marine' anchovies sampled along the western European  
195 coasts (Gironde, Loire, Seine, IJsselmeer). We then quantified the patterns of isolation  
by distance and the effect of latitude on the genetic diversity over the whole range of  
the 'marine' ecotype, and determined whether there are genetically homogeneous  
units. Finally, we investigated the populations' spatial dynamics on a seasonal scale  
between their spawning and overwintering distributions, and proposed a process-  
200 based explanation of these spatial patterns by relating the abundance data from  
scientific surveys to synoptic environmental information.



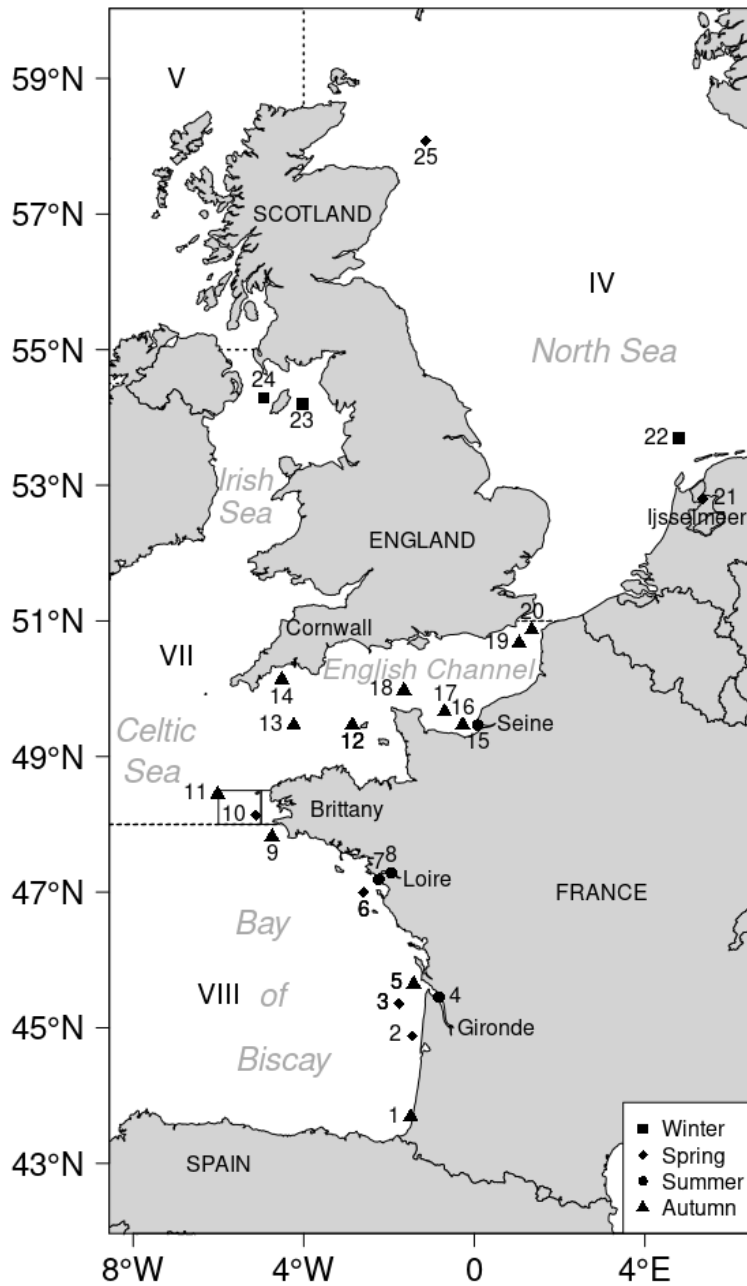


Fig.1. Map with sample locations. Numbers correspond to samples in Table 1, and symbols to different seasons of sampling. Roman numerals stand for ICES subareas. ICES statistical rectangles 25E5 and 25E4 of subarea VII are drawn in front of Brittany from offshore to coast respectively, with samples 10 and 11 located in 25E4

## MATERIALS AND METHODS

### *1) Sampling and data collection*

210 A total of 602 anchovies were collected from 25 locations across different regions from the southern Bay of Biscay to the Irish and North Seas through the English Channel, and from different seasons and years (Table 1, Fig.1). Among those, 470 individuals (20 sampling locations) were collected during IFREMER's surveys or by a professional vessel (sample #23 in Irish Sea). Remaining samples were already  
215 genotyped by Montes et al. (2016) and were combined with our study to extend the sampling coverage in the northernmost range of the species (samples 24 and 25) or to include samples belonging to the estuarine ecotype as known references (samples 4 and 21). IFREMER samples were collected mostly onboard the R/V THALASSA during fish surveys in spring (PELGAS, Doray et al., 2018) and autumn (EVHOE,  
220 Mahe and Laffargue, 1987) in the Bay of Biscay from 2013 to 2017, in autumn in the English Channel in 2014 (CAMANOC, Travers-Trolet et al., 2014) and 2015-2016 (CGFS, Travers-Trolet, 2015, 2016), and in winter in the southern North Sea in 2016 (French IBTS, Verin, 2016). The IFREMER collection was completed by two samples from the Loire estuary and one from offshore the Seine estuary from the NOURDEM  
225 surveys in summer 2017. Samples could only be collected in the English Channel in autumn as no survey was carried out during other seasons and we were not able to collect any from the fishery during winter. The absence of landings during spring and summer strongly indicates that the abundance of anchovy in this area and at that time of the year is negligible .

230 Upon capture, whole fish were stored at -20°C onboard. Once brought in the lab, a small piece of muscle tissue was dissected and stored in 95% ethanol at -20°C.

IFREMER samples were screened for 308 SNPs that had been previously used by Montes et al. (2016), a subset of their markers based on a trade-off between number of samples and number of SNPs. All individuals were screened with TaqMan®  
235 OpenArray™ Genotyping System (Life Technologies) at the Sequencing and Genotyping Service (SGIker) of the University of the Basque Country (UPV/EHU).

**Table 1.** Metadata of samples used in this study. Sampling locations were ordered by marine sub-region and from South to North. Size range is provided when available.

\*Samples collected by Montes et al. (2016). Numbers correspond the the sample numbers in their study

#	Location	Marine region	Sampling month	year	N	Survey / origin	Longitude (°E)	Latitude (°N)	Size range (cm)
1	South Biscay 1	Biscay	October	2015	30	EVHOE	-1.49	43.69	7.5 - 10
2	South Biscay 2	Biscay	May	2012	29	12*	-1.45	44.88	Adults
3	South Biscay 3	Biscay	April	2014	30	PELGAS	-1.76	45.36	12.9 – 18.1
4	Gironde estuary 1	Biscay	September	2011	22	10*	-0.83	45.45	Juveniles
5	South Biscay 4	Biscay	October	2013	9	EVHOE	-1.42	45.64	7.2 – 8.6
6	North Biscay 1	Biscay	May	2014	30	PELGAS	-2.60	47.00	11.0 – 14.9
7	Loire estuary 1	Biscay	July	2017	30	NOURDEM	-2.24	47.19	10.3 - 13.5
8	Loire estuary 2	Biscay	June	2017	9	NOURDEM	-1.94	47.29	10.3 – 12.3
9	North Biscay 2	Biscay	November	2015	30	EVHOE	-4.74	47.82	12.8 - 16.2
10	Brittany 1	Celtic Sea	May	2017	23	PELGAS	-5.12	48.13	15.0 – 18.0
11	Brittany 2	Celtic Sea	November	2016	18	EVHOE	-6.01	48.44	11.4 - 19.0
12	West channel 1	English Channel	September	2014	20	CAMANOC	-2.86	49.46	10.1 - 18.3
13	West channel 2	English Channel	October	2015	30	CGFS	-4.23	49.46	16.0 - 18.9
14	East Channel 4	English Channel	October	2015	30	CGFS	-4.51	50.14	16.5 - 19.8
15	Seine estuary	English Channel	August	2017	27	NOURDEM	0.08	49.47	13.5 – 16.0
16	East Channel 1	English Channel	October	2015	30	CGFS	-0.27	49.47	7.8 - 11.1
17	East Channel 2	English Channel	October	2015	30	CGFS	-0.69	49.66	8.7 - 10.6
18	East Channel 3	English Channel	September	2014	10	CAMANOC	-0.64	49.96	10.2 – 16.0
19	East Channel 5	English Channel	September	2016	10	CGFS	1.05	50.67	15.5 – 18.5
20	East Channel 6	English Channel	September	2016	20	CGFS	1.35	50.86	7.5 - 12.0
21	Ijsselmeer	North Sea	May	2010	27	25*	5.36	52.80	Juveniles
22	North Sea 1	North Sea	February	2016	30	IBTS	4.80	53.69	7.3 – 11.9
23	Irish Sea 1	Irish Sea	March	2010	24	professional	-5.03	54.20	Adults
24	Irish Sea 2	Irish Sea	March	2010	30	24*	-4.94	54.29	Adults
25	North Sea 2	North Sea	May	2009	24	23*	-1.14	58.5	Adults

For each SNP we calculated the minor allele frequency (MAF) using the r-package 'adegenet' (Jombart and Ahmed 2011). We subsequently calculated observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosity, estimated deviations from the Hardy-Weinberg's expectations (HWE) and linkage disequilibrium (LD) between pairs of loci within each sampling site using respectively the r-packages 'hierFSTAT' (Goudet 2005), 'pegas' (Paradis 2010), and the index of association implemented in 'poppr' (Kamvar et al. 2015). In total, 23 loci were subsequently removed because they had MAF values lower than 0.01, consistent departure from the HWE or significant LD (following false discovery rate; Supp. Table 1).

We first measured the overall genetic structure among all sampling locations, using Weir & Cockerham's estimate of  $F_{ST}$  implemented in 'hierFSTAT'. To identify the number of genetic clusters (K) without a priori definition of populations, we used the Bayesian approach implemented in STRUCTURE v2.3 (Pritchard et al. 2000, Falush et al. 2003). Runs were implemented using a standard admixture model with correlated gene frequencies that are less influenced by deviations from the HW expectations (Falush et al. 2003). We tested K values (K ranging from 1 to 7) and implemented 10 replicated runs that consisted in a burn-in period of 200,000 iterations and 500,000 sampled iterations. We systematically inspected the diagnostic plots to ensure that parameters converged. We used the method described in Evanno et al. (2005) to identify the most likely number of genetic clusters; this method uses the rate of change ( $\Delta K$ ) of the log posterior probability of the data ( $\ln(P(K|X))$ ) between successive K across the 10 replicated runs. For each K, an individual probability of membership to each cluster averaged across the 10

265 replicates was estimated using the software CLUMPP 1.1.2 (Jakobsson & Rosenberg  
2007). CLUMPP was run with the 'greedy' alignment algorithm and 100,000  
randomised input orders for each K. Individual average membership coefficients were  
subsequently visualised using DISTRUCT v1.1 (Rosenberg 2004).

To quantify the amount of genetic differentiation explained by these clusters, we  
270 estimated hierarchical  $F_{ST}$  values (no significance test was carried out because the  
groups were identified using STRUCTURE). The most inclusive category of the  
hierarchical structure was whether sampling locations consisted in 'estuarine' or  
'marine' anchovies from the STRUCTURE classification, as these ecotypes were  
associated with major genetic differences in previous studies (Montes et al. 2016; Le  
275 Moal et al. 2016). The 'population' to which samples were associated with was set as  
the second hierarchical level (i.e. whether fish were classified as belonging to the  
Bay of Biscay group or to the northern group). The third level consisted in the  
sampling location within each population.

