Genome-wide SNP based population structure in European hake reveals the need for harmonizing biological and management units

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Genome-wide SNP based population structure in European hake reveals the need for harmonizing biological and management units

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Abstract (<200 words)

Despite its economic importance, the population structure of the European hake, *Merluccius merluccius*, is unresolved, and the species is assessed based on two stocks (northern and southern) separated by the Capbreton Canyon. In order to shed light into the European hake population structure, we used Restriction site Associated DNA sequencing (RAD-seq) to discover and genotype thousands of genome-wide single nucleotide polymorphisms in more than a hundred samples. Our population genetic inferences confirm differentiation of Mediterranean and northeast Atlantic locations and reveal management relevant information within the latter. First, hake in the Norwegian Sea is genetically different from that of the rest of the locations under study and, second, samples from the eastern Bay of Biscay and the northwestern Iberian Peninsula are not genetically different. These results imply that samples from the northern stock belong to different genetic populations, and that samples belonging to locations included in the northern and southern stocks are part of a single genetically homogeneous population. Although the definition of the boundary between the northern and southern stocks and the potential need for additional stocks still requires further analyses, the mismatch between biological and management units should already be considered in further assessments of European hake.

Keywords: RAD-seq; SNPs; stock delimitation; genetic connectivity; European Hake; fisheries management

Introduction

Determining spatiotemporal genetic variation is important for sustainable fisheries management (Hauser and Carvalho, 2008). Yet, intrinsic characteristics of marine fish such as large population sizes, high dispersal capacity and high fecundity often translate into low genetic differentiation even at interoceanic distances (Nielsen and Kenchington, 2001; Nielsen et al., 2009). This often results in a mismatch between biological and management units (Reiss et al., 2009), which can lead to reduced productivity, local reduction of populations and in extreme cases, to local population collapse (Bonfil, 2005). An example of potential mismatch between biological and management units is the European hake (*Merluccius merluccius*) (Reiss et al., 2009), an important commercial fish that inhabits the Northeast Atlantic Ocean and adjacent seas (Korta et al., 2015; ICES, 2018). Within the Northeast Atlantic Ocean, the European hake is managed by the International Council for the Exploration of the Sea (ICES) assuming two stocks separated by the Capbreton Canyon, the northern stock extending northward to Norway (ICES Division 3a, Sub-areas 2, 4, 6 and 7, and Divisions 8a,b,d) and the southern stock, southward to Gibraltar Strait (ICES Divisions 8c and 9a) (ICES, 2011; ICES, 2018). Yet, this separation is not based on clear biological evidences other than the assumption of the barrier effect of the Capbreton Canyon (Casey and Pereiro, 1995). Additionally, in locations from the northern stock (e.g. Bay of Biscay), the main spawning season ranges from January to March, whereas in locations from the southern stock (Galicia and Portugal), spawning occurs all year round with the presence of several other peaks in summer and autumn (Korta et al., 2015; Dominguez-Petit, 2017).

There is still no consensus on the population structure of the European hake (Roldán et al., 1998; Castillo et al., 2005; Pita et al., 2014). Studies based on otolith chemistry, which reflects where fish has been born and grown, have detected differences between samples from southern (represented by northern Portugal) and northern (represented by the Norwegian Sea and West of Scotland) stocks (Swan et al., 2006), whereas others (Tanner et al., 2012) found no significant differences between samples from the northern (represented by the Celtic Sea and the Armorican Shelf) and the southern stock (represented by samples from the Portugal and Galician Shelf). Early studies based on

microsatellite genetic markers resulted in conflicting results. For example Lundy et al. (1999) found differentiation within the northern (e.g. Celtic Sea and Norwegian Sea) and southern (e.g. Cantabrian Sea and southern Portugal) stocks but no differentiation between the two stocks (e.g. Celtic Sea and Bay of Biscay). Castillo et al. (2005) confirmed the lack of differentiation between samples from the Bay of Biscay (ICES divisions 8c vs. 8a,b,d) belonging to the two stocks but found no differentiation between the Cantabrian Sea and Portuguese coast samples (ICES divisions 8 and 9). More recent studies using microsatellites have confirmed the lack of differentiation between Bay of Biscay samples belonging to northern and southern stocks and suggested a possible differentiation of North Sea samples (Pita et al., 2011; Pita et al., 2014).