### 280 3) *Patterns of isolation-by-distance and latitudinal variation in gene diversity*

We tested the hypothesis that there is a pattern of isolation-by-distance using a  
Mantel test implemented in 'ade4' (Chessel et al. 2004). As landmasses are barriers  
to movements and gene flow in marine species, we calculated a least-cost path  
285 (LCP) distance between each sampling location using the r-package 'MarMap' (Pante  
& Simon-Bouhet 2013), constraining the LCP to avoid landmasses and areas deeper  
than 300 m (anchovies are hardly ever captured beyond the shelf of the Bay of  
Biscay). The Mantel test was performed using transformed  $F_{ST}$  values (i.e.  $F_{ST} / (1 - F_{ST})$ ) as a function of the least cost path distance between pairs of sampling

290 locations. The significance of pairwise  $F_{ST}$  values and the Mantel test were obtained  
by comparing the observed values with a distribution of 999 permutations of  
individuals among sampling locations (for  $F_{ST}$ ) or pairwise genetic/geographic  
distances (for the Mantel test). The overall test was carried out without sampling  
locations identified as 'estuarine' by STRUCTURE (Gironde 1, Loire 1, Loire 2,  
295 IJsselmeer). The same test was subsequently carried out within each population,  
considering only the marine sampling locations, to determine whether these form  
homogeneous genetic units, or whether genetic drift occurs within each predefined  
population.

To test the effect of the latitude on the genetic diversity and genetic structure of  
300 anchovy, we focused on samples identified by STRUCTURE as belonging to the  
marine ecotype (7 and 14 sampling locations in the Bay of Biscay and the northern  
group respectively, see Results). For each sampling location, we calculated both the  
observed heterozygosity ( $H_o$ ) and gene diversity ( $H_s$ ) using 'hierFSTAT'. These  
parameters were used as response variables in linear models that included the  
305 latitude as the explanatory variable. We visually checked the assumptions of  
normality of the residuals and homoscedasticity. We then used a partial redundancy  
analysis (RDA) to quantify the effect of the latitude accounting for the broad scale  
difference of the two populations. We carried out a principal coordinate analysis on  
the matrix of pairwise  $F_{ST}$  values, of which we extracted the first three components  
310 that cumulatively explained 95% of the total variance in pairwise genetic  
differentiation. We then quantified the variance of these three components in an RDA  
due to the latitude (partialling out the population assignment), and that due to the  
population assignment (partialling out the latitude). This approach enabled us to  
determine whether the northward expansion when anchovy settled in the northern

315 areas was associated to the formation of genetically homogeneous units that progressively genetically drifted from the Bay of Biscay population (in which case the variance explained by the population assignment should be far greater than that explained by the latitude) or whether the northern expansion occurred in a stepping stone manner with several small spawning sites that were partially isolated from one another (in which case, the effect of latitude should be far greater than that of the population assignment). These analyses were performed using the R package “vegan” (Oksanen et al. 2018) and significance tests of the variance partitioning were implemented using permutation test (999 permutations).

325 4) *Seasonal variability in anchovy spatial distribution and relationship with the environment*

We gathered all available information from scientific surveys to better describe the variation in anchovy’s seasonal distribution in western European waters and understand where English Channel individuals observed in autumn come from. Over the French shelf of the Bay of Biscay, French acoustic survey PELGAS (since 2000, Doray et al., 2018a) occurs every year in spring during the anchovy spawning season. In the Western English Channel, the English acoustic survey PELTIC is organised every year since 2011 in the UK waters around the Cornwall peninsula. It occurred in May-June in 2011, and in autumn subsequently partly because of the absence of anchovy in spring. There are small pelagic fish surveys in the North Sea and waters around UK and Ireland, but mostly dedicated to herring. Furthermore, run once a year they do not provide seasonal comparison. In spite of their focus on demersal fish, the coordinated International Bottom Trawl Surveys (IBTS) deliver



340 standardised Catch Per Unit Effort (CPUE) rich of information for anchovy abundance  
and distribution over the North-western Europe (Alheit et al., 2012; Petitgas et al.,  
2012) and they allow the seasonal comparison over the whole North Sea. We  
therefore used the following IBTS surveys: NS-IBTS during Q1 for Winter and Q3 for  
Summer (1990-2017) in the North Sea, Scottish SWC-IBTS during Q1 and Q4 for  
345 Autumn-Winter (1995-2017) around Scotland, North Ireland NIGFS during Q1 and  
Q4 for Autumn-Winter (2005-2017) in the North Irish Sea, the Irish IGFS during Q4  
for Autumn (2003-2017) in the south and west of Ireland, French CGFS during Q4 for  
Autumn (1990-2017) in the Eastern Channel, EVHOE during Q4 for Autumn (1997-  
2017) in the Bay of Biscay. For the western Channel, we used data from the recent  
350 surveys CAMANOC (Q4, 2014) and CGFS (Q4, 2015-2016) that used the same IBTS  
protocol. All CPUE have been extracted from the dedicated database DATRAS  
(<http://www.ices.dk/marine-data/data-portals/Pages/DATRAS.aspx>), or compiled  
when only raw information was available. We used the raw CPUE (in abundance per  
haul per hour) that we log-transformed. In the Bay of Biscay, spring distribution is  
355 available as biomass (tons per nautical miles) estimated from acoustics during the  
PELGAS dedicated survey (Doray et al., 2018a). This biomass data is gridded (Doray  
et al., 2018b) and averaged over the time-series 2000-2016.

We used monthly climatologies (pluriannual monthly averages) of Sea Surface  
Temperature (SST) and surface chlorophyll-a from satellite remote-sensing data, as  
360 well as Sea Surface Salinity (SSS) from hydrodynamic modelling. SST was obtained  
from the Ifremer dataset derived from AVHRR/Pathfinder daily products interpolated  
by kriging (Saulquin and Gohin, 2010) for the period 1986-2009 over whole European  
waters. Chlorophyll-a is obtained from daily standard remote-sensing reflectances of  
MODIS/Aqua between 2003 and 2010, after processing as detailed in Gohin (2011).

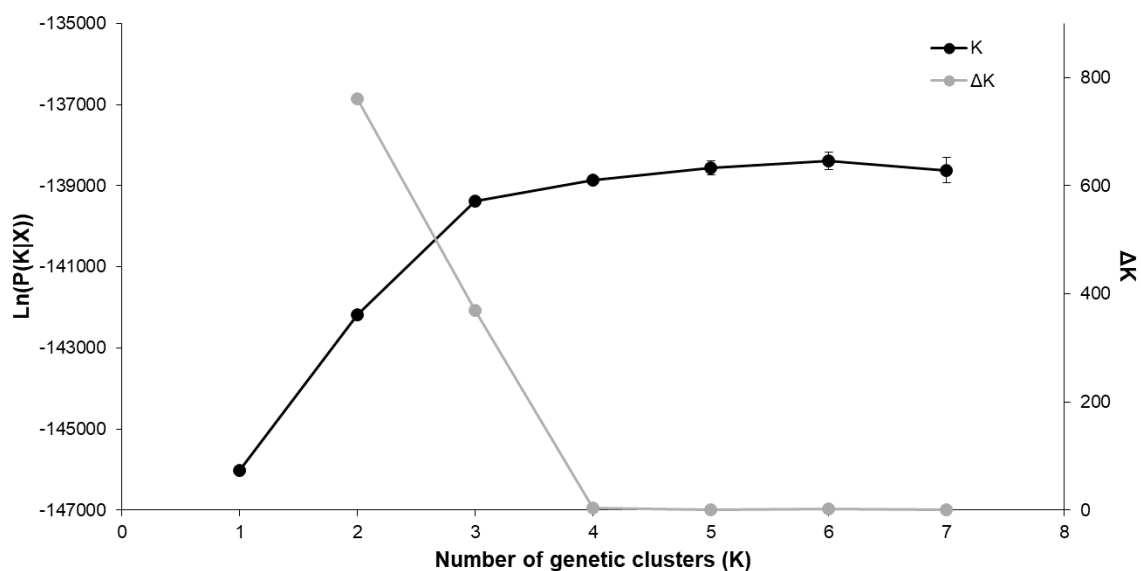
365 SSS was obtained from the FOAM (Forecasting Ocean Assimilation Model) reanalysis between 1993 and 2017 over the North West European Shelf, as delivered on the Copernicus website ([marine.copernicus.eu](http://marine.copernicus.eu)).

To quantify the ecological factors driving changes in anchovy spatial distribution on the seasonal scale, we described its habitat preferences by relating fish abundances  
370 and environment covariates using quotient analysis (Bernal et al., 2007; Ibaibarriaga et al., 2007). Here environment covariates are divided in equally sized bins and the quotient is calculated as the proportion of CPUEs (or biomass for May in the Bay of Biscay) per covariate bin divided by the proportion of covariate observations per bin. A quotient deviating from 1 suggests habitat avoidance ( $<1$ ) or preference ( $>1$ ). The  
375 chlorophyll-a covariate was log-transformed before binning to balance the number of environmental observations per bin. Additionally, the confidence intervals of the null hypothesis of even fish distribution was computed by bootstrapping. More specifically, we created a large number ( $n=999$ ) of random sampling from the observed CPUE values that we affected to the unchanged dataset of covariates.  
380 Quotient values were calculated for each of the 999 pseudo sampling, from which we calculated the percentiles 0.025 and 0.975. We limited this analysis to the North Sea and the Bay of Biscay, the two regions that provided fish abundance at two distinct seasons, one being the reproductive.

## RESULTS

### 385 1) Genetic differentiation across sampling locations

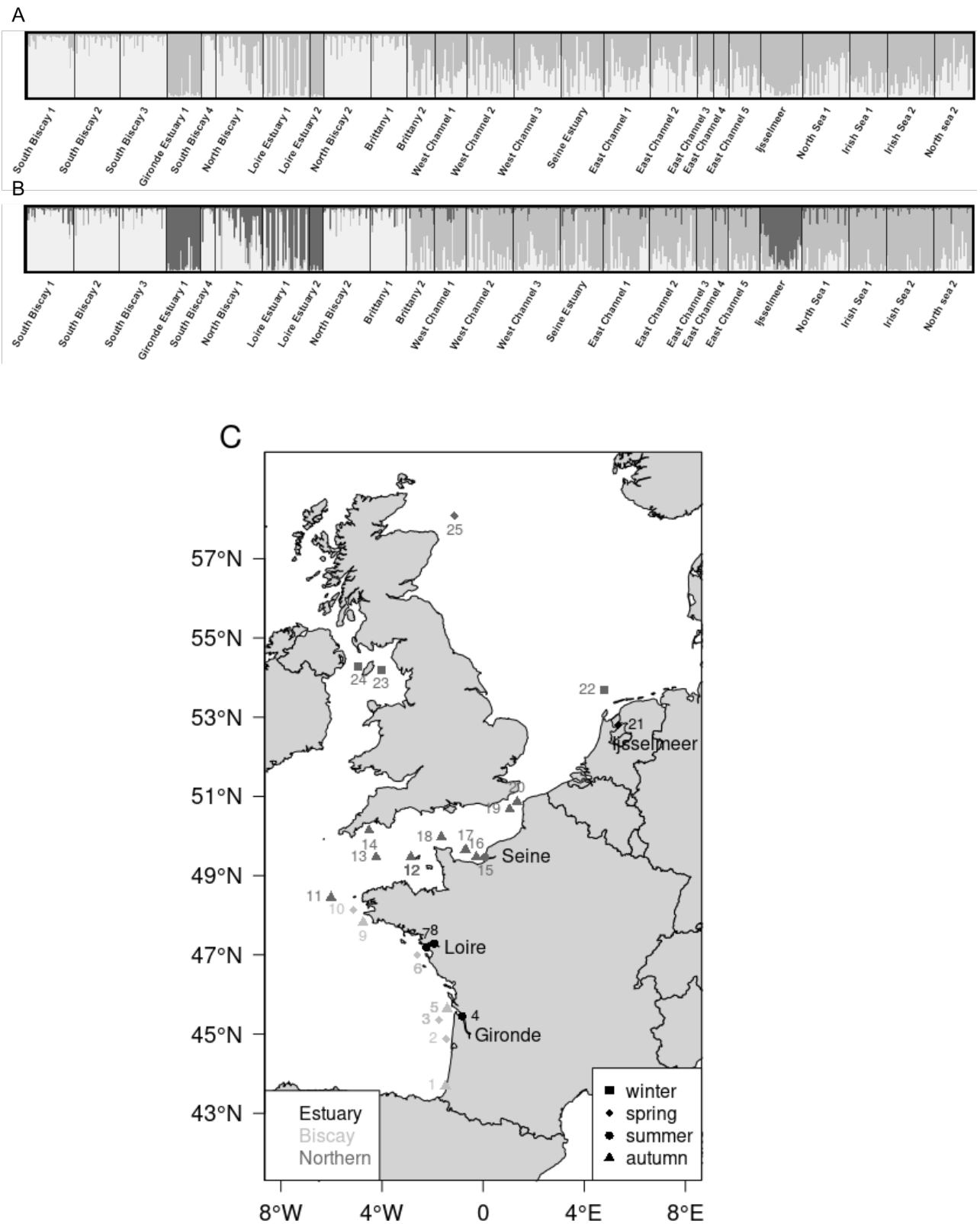
Across the 25 sampling locations, there was a significant overall genetic differentiation ( $F_{ST} = 0.047$ , 95% CI: 0.038-0.056). The mean log likelihood of the data ( $\ln P(K|X)$ ) from 10 replicate runs increased substantially between K1 and K3 at which it almost reached its maximum (Fig 2). Following K3, there was an almost  
390 stable  $\ln P(K|X)$  with an increase in its standard deviation. The estimate of  $\Delta K$  showed highest values at K2 then K3, with negligible values at  $K > 3$  suggesting that the data was best explained with 2 or 3 distinct genetic clusters.



395 *Figure 2. Changes in the log posterior likelihood of the data ( $\ln P(K|X)$ ) with increasing number of clusters  $K$  and the rate of change ( $\Delta K$ ) between consecutive  $K$  averaged over 10 replicates.*

At K2, there was a clear difference between marine sampling locations in the Bay of Biscay and all the other populations (estuarine locations and marine sampling  
400 locations from the English Channel, the Irish Sea, and the North sea) with a clear boundary near the tip of the Brittany peninsula (Fig. 3A,C) that separates between the two samples of the Celtic Sea. At K3, these two broad clusters remained, only

one individual assigned to the Northern group was sampled in the Bay of Biscay (0.05%, N = 181) while 29 of the anchovies sampled in the Northern group were  
405 assigned to the Bay of Biscay cluster (8.71%, N = 333), suggesting a directional gene flow (Fig. 3B). A third genetic cluster appeared that consisted in most of the anchovies sampled in estuarine locations (Fig. 3B; Table 1), indicating that there was indeed a strong genetic similarity between anchovies sampled in Gironde, Loire and IJsselmeer and that these anchovies were genetically distinct of the neighbouring  
410 marine anchovies. Yet, the delineation of estuarine vs marine anchovies is not trivial as some samples collected near the mouth of estuaries or in associated river plumes are clearly marine anchovies (South Biscay 4, Seine estuary) or admixed with marine anchovies (North Biscay 1, Loire estuary 1). The median assignment to one or the other cluster in sample 'Loire estuary 1' was 92% (IQR: 82-97%) suggesting that this  
415 admixture was primarily due to the formation of mixed schools of estuarine and marine anchovies rather than an introgression of one cluster into the other one. Noteworthy the two sampling locations of the Celtic Sea near the tip of the Brittany peninsula assigned to different genetic clusters as individuals collected in spring (Brittany 1) were genetically similar to marine anchovies of the Bay of Biscay, while  
420 individuals collected in autumn (Brittany 2) were grouped with the Northern anchovies (Fig. 3B).



425 *Figure 3. Proportion of individual's ancestry assigned to the different genetic clusters for K2 (panel A) and K3 (panel B), and the cluster mapping for K3 (panel C). Each line (panels A,B) corresponds to a single individual partitioned into K segments according to the coefficients of their relative cluster membership. On panel C, the cluster for each sample is set based on*

the dominant membership to each cluster among individuals, but samples 6 and 7 are

430

admixed between Biscay and estuarine clusters.

Hierarchical fixation indices clearly showed that although the geographic distance between ecotypes is small, their genetic differentiation is substantial ( $F_{ST} = 0.033$ ; Table 2). There was a clear genetic structure between anchovies sampled in the Bay of Biscay and in the northern European waters (English Channel, Irish Sea and North Sea;  $F_{ST} = 0.076$ ). This differentiation was consistent across ecotypes ( $F_{ST} = 0.044$ ) indicating that the North-South divide mattered both for estuarine and marine anchovies. Finally, the genetic differentiation between the sampling locations within each population was more limited ( $F_{ST} = 0.016$ ) compared to the differentiation between sampling locations within ecotypes ( $F_{ST} = 0.059$ ).

440 **Table 2.** Hierarchical indices of fixation ( $F_{ST}$ ) of anchovy partitioned according to their ecotype (estuarine vs. marine), population assignments (Bay of Biscay vs. English Channel/Irish Sea/North Sea), and their sampling location. The indices are calculated between the partitions of each type presented in columns, for all individuals (total) or for individuals within each partition presented in rows.

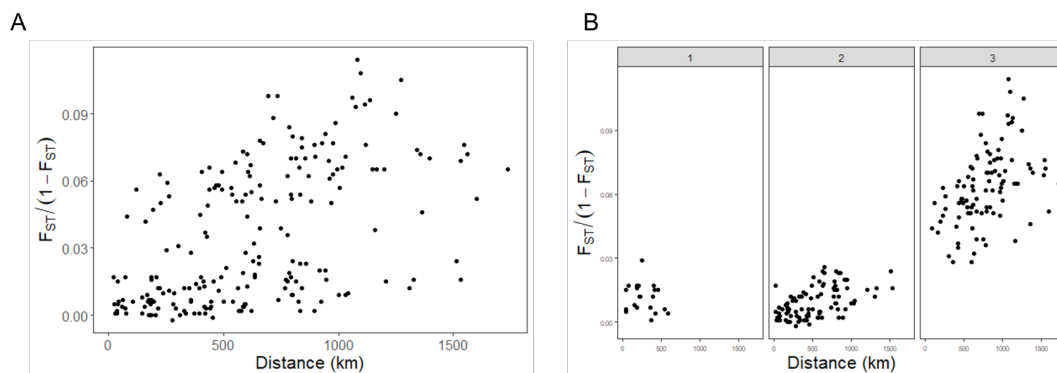
	Ecotype	Population	Sampling locations	Individuals
Total	0.033	0.076	0.090	0.140
Ecotype	-	0.044	0.059	0.110
Population	-	-	0.016	0.069
Sampling locations	-	-	-	0.054

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## 2) Pattern of isolation-by-distance and variation in genetic diversity along the latitudinal gradient

Across all sampling locations of the marine ecotype, there was a significant isolation-by-distance pattern ( $r = 0.531$ ;  $P = 0.001$ , Fig 4A). The amount of variance in genetic distances explained by the distances between sampling locations was relatively small as standardised pairwise  $F_{ST}$  values between sampling locations distant of less than 100 km and more than 1,000 km ranged from 0.001 to 0.044 and 0.009 to 0.114,

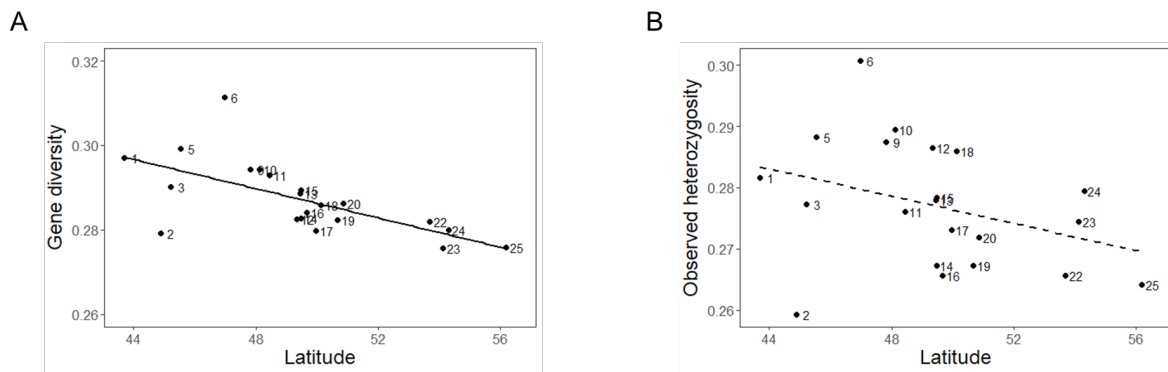
respectively (Fig. 4A). There was no significant isolation-by-distance within the Bay of  
 455 Biscay ( $r = -0.204$ ,  $P = 0.800$ , Fig. 4B), hence the overall pattern was primarily due to  
 the pattern of isolation-by-distance within the Northern group ( $r = 0.530$ ,  $P = 0.004$ )  
 associated with the strong differentiation between populations (Fig. 4B). Indeed,  
 pairwise  $F_{ST}$  values were particularly low across anchovies sampled in the English  
 Channel and the southern location of the North Sea (within sites 11-22 (without site  
 460 21): mean  $F_{ST}$ : 0.005; range: 0.002 to 0.013, Suppl. Mat.), but their pairwise  $F_{ST}$   
 values with anchovies sampled in Irish Sea and northern part of the North Sea were  
 substantially higher (between sites 11-22 (without site 21) and 23-25: mean: 0.016;  
 range: 0.002 to 0.026).