Despite the advent of High Throughput Sequencing technologies and their demonstrated potential to study population structure in marine fish (Bernatchez et al., 2017), so far only one study has applied this technique to study population connectivity in the European hake (Milano et al., 2014), and is only based on 381 single nucleotide polymorphisms (SNPs), a very low number compared to the tens of thousands used in other studies aimed at resolving population structure of marine fish (Rodríguez-Ezpeleta et al., 2016; Rodríguez-Ezpeleta et al., 2017). Milano et al. (2014) did not find differentiation between northern and southern stocks when using neutral markers but found differentiation within the southern (between Galicia and North Portugal) and within the northern (between North Sea and the other locations) stocks when using 7 outlier loci. This result was further confirmed by another study based on a subset of this dataset (Westgaard et al., 2017); yet, neutral markers did not show the differentiation observed with outlier loci, even when using a bigger sample size, meaning that additional and more powerful analyses are needed to better resolve the European hake population structure.

Here, we have discovered and genotyped genome-wide SNP markers in European hake to (i) define the population structure of this commercially important species along the Mediterranean-Norwegian gradient, (ii) investigate the possibility of seasonal genetic changes within southern stock samples, and (iii) test if the current assessment and management units and population structure of European hake coincide. In order to

reach these goals, we generated Restriction-site-Associated DNA sequencing (RAD-seq) data from 111 European hake individuals that span four geographic areas where this species inhabits: the Norwegian Sea, the eastern Bay of Biscay, the northwestern Iberian Peninsula (including samples from both spawning seasons) and the Mediterranean Sea (Balearic Sea), enabling the opportunity to explore population genetic structure and scan for selective divergences in this species. Our study reveals a mismatch between biological populations and management units in the European hake, which should be considered in the assessment of this commercially important species.

Materials and methods

Sampling, DNA extraction and RAD-seq library preparation

European hake samples from the Mediterranean Sea, the northwestern Iberian Peninsula (southern stock), the eastern Bay of Biscay (northern stock) and the Norwegian Sea (northern stock) were obtained from scientific surveys and commercial fisheries (**Figure 1; Table S1**). Samples include fish in spawning and pre-spawning condition, assumed to be at or close to where they will spawn, and juveniles of age 0 (less than 20 cm), assumed to be close to where they were born (de Pontual et al., 2013). From each specimen, a ~1 cm³ muscle sample was excised and stored in 96% molecular grade ethanol at -20 °C prior to DNA extraction. Genomic DNA was extracted from about 20 mg of muscle tissue using the Wizard® Genomic DNA Purification kit (Promega, WI, USA) following manufacturer's instructions. Extracted DNA was suspended in Milli-Q water and concentration was determined with the Quant-iT dsDNA HS assay kit using a Qubit® 2.0 Fluorometer (Life Technologies). DNA integrity was assessed by electrophoresis. RAD-seq libraries were prepared following the methods of Etter et al. (2011). Between 300 and 400 ng of genomic DNA per sample was digested using the *Sbf*I restriction enzyme and then ligated to modified Illumina P1 adapters containing 5bp sample-specific barcodes. Pools of 32 DNA samples were sheared using the Covaris® M220 Focused-ultrasonicator™ Instrument (Life Technologies) and size-selected to 300-500 pb by cutting DNA fragments migrated through an agarose gel. Following the ligation

of the Illumina P2 adaptors, each genomic library was amplified using 12 PCR cycles. Finally, the pools were paired-end sequenced (100 bp) on an Illumina HiSeq2000.