465 *Figure 4. Pattern of isolation-by-distance of anchovy in western European seas across all 21*  
*sampling locations of the marine ecotype (panel A) and split according to their population*  
*assignment (Bay of Biscay or Northern group; panel B). The geographical distance was*  
*calculated as a least cost path avoiding landmasses and area deeper than 300m. Groups in*  
*panel B are: within marine sampling locations of the Bay of Biscay (1), within marine*  
 470 *locations of the northern group (English Channel, Irish Sea, North Sea, 2), between marine*  
*locations of the Bay of Biscay and the Northern group (3).*

The gene diversity ( $H_S$ ) within each marine sampling site declined with increasing  
 latitude ( $\beta = -0.0017 \pm 0.0005$  (SE),  $t = -3.695$ ,  $P = 0.002$ , Fig. 5A) while the observed  
 475 heterozygosity ( $H_O$ ) only tended to decline ( $H_O$ :  $\beta = -0.0011 \pm 0.0007$  (SE),  $t = -1.635$ ,  
 $P = 0.119$ , Fig. 5B). Overall, the population assignment explained 4.5 times more of  
 the variance in pairwise genetic structure (18%) than the latitude itself (4%; Table 3)

confirming that the two populations are genetically distinct units and that within the Northern group there are signs of isolation-by-distance.



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Figure 5. Decline in within sample gene diversity (panel A) and observed heterozygosity (panel B) with latitude. Identification numbers for each sampling location and their corresponding information can be found in Table 1. Only samples assigned to the marine ecotype are considered.

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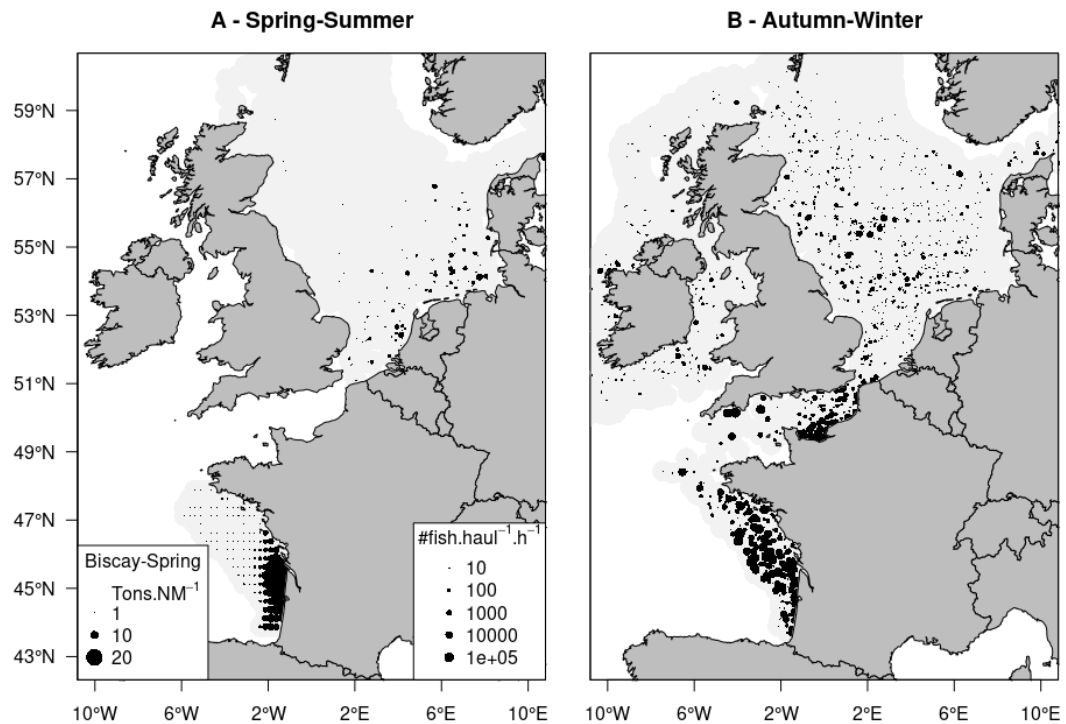
Table 3. Results of the distance-based redundancy analysis quantifying the relative contribution of population assignment and latitude on the variance in pairwise genetic differentiation on anchovies. The total inertia (variance) was partitioned to quantify the effect of population assignment (conditioned by latitude), the effect of the latitude (conditioned by the population assignment), and their shared effect. Only samples assigned to the marine ecotype are considered.

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Source of variation	Inertia (x10 <sup>-3</sup> )	Percentage
population assignment	0.24	0.18
Latitude	0.06	0.04
Shared inertia	0.77	0.58
Residual	0.26	0.20
Total	1.33	1.00



### 3) Seasonal variability in anchovy's distribution



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Fig.6 Anchovy distribution at two different seasons. Filled circles are CPUEs (in number of fish per haul per hour, log-transformed) except for spring in the Bay of Biscay (biomass in tons per nautical miles as Elementary Sampling Distance Unit averaged over 2000-2016). Grey background is the sampled domain revealing the areas/seasons without anchovy. See text for origin of survey data.

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Anchovy were much more concentrated during the reproductive season (Fig. 6A) than during the overwintering season when anchovies were spread over most of the northwest European shelf (Fig. 6B). More specifically, in the Bay of Biscay and over the French shelf, anchovy was widespread in autumn and occurred as far north as Brittany, while in spring it was much more concentrated in the south of the Bay. Similarly, anchovies in the northern areas in winter were recorded in the entire North Sea, the Irish Sea, and the English Channel. In the North Sea, highest abundances occurred in the central to western part. In summer, the distribution of Northern anchovies was primarily concentrated in the south-eastern North Sea, but there were no survey information around Scotland and Ireland during this season. Noteworthy,

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the small pelagic fish survey PELTIC (May-June 2011) reported no anchovy in the western Channel (ICES, 2011). In the Eastern Channel, the presence of anchovy was recorded at least in the Bay of Seine (one sample in August 2017 analysed in this study), but we lacked quantitative and wider distribution information.

#### 4) *Seasonal description of anchovy's habitat*

The quotient analysis (Fig. 7) confirmed the higher aggregations in spring in the Bay of Biscay and in summer in the North Sea, and showed that this pattern was linked with habitat preferences based on the covariates mapped on Fig. 8. Indeed, quotient values were generally much higher in summer (Q3) in the North Sea (Fig. 7abc) and May in the Bay of Biscay (Fig. 7ghi), than in winter (Q1, Fig. 7def) and November (Fig.7jkl), respectively. This revealed a much higher habitat selection during the spawning season in both regions, and these preferences (or avoidances) were more frequently statistically significant.

More specifically in the North Sea in summer, anchovies clearly preferred higher temperatures, and avoided lower temperatures, with a limit around 17°C (Fig. 7a). They also significantly preferred low salinity waters (<34 pss) and avoided higher salinity waters (Fig. 7b), and finally significantly preferred chlorophyll rich than chlorophyll poor waters (Fig. 7c). This preferred habitat corresponded to the south-eastern North Sea, which is its warmest and less saline part, with highest concentrations of chlorophyll-a (Fig. 8 g,h,i). In the North Sea in winter (Q1), temperature and salinity were more homogeneous (Fig. 8d,e), and the only significant pattern based on the quotient values was the avoidance of very cold (<4.5°C) and less saline (<34 pss) waters, that again corresponded to the south-eastern North Sea (Figs. 6b and 8a,b).

In the Bay of Biscay, anchovy in spring significantly preferred high temperatures (significantly above 14.8°C) and avoided low temperatures (significantly below 14.2°C), preferred salinities between 33.5 and 35 even though this was not  
540 significant, and significantly preferred chlorophyll rich waters (Fig. 7g,h,i). This combination of covariate preferences corresponded to the coastal habitat in front and south of the Gironde estuary in the south-eastern Bay of Biscay (Fig. 8d,e,f) and was consistent with the preferences observed in northern anchovies for warm, low saline and rich coastal waters. In the Bay of Biscay in autumn, there was no significant  
545 habitat preference for anchovy given the environment of Fig. 8j,k,l, but the relative avoidance of temperature below 14°C occurring offshore in the north of the bay.

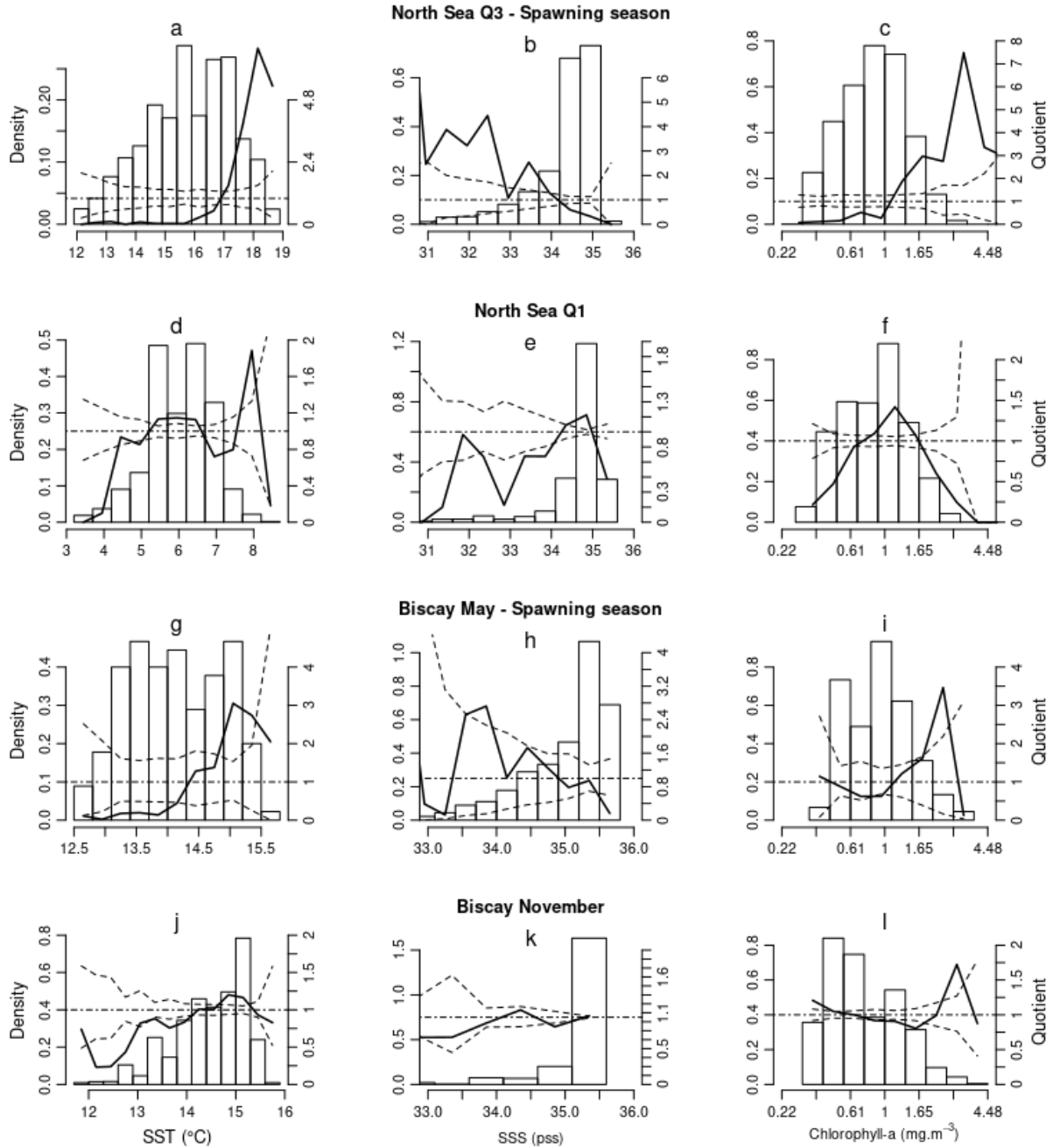


Fig. 7. Results of the quotient analysis. The quotient (continuous line, right axis of each plot) of fish CPUE or biomass (May in Biscay, see text) was calculated for three environment covariates (left to right: Sea Surface Temperature, Sea Surface Salinity and surface chlorophyll-a) and two different regions and seasons. Histograms represent the number of observations within each bin of the covariate, normalised as densities so that the surface of each histogram sum to 1. The horizontal dot-dashed line represent the null hypothesis of evenly distributed fish, and the dashed lines its 95% confident interval.

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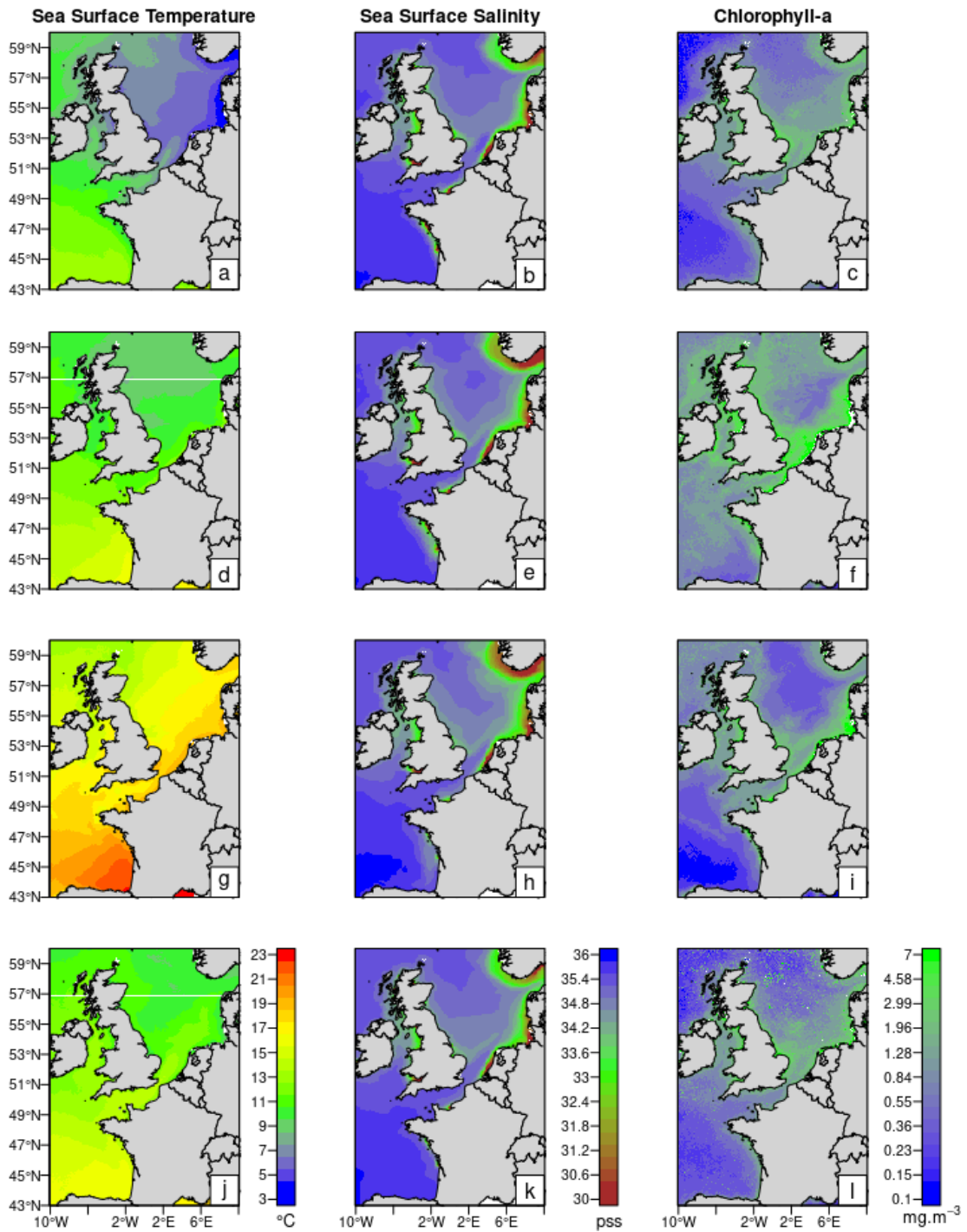


Fig.8. Monthly climatologies of Sea Surface Temperature (SST, °C, left panels), Sea Surface Salinity (SSS, pss, center panels) and surface chlorophyll-a ( $\text{mg.m}^{-3}$ , right panels) for the months of February (a,b,c), May (d,e,f), August (g,h,i) and November (j,k,l), from top to bottom.

## DISCUSSION

### 565           1) *Identification of the stocks*

Clearly identifying the boundaries between management units in different seasons while fish distribution changes substantially is a major challenge in fisheries research. To address this issue in the anchovy inhabiting western European waters, we carried out a thorough sampling of this species both in its known stronghold (the Bay of Biscay), its northern and unmanaged population (the North Sea) and in the English Channel to determine whether there is a clear boundary between these different units and whether anchovies captured in the English Channel belong to the Bay of Biscay or the Northern stocks. Identifying the correct number of clusters is not straightforward as the  $\Delta K$  method often leads to an overestimate of  $K_2$  (the likelihood of models testing whether there is no genetic cluster is often usually particularly low; Evanno et al. 2005) and the presence of isolation-by-distance can lead to an overestimation of  $K$  (Frantz et al. 2009). However, the presence of a clear plateau when  $K$  is greater than three clearly indicates that our samples comprise 2 or 3 clusters. At  $K_2$ , STRUCTURE identified the Bay of Biscay (excluding estuarine samples) and all the northern groups (including all estuarine samples) as distinct units. At  $K_3$ , the software was able to differentiate the estuarine ecotype (Gironde, Loire, IJsselmeer) and both marine populations of Bay of Biscay and marine Northern. These differences can be explained by the overriding differentiation between anchovies of the Bay of Biscay and those of the Northern group, the difference between marine and estuarine ecotypes being less pronounced especially in the Bay of Biscay where some sampling sites clearly had mixed ecotypes. Nevertheless, this result consolidates the temporal stability of the genetic pattern found in previous analyses that suggested that anchovies from the North Sea and the English Channel were genetically different from those of the Bay of Biscay (Zarraonaindia et al., 2012).

590 The Bay of Biscay forms a remarkably genetically homogeneous entity, as in spite of  
the large distance between the sampling locations, their genetic differentiation was  
limited (Fig 4B-1). This confirms previous genetic results in the Bay of Biscay  
(Montes et al., 2016; Zarraonaindia et al., 2012), but our analyses is clearly more  
robust given the more widespread sampling in space (samples collected as far north  
595 as the Iroise Sea at the tip of Brittany) and time (samples collected both in spring and  
late autumn). The population structure found for anchovy in north-western European  
waters is remarkable, considering the low levels of differentiation generally found in  
marine populations due to large effective sizes and only a recent divergence, which  
suggests that the migration and/or drift equilibrium has been reached between the  
600 Bay of Biscay and the northern populations. Silva et al. (2014) proposed the  
colonisation of the northern areas from the Bay of Biscay after the Last Glacial  
Maximum (~18kyr), while Zarraonaindia et al. (2012) suggested that only after the  
reopening of the land bridge between the British Isles and the continent (~7500yr)  
was the northward dispersal possible.

605 For the first time we described an estuarine population in the Loire estuary, which  
with the Gironde are the two largest estuaries of the Bay of Biscay in terms of river  
run-offs. The Loire estuarine population adds to the ones already described in North-  
western Europe: Gironde (Montes et al., 2016), Adour (Le Moan et al., 2016), and  
Ijsselmer (Montes et al., 2016) in the Netherlands. However, we were not able to  
610 sample 'estuarine' anchovies in the Seine estuary. The genetic analyses clearly  
showed that the anchovies collected at the mouth of the estuary (#14, Fig. 1)  
belonged to the marine ecotype of the Northern group. These observation and result  
suggest that there may be no estuarine population in the Seine estuary (the  
NOURDEM survey sampled extensively the Seine estuary and found no anchovies  
615 upstream of this location). Based on the distances between estuaries, connectivity

seems possible between Gironde and Loire, especially because their low salinity plumes sometimes connect along the coast, bridging a marine high salinity gap that estuarine anchovies would otherwise probably avoid. However, connectivity does not seem an option between the Bay of Biscay estuaries and IJsselmeer in the Netherlands. In support to this, none first generation estuarine migrant was found among our marine samples besides the mixed samples within river plumes. Thus common historical evolutionary processes, or insufficient time to create genetic differences since their separation, may more likely be the reasons for such similarity between remote estuaries.

625

## 2) Gene flow and connectivity between the populations

Our analyses clearly showed that western Brittany waters acted as the boundary between anchovies of the Bay of Biscay and those of the Northern group, despite a convergence in autumn in the Celtic Sea, west of Brittany, of part of both the Bay of Biscay and northern populations. The region to the west-northwest of Brittany has been described as a biogeographical transition zone between the warm-temperate and cold-temperate water masses, or Lusitanian-Boreal (to the south) and Boreal-Lusitanian (to the north) marine provinces (Cox and Moore, 2000; Dinter, 2001). Although these assemblages were mostly set based on invertebrate or macrophyte taxa, it also represented a boundary in the distribution range of fish species. For example, observations of boreal species such as Atlantic cod (*Gadus Morhua*), Haddock (*Melanogrammus aeglefinus*) or Atlantic herring (*Clupea harengus*) are very rare within the Bay of Biscay (Froese and Pauly, 2019) while they are targeted by fisheries within and northward of the Celtic Sea – English Channel area. However at the level of populations, there exists surprisingly no other description of fish species with a clear genetic boundary west of Brittany. This is likely due to the low sampling



effort generally performed in the English Channel in genetic studies. For example, Charrier et al. (2007) revealed a clear genetic distinction for whiting (*Merlangius merlangus*) between the Bay of Biscay population and individuals sampled to the north of the English Channel, unfortunately without samples in the later area. A similar result was found for sole (*Solea solea*), with no clear assignment of the unique sample from the western English Channel to either the Bay of Biscay or northern populations (Cuveliers et al., 2012). Only for an invertebrate species a sharp genetic break was also found west of Brittany (Jolly et al., 2005). The 'Iroise Sea' at the west of Brittany is a particularly interesting area in terms of hydrodynamics with strong tidal currents all year round and a complex frontal structure named the Ushant front from May to October (Pingree, 1980; Le Boyer et al., 2009) that sets a clear hydrological discontinuity between the Bay of Biscay and the western English Channel and may act as a barrier to larval dispersal (Gailord and Gaines, 2000; Le Fèvre, 1986) or adult movement.