RAD-tag assembly and SNP calling

Generated RAD-tags were analyzed using *Stacks* version 2.1 (Catchen et al., 2013). Quality filtering and demultiplexing was performed using the *Stacks* module *process_radtags*, truncating all reads to 90 nucleotides after sequence quality verification in FastQC (Andrews, 2010) in order to avoid lower quality bases at the end of the read. The module *clone_filter* was applied to reads whose forward and reverse pairs passed quality filtering to remove PCR duplicates, and only samples with at least 400,000 clone and quality-filtered reads remaining were kept. The module *ustacks* was then used to assemble orthologous tags (stacks) per individual, with a minimum coverage depth required to create a stack (parameter -m) of 5, and a maximum nucleotide mismatches allowed between stacks (parameter -M) of 2. RAD loci were then assembled using the module *cstacks* with a maximum of 2 mismatches allowed between sample tags when generating the catalog (parameter -n). Matches to the catalog for each sample were searched using *sstacks*. The module *populations* was used to export from the catalog the SNPs present in RAD loci found in at least 75% of the individuals. The software *PLINK* version 1.07 (Purcell et al., 2007) was used to further filter the data. The initial filtering steps were based on a maximum of 10% of missing values per locus, and a maximum of 25% of missing values per individual. Subsequently, SNPs with a minimum allele frequency (MAF) smaller than 0.05 and which failed the Hardy Weinberg equilibrium (HWE) test at $p < 0.05$ in at least two areas of study were excluded from downstream analyses. Only the first filtered SNP of each RAD-tag was kept. Additionally, an alternative dataset with $M=4$ and $n=6$ (keeping the rest of the parameters unchanged) and another with MAF threshold of 0.1 (keeping the rest of the parameters unchanged) were also explored for consistency as suggested (Díaz-Arce and Rodríguez-Ezpeleta, 2019). The genotype files are available at X [to be added upon acceptance].

Identification of outlier loci

Outlier loci, potentially under selection, were identified using the independent approaches used by *Bayescan* version 2.0 (Foll and Gaggiotti, 2008) and *Arlequin* version 3.5.2.2 (Excoffier and Lischer, 2010) using the whole dataset and only the Bay of Biscay (Western and Eastern) samples. In *Bayescan*, 20 pilot runs of 5,000 iterations each were run, with a burn-in of 50,000 iterations, 5,000 samples with a thinning interval of 10 and a prior odd increased to 100 in order to minimize potentially false positives in a dataset with > 10,000 loci. Using these parameters, loci with an α -value significantly > 0 (indicative of diversifying selection) and q-values < 0.05 were considered "outliers". In addition, in the hierarchical Fdist model implemented in *Arlequin*, loci with significantly higher F_{ST} values ($p < 0.001$) were considered "outliers". These stringent parameters were used to account for potential false positive outliers discovery (Narum and Hess, 2011; Ahrens et al., 2018). Outputs from *Bayescan* and *Arlequin* were intersected in order to create a consensus panel of shared loci potentially under selection which were annotated by matching the SNP flanking regions against non-redundant database of Genbank (www.ncbi.nlm.nih.gov/genbank/) using BLAST (Altschul et al., 1990). In addition, a multivariate approach to outlier loci discovery was also applied using the package *pcadapt* (Luu et al., 2016) in R version 3.5.0 (R Core Team, 2018).

Genetic diversity and structure analyses

Analyses were performed on the whole SNP dataset, on the neutral SNP dataset and on the outlier SNP dataset. Expected and observed heterozygosity per group (H_e and H_o respectively), pairwise average F_{ST} among groups and distributions of F_{ST} and F_{IS} values were computed with *Arlequin* version 3.5.2.2 (Excoffier and Lischer, 2010). Principal component analyses (PCA) and a three-cluster based discriminant analyses of principal components (DAPC) (Jombart et al., 2010) were performed without any *a priori* assignment of individuals to populations using the package *adeigenet* (Jombart and Ahmed, 2011) in R version 3.5.0 (R Core Team, 2018). The best number of PCs to retain for the DAPC analysis was inferred using the cross-validation test. The genetic ancestry of each individual was estimated using the admixture model as implemented in the Bayesian clustering approach in STRUCTURE version 2.3.4 (Pritchard et al., 2000) without any prior population assignment. Estimations were obtained from the 300,000

iterations that followed a burn-in period of 100,000 iterations. Each value of potential ancestral population (K from 1 to 5) was examined with CLUMPP (Jakobsson and Rosenberg, 2007) to identify common modes, and results were plotted using DISTRUCT (Rosenberg, 2004). The value of K that better fits the data was identified according to the Evanno method (Evanno et al., 2005) as implemented in StructureHarvester (Earl and vonHoldt, 2012). Additionally, ten subsets of 300 randomly selected SNPs were analyzed with $K=3$ in order to investigate the effect of using a smaller dataset of genome-wide representative SNPs.