Putatively first generation migrants were extremely rare between the two populations (0.05% in the Bay of Biscay and 8.7% in the north), confirming that gene flow is limited. These 'migrants' were not part of the samples closest to the boundary between the two populations, but from a marine sample in front of the Loire on one hand, and from samples throughout the English Channel on the other hand, suggesting that they are well integrated in the local population. We recognise that these first generation migrants could be artifacts, i.e. outlier individuals that were misassigned by STRUCTURE due to a peculiar genetic diversity. If real 'migrants', the difference found between regions might be explained by two factors. First, the difference in population abundance between the two populations can create a bias, even in the case of similar absolute numbers of adults migrating, or larvae connecting through passive drift, between the two populations. Second, there could

be a real directional gene flow, especially when considering connectivity occurring during the early life stages of this species. Indeed, using a larval drift model and generic species, Ayata et al. (2010) concluded that the Iroise Sea 'acts as a partly-permeable one-way barrier for connectivity: northwards larval exchanges are scarce, whereas southwards larval exchanges are unlikely'. Furthermore, with the same type of model but applied to the European anchovy, Huret et al. (2010) showed that eggs spawned in the north of the Bay of Biscay can potentially end up in the western English Channel, but only under specific oceanographic conditions such that the spatio-temporal pattern of anchovy spawning may effectively strongly limits this connectivity (Petitgas et al., 2012). As no spawning has ever been reported in the western English Channel, larval drift from the northern population (southern North Sea spawning habitat) to the Bay of Biscay seems impossible due to a combination of distance and eastward residual circulation in the English Channel (Pingree and Maddock, 1977). The large spatio-temporal diversity of samples collected in our study and the clearly limited gene flow between the anchovies of the Bay of Biscay and the Northern group, confirms the conclusion of Petitgas et al. (2012) on a local outburst rather than an allopatric origin to explain the recent increase in abundance of the North Sea anchovy.

The sharp genetic discontinuity that we found between the two groups indicated that they are unlikely to interbreed at the tip of Brittany although their spatial ranges slightly overlap in autumn. This can easily be explained by the fact that the Bay of Biscay population stops spawning in August (Huret et al., 2018; Motos et al., 1996) and that no spawning has ever been reported in the western English Channel. Hence, anchovies observed in that area spawn elsewhere and solely occupy this area for other purposes than breeding (see section 5 below). Despite the potential oceanographic boundary of the Iroise Sea at the tip of Brittany as described above, it

is still surprising that no more adult individuals from the Bay of Biscay venture in the  
695 more plankton productive English Channel in summer, and that no more individuals  
from the English Channel venture south following a positive water temperature  
gradient, especially in spring when searching for a suitable spawning habitat. Overall,  
this suggests a strong spawning site fidelity for both populations.

Concerning the differences between 'marine' and 'estuarine' anchovies, Montes et al.  
700 (2016) proposed three potential mechanisms to explain their reproductive isolation  
despite an *a priori* possibility for hybridisation and an overlap during their spawning  
season. First, in these overlapping areas, spawning behaviour and mate selection,  
for example based on size, may isolate the phenotypically divergent ecotypes.  
Second, gamete incompatibility may prevent reproduction between them, which was  
705 supported in their study by differences between ecotypes at two outlier genes,  
apparently under natural selection and involved in a critical stage of fertilisation  
process. Third, if hybridisation was to occur, low hybrid fitness could prevent them  
from reaching reproductive maturity, which was also supported by the different  
frequencies between ecotypes of the outlier genes associated to metabolic  
710 pathways.

### 3) *Is the Northern population genetically homogeneous ?*

There is a low but clear decline in gene diversity with the latitude (the observed  
heterozygosity only tended to decline), and some of the variance in pairwise genetic  
715 structure was explained by the latitude itself. These patterns are mostly explained by  
the northernmost samples of the Irish and North seas suggesting that the Northern  
group is not genetically homogeneous. The Northern group was probably founded  
after the last glacial maximum (~18ky) with large numbers of anchovies enabling it to

retain most of the genetic diversity of the Bay of Biscay population (Silva et al. 2014).  
720 Gene flow between anchovies of the Bay of Biscay and the Northern group  
subsequently declined enabling the action of genetic drift that led to the sharp genetic  
differences we found between anchovies of the Bay of Biscay and those of the  
Northern group. The low decline in genetic diversity and the significant isolation-by-  
725 distance pattern within the Northern group could then be due to more recent or  
ongoing founder effect at the northern edge of the distribution area of European  
anchovy. Although samples collected in the English Channel were clearly genetically  
similar to those of the southern North Sea, these were distinct to anchovies sampled  
in the Irish Sea and north-western North Sea. As the sample from the north-western  
North Sea was collected in May, just before the known spawning season in the south-  
730 eastern North Sea (Alheit et al., 2012), it is therefore clearly possible that  
northernmost locations represent distinct spawning units, but further research are  
needed to confirm this result.

#### *4) A contraction of anchovy's distribution during spawning*

735 Distributions of anchovies inhabiting the Bay of Biscay and the North Sea had a  
similar seasonal pattern, with a clear contraction during the spawning season and a  
spreading over a much larger area for overwintering. With aggregated abundance  
data over multiple years and climatologies for environment covariates, our objective  
was not to model precisely anchovy habitat as it was done already for the spawning  
740 season (Ibaibarriaga et al., 2013; Petitgas et al., 2014; Planque et al., 2007). Our  
objective was rather to explain the pattern of variability in anchovy distribution  
between seasons. Anchovy's spawning has extensively been studied in the Bay of  
Biscay, spanning from April to August with a peak in May-June (Huret et al., 2017;  
Motos et al., 1996) with some affinity for productive areas such as plumes

745 (Koutsikopoulos and Le Cann, 1996) and highest local temperatures (Planque et al., 2007). As the season progresses spawning was described to extend further north, and autumn distribution is well spread throughout all the Bay of Biscay (ICES, 2010b; Uriarte et al., 1996).

Using for the first time the EVHOE bottom trawl survey in autumn in addition to the  
750 small pelagic survey in spring, our results confirm previous findings, with a contraction of the spawning habitat in warmer and more productive areas corresponding in spring to the plumes in the south-eastern Bay of Biscay, and a large spreading in autumn as well as probably winter (Figs. 6,7,8). Using a bioenergetic model, Politikos et al. (2015) showed that this spawning aggregation was favorable  
755 for adult anchovy bioenergetics and eventually fecundity, and temperature and productivity are obviously key elements for the growth and survival of early life stages.

The North Sea anchovy habitat has not yet received as much attention, but the southern North Sea is known as the main spawning grounds with specific locations  
760 such as the Schelde estuary, the Wadden Sea and German Bight hosting a significant anchovy biomass during the spawning season (from June to August with a peak in July; Alheit et al., 2012). Similarly to the Bay of Biscay, our results suggest that the spawning aggregation in the Northern group is strongly driven by temperature, salinity, and productivity gradients, attracting anchovy to the south-  
765 eastern North Sea from a much more widespread distribution in winter (Figs. 6,7,8).

The winter distribution clearly shows anchovy's avoidance of the coldest areas of the southern North Sea. The north-western North Sea is directly under the influence of the North Atlantic warm waters that temperate their winter cooling, while the south-eastern North Sea is both shallow and influenced by river discharges and thus more  
770 prone to rapid cooling (Rodhe, 1998). The warming of the North Sea waters is also

quicker in this area in summer, and as such represents an anomaly in the latitudinal gradient of temperature in addition to being very productive (Fig. 8g,i). First individuals settling north of the Bay of Biscay certainly had to reach this particular habitat for spawning and be able to complete their life cycle. This area also likely served as a refuge for a remnant population during periods of adverse environmental conditions but clearly, this population is a bridgehead of the European anchovy, ready to expand when conditions become more favorable (Alheit et al., 2012; Petitgas et al., 2012).

780           5) *Autumn anchovy in English Channel coming from the south-eastern North Sea spawning aggregation?*

There are evidences that anchovy is absent in spring-summer in the English Channel, in particular in its western part. More than a century ago, Cunningham (1895) mentioned its presence in autumn-winter but could not find any in spring. Later, Arbault and Lacroix (1971) found no anchovy egg in the Celtic Sea during May and July 1968, although this corresponds to its spawning season. Again, no anchovy eggs were reported off Plymouth in June-July 1969 and 1970 (Demir and Southward, 1974). More recently, the PELTIC survey that occurred in May-June 2011 on its first year, could not find any adult anchovy (ICES, 2011), and only one egg was reported offshore Brittany thus likely spawned by the few individuals of the Bay of Biscay population that remains in the north in spring.

From this statement, one can ask 'where do the observed autumn anchovies come from, and why do they come in this specific area?' Our genetic results clearly showed that they belong to the northern group, and strongly suggest that they are part of a spreading out from the spawning grounds of the south-eastern North Sea, based on

the strong homogeneity found between the English Channel and the southern North Sea within the identified northern group, thereby confirming the assumption proposed by Cunningham (1895) and Redeke (1916) a long time ago. Our quotient plots for the northern group only encompass the North Sea because fish distribution data only covers one season elsewhere. Nonetheless one can hypothesise that part of this population that spread around within the North Sea to escape its coldest coastal waters in winter, could also be attracted by the warmest, Atlantic influenced waters of the English Channel. This migration would occur in autumn when the gradient of temperature reverse between the southern North Sea and the northern North Sea or English Channel. The gradient of chlorophyll-a also generally decreases which reduces anchovies' benefits of remaining in the south-eastern North Sea. Conversely, as the spawning season approaches, anchovies leave the English Channel guided by a positive gradient of both temperature and food towards the south-eastern North Sea (Fig. 8D-I). We do not have yet evidences of spawning in the eastern Channel, but it may occur in specific areas such as the Bay of Seine, whose environmental properties resembles those of the south-eastern North Sea in summer. This would represent a shorter distance to migrate back and forth for western Channel anchovies.

#### 815           6) *Implication for management*

Our results show that the northern delineation of the Bay of Biscay stock, currently set at 48°N, is justified but may be refined. Indeed, the assessment group traditionally considered that the portion of the catch in the coastal Celtic Sea in front of Brittany (rectangles 25E4 and 25E5 of subarea VII, see Fig.1) consists of individuals from the Bay of Biscay stock. Our genetic analyses validate this choice as the most coastal sampling location in spring (#10 in the 'Iroise Sea', see Fig.4C) was

assigned to the Bay of Biscay group. However, the sampling location 11, just few miles offshore but collected in autumn, belongs to the northern group. Hence, during the spring anchovy biomass assessment, the small biomass observed on some  
825 years along the coast north of 48°N does belong to the Bay of Biscay stock; anchovies of the northern stock have left this area towards the North Sea. Moreover, it is likely that the autumn catches from the coastal area west of Brittany again belong to the Bay of Biscay anchovies. Yet, as northern anchovies are present in the western Channel and Celtic Sea, we cannot exclude that some of them come along  
830 the coast inside the fishing area. Additional samples from this transitional area are needed to robustly test this hypothesis, and, if rejected, then the northern boundary of the Bay of Biscay stock could be shifted to include the Iroise Sea at the tip of Brittany.

The spatial overlap of marine and estuarine ecotypes in river plumes may be  
835 problematic with respect to the biomass assessment of marine anchovies. Indeed, the estuarine ecotype has a strong affinity to low salinity waters in European waters (Montes et al., 2016; Zarraonaindia et al., 2012), and their core habitat are located within the estuaries. However, in spring in the Bay of Biscay, when river discharges are at their maximum after wet winters, estuarine anchovies may expand together  
840 with the river plumes that spread along the coast outside estuaries. In Montes et al. (2016) six of the twelve samples collected outside the Gironde estuary were mixed group holding both marine and estuarine ecotypes (another sampling location at the mouth of the estuary consisted in pure estuarine anchovies), and estuarine anchovies could be sampled further away from the estuary in spring than in autumn.  
845 We did not collect any mixed samples among the two spring and unique autumn samples made outside the Gironde estuary. However, we identified two mixed samples with anchovies from the newly identified population of the Loire estuary, in



spring, one at the mouth (#7, Fig.4C) and one in the river plume (#6). Scientific surveys (PELGAS and BIOMAN) occur specifically in spring in the region and they  
850 logically sample the plumes for which spawning marine anchovy has some affinity (Figs. 6 and 7). Parameters such as age-length keys and maturity are pivotal in assessment methodologies of the population biomass (ICES, 2017). As growth patterns differ between the two ecotypes (Guerault and Avrilla, 1976; Aldanondo et al., 2010), as well as potentially the reproductive traits, the assessment process may  
855 be biased by the presence of estuarine anchovies in the sampled hauls. An assessment of this bias should be performed if a simple visual separation of the two ecotypes is too uncertain or not feasible operationally. In the same way, marine and estuarine anchovies may overlap in the North Sea, especially during summer when marine anchovies gather in the low saline south-eastern North Sea close to Belgian,  
860 Netherlands and German large estuaries. Our quotient plot results reveals an affinity of North Sea anchovies for water salinities lower than 33 pss, salinities in which they are barely observed in the Bay of Biscay. Refined genetic studies along the North Sea southern coast should be performed in order to evaluate the proportion of estuarine anchovies within the spawning habitat of the marine ecotype.

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880 **Suppl. Table 1.** Summary statistics of the 308 SNPs used in this study calculated in three  
predefined broad groups: coastal, offshore Bay of Biscay, offshore northern area (the English  
Channel, the Irish Sea, the North Sea). Ho: mean observed heterozygosity across all  
885 sampling locations; He: mean expected heterozygosity across all sampling locations; HWd:  
number of sampling locations in which each loci deviated from the Hardy-Weinberg  
expectations; MAF: minor allele frequency, loci with MAF < 0.01 over the entire study are  
were excluded.

Locus	Ho	Hs	HWd (sites)	MAF	Exclusion
X01597_544	0.27	0.42	9	0.299	-
X03047_470	0.44	0.48	1	0.430	-
X03273_425	0.31	0.31	1	0.186	-
X03345_524	0.32	0.38	2	0.361	-
X03543_182	0.04	0.03	0	0.016	-
X03581_48	0.26	0.27	3	0.163	-
X04911_237	0.48	0.47	2	0.399	-
X05056_415	0.42	0.44	1	0.443	-
X05339_649	0.37	0.39	0	0.261	-
X05521_139	0.49	0.49	0	0.422	-
X05837_299	0.01	0.01	0	0.003	MAF
X05840_191	0.41	0.39	0	0.397	-
X06078_113	0.31	0.29	0	0.175	-
X07202_225	0.43	0.44	0	0.334	-
X08788_1119	0.33	0.38	2	0.270	-
X08794_814	0.42	0.44	5	0.349	-
X08799_2280	0.15	0.16	1	0.094	-
X08816_261	0.40	0.42	3	0.291	LD
X08817_1665	0.45	0.49	2	0.455	-
X08821_838	0.01	0.01	0	0.005	MAF
X08834_1249	0.00	0.00	0	0.001	MAF
X08841_1676	0.26	0.29	2	0.188	-
X08846_648	0.46	0.48	1	0.459	-
X08852_1221	0.37	0.40	1	0.316	LD
X08861_947	0.51	0.46	4	0.424	-
X08875_1272	0.27	0.29	1	0.176	-
X08898_1484	0.38	0.40	1	0.276	-
X08906_470	0.34	0.38	3	0.329	-
X08911_1337	0.49	0.49	0	0.425	-
X08912_590	0.17	0.19	1	0.105	-
X08914_377	0.38	0.38	3	0.308	-
X08920_497	0.01	0.01	0	0.003	MAF
X08932_629	0.31	0.36	3	0.498	-
X08984_532	0.47	0.41	9	0.299	LD
X08985_993	0.33	0.35	1	0.223	-
X08993_795	0.43	0.49	2	0.481	-
X09006_1435	0.34	0.38	2	0.250	-
X09043_1099	0.31	0.35	3	0.485	LD
X09045_299	0.34	0.34	2	0.313	-
X09058_237	0.01	0.03	1	0.012	-
X09095_1129	0.48	0.46	2	0.498	-
X09096_722	0.50	0.49	4	0.481	-
X09107_368	0.06	0.07	1	0.034	-
X09111_1359	0.23	0.24	0	0.145	-
X09153_640	0.48	0.48	1	0.426	-
X09160_940	0.47	0.47	1	0.383	-
X09178_827	0.34	0.37	3	0.404	LD
X09192_804	0.48	0.49	1	0.446	-

X09207_399	0.06	0.07	2	0.039	-
X09207_789	0.42	0.43	2	0.344	-
X09226_650	0.35	0.37	2	0.266	-
X09227_165	0.07	0.08	1	0.043	-
X09236_194	0.76	0.43	19	0.438	Hwe
X09279_638	0.35	0.40	3	0.301	-
X09281_860	0.40	0.40	5	0.431	-
X09282_621	0.33	0.40	4	0.394	-
X09321_825	0.07	0.08	3	0.038	-
X09327_303	0.06	0.07	1	0.039	-
X09372_271	0.20	0.22	2	0.123	-
X09375_117	0.03	0.03	0	0.013	-
X09383_316	0.44	0.44	2	0.326	-
X09390_450	0.43	0.45	1	0.362	-
X09419_427	0.05	0.06	0	0.034	-
X09456_504	0.15	0.15	1	0.085	-
X09461_83	0.21	0.24	2	0.145	-
X09481_410	0.24	0.25	1	0.159	-
X09490_1093	0.45	0.45	0	0.348	-
X09494_827	0.38	0.38	2	0.291	-
X09503_988	0.50	0.50	2	0.479	-
X09505_542	0.01	0.01	0	0.008	MAF
X09513_880	0.40	0.49	4	0.467	-
X09538_502	0.47	0.48	1	0.438	-
X09543_535	0.45	0.46	2	0.372	-
X09560_634	0.16	0.16	2	0.092	-
X09567_523	0.08	0.10	5	0.048	-
X09602_685	0.12	0.11	2	0.059	-
X09632_809	0.11	0.10	0	0.059	-
X09696_245	0.06	0.07	5	0.038	-
X09709_113	0.28	0.29	0	0.184	-
X09712_527	0.05	0.04	0	0.025	-
X09724_89	0.02	0.03	2	0.015	-
X09738_452	0.08	0.10	5	0.051	-
X09743_231	0.30	0.44	6	0.323	-
X09748_471	0.23	0.22	0	0.124	-
X09764_372	0.03	0.03	0	0.012	-
X09772_525	0.00	0.00	0	1.000	-
X09780_701	0.00	0.00	0	1.000	-
X09813_652	0.40	0.41	1	0.343	LD
X09834_192	0.35	0.37	1	0.287	-
X09841_948	0.44	0.45	1	0.353	-
X09845_779	0.05	0.05	0	0.027	-
X09849_297	0.47	0.47	2	0.457	-
X09851_624	0.33	0.33	1	0.279	-
X09864_268	0.14	0.15	1	0.108	-
X09871_575	0.04	0.04	0	0.016	-
X09896_551	0.11	0.13	3	0.079	-
X09921_169	0.34	0.40	3	0.380	-
X09950_363	0.36	0.36	3	0.236	-
X10005_820	0.00	0.00	0	1.000	-
X10019_1019	0.23	0.26	3	0.157	-
X10032_732	0.07	0.09	4	0.040	-
X10043_585	0.32	0.37	4	0.368	-
X10058_1013	0.33	0.37	3	0.402	LD
X10068_758	0.34	0.41	3	0.329	LD
X10075_728	0.10	0.15	4	0.076	-
X10080_692	0.14	0.19	3	0.136	-
X10113_376	0.26	0.30	4	0.197	-
X10124_576	0.45	0.45	1	0.350	-
X10128_444	0.22	0.24	1	0.176	-



X10153_343	0.00	0.00	0	1.000	-
X10182_173	0.42	0.49	1	0.427	-
X10193_287	0.02	0.03	0	0.010	MAF
X10205_544	0.32	0.34	1	0.238	-
X10237_141	0.18	0.19	0	0.107	-
X10239_215	0.14	0.13	0	0.066	-
X10334_161	0.40	0.41	1	0.306	-
X10345_230	0.43	0.48	0	0.419	-
X10366_160	0.30	0.29	1	0.184	-
X10404_730	0.34	0.40	3	0.421	-
X10421_445	0.21	0.22	0	0.130	-
X10450_563	0.29	0.26	0	0.156	-
X10457_564	0.39	0.38	1	0.271	-
X10461_739	0.12	0.13	3	0.074	-
X10478_384	0.15	0.20	4	0.099	-
X10478_970	0.30	0.38	4	0.273	-
X10484_817	0.02	0.03	2	0.013	-
X10539_125	0.46	0.48	2	0.413	-
X10572_547	0.29	0.34	1	0.238	-
X10584_740	0.18	0.23	6	0.132	-
X10601_603	0.18	0.21	2	0.123	-
X10602_675	0.41	0.44	1	0.325	-
X10620_734	0.40	0.45	2	0.340	-
X10627_879	0.04	0.05	2	0.024	-
X10662_571	0.05	0.05	1	0.023	-
X10689_211	0.45	0.50	2	0.492	-
X10719_256	0.35	0.38	1	0.263	-
X10779_629	0.16	0.18	1	0.109	-
X10790_724	0.01	0.01	0	0.006	MAF
X10806_153	0.39	0.38	0	0.267	-
X10823_224	0.42	0.46	1	0.346	-
X10837_384	0.12	0.13	3	0.067	-
X10884_599	0.39	0.41	3	0.314	-
X10887_78	0.42	0.44	1	0.465	-
X10927_290	0.14	0.15	3	0.080	-
X10932_78	0.28	0.31	3	0.198	-
X10977_388	0.27	0.28	1	0.182	-
X11029_665	0.32	0.36	2	0.264	LD
X11043_621	0.10	0.10	0	0.052	-
X11045_387	0.38	0.46	3	0.347	-
X11055_434	0.08	0.09	2	0.044	-
X11061_716	0.14	0.14	0	0.086	-
X11095_384	0.31	0.32	2	0.200	-
X11108_448	0.35	0.39	2	0.285	-
X11214_334	0.49	0.49	0	0.421	-
X11242_689	0.39	0.37	3	0.248	-
X11294_128	0.02	0.03	1	0.014	-
X11303_506	0.46	0.47	1	0.397	-
X11405_637	0.08	0.10	4	0.050	-
X11416_126	0.23	0.24	2	0.159	-
X11499_395	0.18	0.19	2	0.112	-
X11502_593	0.47	0.49	1	0.478	-
X11510_530	0.32	0.35	3	0.492	LD
X11626_688	0.36	0.38	1	0.429	-
X11688_328	0.22	0.21	0	0.116	-
X11711_568	0.03	0.03	0	0.015	-
X11722_296	0.45	0.46	0	0.357	-
X11777_324	0.32	0.38	6	0.435	LD
X11782_536	0.25	0.24	0	0.132	-
X11791_524	0.27	0.29	1	0.181	-
X11914_189	0.28	0.32	2	0.205	-

X11919_505	0.02	0.03	1	0.012	-
X11939_264	0.24	0.25	2	0.145	-
X11965_96	0.15	0.15	2	0.083	-
X11980_453	0.39	0.41	0	0.305	-
X11993_334	0.31	0.34	2	0.496	LD
X12008_538	0.07	0.09	6	0.047	-
X12019_326	0.39	0.49	3	0.462	-
X12083_486	0.30	0.30	0	0.218	-
X12122_724	0.00	0.00	0	1.000	-
X12135_197	0.44	0.50	3	0.464	-
X12144_233	0.22	0.23	1	0.136	-
X12312_319	0.25	0.29	2	0.182	-
X12317_510	0.35	0.36	1	0.237	-
X12382_526	0.37	0.40	3	0.283	-
X12437_210	0.27	0.26	3	0.184	-
X12439_304	0.00	0.00	0	1.000	-
X12446_189	0.36	0.38	1	0.299	-
X12459_84	0.19	0.21	3	0.118	-
X12532_591	0.41	0.41	3	0.305	-
X12567_537	0.46	0.48	1	0.405	-
X12646_317	0.08	0.14	5	0.073	-
X12658_528	0.31	0.32	2	0.229	-
X12672_206	0.03	0.03	0	0.020	-
X12680_272	0.31	0.33	4	0.249	-
X12713_425	0.31	0.34	3	0.230	-
X12781_398	0.28	0.28	3	0.215	-
X12833_193	0.38	0.41	3	0.331	LD
X12834_74	0.12	0.12	0	0.070	-
X12855_124	0.01	0.02	2	0.006	MAF
X12873_197	0.22	0.24	2	0.136	-
X12918_432	0.19	0.21	1	0.114	-
X12998_366	0.36	0.40	2	0.322	LD
X13069_588	0.20	0.23	1	0.139	-
X13098_472	0.16	0.17	3	0.143	-
X13127_346	0.46	0.47	2	0.377	-
X13144_589	0.35	0.22	8	0.190	-
X13163_324	0.25	0.20	5	0.149	-
X13244_434	0.41	0.42	1	0.303	-
X13299_460	0.27	0.26	2	0.153	-
X13323_578	0.42	0.42	1	0.296	-
X13451_592	0.43	0.47	2	0.398	-
X13524_148	0.39	0.41	3	0.335	LD
X13569_332	0.38	0.37	2	0.258	-
X13625_105	0.31	0.39	5	0.421	-
X13626_152	0.06	0.09	2	0.047	-
X13703_470	0.34	0.37	2	0.396	LD
X13719_283	0.41	0.43	2	0.323	-
X13903_340	0.37	0.38	0	0.261	-
X13905_323	0.33	0.37	1	0.272	-
X13909_340	0.04	0.04	1	0.022	-
X13953_240	0.41	0.47	3	0.395	-
X13983_433	0.27	0.33	4	0.221	-
X14158_552	0.30	0.31	2	0.182	-
X14185_525	0.12	0.15	0	0.095	-
X14273_522	0.25	0.27	3	0.174	-
X14317_289	0.04	0.04	1	0.019	-
X14370_397	0.18	0.20	2	0.117	-
X14371_319	0.25	0.31	3	0.200	-
X14391_161	0.31	0.41	4	0.301	-
X14403_126	0.36	0.40	3	0.351	LD
X14713_393	0.12	0.14	0	0.083	-

X14832_383	0.42	0.37	3	0.497	-
X14900_404	0.45	0.48	2	0.436	-
X14931_283	0.42	0.42	1	0.388	LD
X14961_184	0.20	0.26	5	0.155	-
X15159_307	0.06	0.07	2	0.036	-
X15283_412	0.17	0.17	2	0.106	-
X15306_404	0.40	0.40	0	0.334	LD
X15336_423	0.34	0.38	1	0.479	LD
X15347_281	0.22	0.22	1	0.130	-
X15544_184	0.33	0.43	4	0.473	-
X15582_400	0.46	0.50	2	0.473	-
X15585_247	0.35	0.42	2	0.324	-
X15643_532	0.41	0.47	3	0.383	-
X15646_218	0.00	0.00	0	0.002	MAF
X15669_206	0.48	0.50	2	0.496	-
X15726_130	0.09	0.08	0	0.044	-
X15748_406	0.28	0.31	3	0.203	-
X15751_465	0.00	0.00	0	0.002	MAF
X15762_364	0.12	0.16	4	0.086	-
X15997_474	0.32	0.33	3	0.216	-
X16005_81	0.01	0.01	0	0.006	MAF
X16035_158	0.21	0.23	2	0.128	-
X16066_298	0.29	0.33	1	0.196	-
X16105_487	0.27	0.28	1	0.169	-
X16208_135	0.06	0.08	3	0.038	-
X16254_137	0.18	0.20	2	0.194	-
X16325_155	0.37	0.40	3	0.339	LD
X16396_134	0.37	0.43	2	0.340	-
X16535_123	0.40	0.47	3	0.385	-
X16577_226	0.44	0.45	2	0.487	-
X16603_434	0.07	0.07	0	0.039	-
X16681_268	0.42	0.44	1	0.347	-
X16817_168	0.46	0.47	1	0.409	-
X17006_155	0.37	0.40	3	0.322	-
X17035_281	0.21	0.21	0	0.118	-
X17311_200	0.25	0.25	1	0.155	-
X17334_71	0.47	0.49	1	0.471	-
X17438_37	0.35	0.38	3	0.261	-
X17522_192	0.27	0.27	2	0.152	-
X17577_212	0.53	0.48	6	0.428	-
X17821_110	0.15	0.16	1	0.088	-
X17829_160	0.44	0.48	4	0.458	-
X17848_61	0.09	0.12	3	0.066	-
X18075_369	0.39	0.45	3	0.450	LD
X18203_289	0.49	0.46	3	0.409	-
X18293_90	0.48	0.46	0	0.361	-
X18677_177	0.10	0.10	0	0.058	-
X18747_319	0.44	0.50	2	0.437	-
X18748_228	0.37	0.46	3	0.395	-
X18928_162	0.01	0.01	0	0.007	MAF
X18932_292	0.39	0.40	1	0.284	-
X19017_210	0.37	0.41	3	0.339	LD
X19190_426	0.35	0.37	2	0.239	-
X19256_208	0.05	0.05	0	0.025	-
X19285_138	0.04	0.07	2	0.034	-
X19314_207	0.37	0.38	3	0.409	-
X19364_189	0.12	0.13	3	0.080	-
X19688_182	0.30	0.30	0	0.189	-
X19769_93	0.42	0.46	2	0.382	-
X19801_36	0.21	0.22	1	0.137	-
X19862_253	0.07	0.08	0	0.048	-

X19931_290	0.39	0.39	4	0.316	-
X20051_166	0.01	0.01	0	0.007	MAF
X20143_77	0.44	0.44	1	0.456	-
X20366_139	0.21	0.32	7	0.193	-
X20370_220	0.21	0.22	2	0.132	-
X20576_112	0.42	0.42	2	0.354	-
X20596_142	0.25	0.25	4	0.148	-
X20897_88	0.18	0.22	2	0.119	-
X20947_178	0.29	0.30	1	0.192	-
X21300_138	0.29	0.31	2	0.198	-
X21652_168	0.16	0.17	2	0.090	-
X21904_186	0.39	0.42	2	0.307	-
X21994_350	0.06	0.06	0	0.027	-
X22028_308	0.37	0.42	3	0.308	-
X22056_332	0.21	0.24	2	0.139	-
X22375_80	0.03	0.04	2	0.020	-

**Suppl. Table 2.** Pairwise  $F_{ST}$  values among the 25 sampling locations of the European anchovy in western European seas used in this study. Description of the sampling locations can be found in Table 1.

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	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	
1	-																									
2	0.008	-																								
3	0.007	0.014	-																							
4	0.129	0.144	0.148	-																						
5	0.012	0.017	0.005	0.127	-																					
6	0.012	0.028	0.017	0.092	0.016	-																				
7	0.055	0.075	0.066	0.034	0.050	0.023	-																			
8	0.151	0.178	0.163	0.029	0.145	0.097	0.027	-																		
9	0.006	0.015	0.001	0.136	0.007	0.017	0.060	0.157	-																	
10	0.004	0.015	0.004	0.135	0.017	0.012	0.055	0.155	0.006	-																
11	0.055	0.054	0.053	0.114	0.055	0.030	0.045	0.119	0.053	0.042	-															
12	0.065	0.073	0.063	0.113	0.067	0.035	0.049	0.122	0.056	0.060	0.011	-														
13	0.049	0.051	0.049	0.109	0.051	0.028	0.039	0.114	0.045	0.040	0.006	0.006	-													
14	0.066	0.071	0.065	0.110	0.069	0.037	0.038	0.107	0.060	0.054	0.006	0.010	0.001	-												
15	0.054	0.058	0.062	0.111	0.066	0.031	0.049	0.122	0.055	0.053	0.011	0.003	0.000	0.005	-											
16	0.048	0.051	0.051	0.117	0.059	0.027	0.046	0.122	0.047	0.043	0.003	0.006	-0.002	0.004	0.001	-										
17	0.071	0.073	0.074	0.101	0.078	0.042	0.044	0.119	0.062	0.060	0.004	0.005	0.005	0.003	0.007	0.002	-									
18	0.049	0.049	0.052	0.118	0.060	0.034	0.051	0.131	0.050	0.047	0.012	0.008	0.001	0.012	0.002	0.003	0.010	-								
19	0.070	0.079	0.075	0.097	0.072	0.037	0.035	0.109	0.068	0.064	0.004	0.011	0.003	0.004	0.009	0.007	0.001	0.013	-							
20	0.061	0.062	0.060	0.115	0.065	0.034	0.043	0.117	0.058	0.048	0.002	0.006	-0.001	0.000	0.006	0.000	0.000	0.006	0.001	-						
21	0.081	0.094	0.098	0.001	0.076	0.049	0.014	0.034	0.090	0.086	0.054	0.060	0.052	0.051	0.053	0.058	0.047	0.060	0.041	0.055	-					
22	0.064	0.065	0.068	0.105	0.069	0.037	0.041	0.105	0.061	0.058	0.009	0.007	0.002	0.002	0.005	0.005	0.002	0.006	0.001	0.001	0.051	-				
23	0.083	0.086	0.085	0.117	0.089	0.048	0.049	0.126	0.081	0.071	0.018	0.016	0.012	0.012	0.015	0.015	0.016	0.017	0.002	0.006	0.059	0.012	-			
24	0.095	0.088	0.097	0.114	0.102	0.067	0.068	0.133	0.089	0.090	0.026	0.022	0.019	0.022	0.023	0.023	0.017	0.020	0.020	0.016	0.067	0.016	0.017	-		
25	0.061	0.049	0.067	0.117	0.071	0.044	0.060	0.140	0.061	0.061	0.014	0.020	0.010	0.019	0.009	0.009	0.012	0.009	0.023	0.013	0.061	0.014	0.024	0.015	-	