Results

SNP datasets

Of the 111 analyzed samples, only one was removed for having too few reads. Considering the remaining samples, between 402 599 and 3 053 042 quality-filtered RAD-tags per individual were obtained (average of 1 365 352). Ninety percent of the reads were used to build the stacks, with a range per individual between 81% and 95%, and the average effective per-sample coverage is 34.1x (stdev=12.8x, min=11.3x, max=72.9x). The number of RAD-loci present in at least 75% of the individuals was 29 090, totaling 452 702 SNPs. After filtering for missing data, minor allele frequency and Hardy-Weinberg equilibrium and keeping a single SNP per tag, the number of RAD-loci and SNPs remaining for analysis was 14 120 (**Figure S1**). The amount of missing data was less than 5% overall, and datasets with 0 and 1% of missing data, albeit composed of fewer SNPs produced equivalent results (not shown). Of the remaining SNPs, 147 (1% of the total) and 217 SNPs (1.5% of the total) were considered outliers according to the Bayesian approach implemented in *Bayescan* and the hierarchical Fdist model implemented in *Arlequin* when using the whole dataset (**Figure S2**), and 116 of them were common to both approaches. No outlier loci were found when using only the northwestern Iberian Peninsula and the Eastern Bay of Biscay samples. Additionally, 347 SNPs (2.4% of the total) were considered outliers according to the multivariate approach implemented in *pcadapt*. Of these loci, 108 were included in the 116 shared outlier

datasets by *Bayescan* and *Arlequin*. Annotation of each of the outlier SNPs is available in **Table S2**.

Genetic diversity and population structure

Ranges of observed and expected heterozygosity per groups were similar, both when using the whole or the only outlier SNP dataset, indicating no deficits of heterozygous genotypes in all samples (**Table 1**). STRUCTURE analyses using the best K values according to the Evanno method for the whole or the outlier only datasets (**Table S3**) resulted in three well differentiated clusters: i) the Mediterranean Sea samples, ii) the northwestern Iberian Peninsula and the eastern Bay of Biscay samples and iii) the Norwegian Sea samples (**Figure 2**), with no further structure observed when including only the eastern Bay of Biscay and the northwestern Iberian Peninsula samples. Analyses based on higher values of K resulted in no further differentiation (**Figure S3**). Analyses based on ten subsets of 300 randomly selected SNPs datasets provided varying results (**Figures S4**): same differentiation pattern (five subsets), no differentiation within the Atlantic (four subsets) differentiation between the Mediterranean plus Norwegian Sea samples and the northwestern Iberian Peninsula plus the eastern Bay of Biscay samples (one subset). The Analysis of Principal Components (PCA) on the whole dataset were congruent with the STRUCTURE analyses, revealing the same three well differentiated groups (**Figure 3**), even with alternative RAD-tag assembly parameter and MAF threshold values (**Figure S5**), and with the Discriminant Analysis of Principal Component (DAPC) when using three clusters (**Figure S6**).

The locus-by-locus F_{ST} and F_{IS} were distributed around zero (**Figure S7**) suggesting a balanced amount of heterozygosity and homozygosity along the loci, which is congruent with the low number of outliers detected. Pairwise F_{ST} values among the three differentiated areas were significant ranging from 0.023 to 0.058, from 0.443 to 0.498 and from 0.014 to 0.042 (**Table 2, S4 and S5**) when using all, only the outlier SNPs and only the neutral SNPs respectively. Low and not significant differentiation was detected within samples from southern Europe, even when using only adult samples or only

outlier SNPs suggesting that all samples from the Northern Spanish coast are part of a single population with no seasonal demographic changes.

Discussion

Genome-wide population structure of European hake does not support current stock delineation

Our genome-wide marker-based population structure inference of European hake supports differentiation between Mediterranean and Northeast Atlantic hake. Within the Northeast Atlantic, our study showed a clear differentiation between the Norwegian Sea and southern Europe, but did not find differences between samples of the Bay of Biscay and northwestern Iberian Peninsula, belonging to the northern and southern stocks. These results confirm previous findings using microsatellites that found no differentiation between samples within the Bay of Biscay and Celtic sea, but found differentiation between these regions and the Norwegian Sea (Lundy et al., 1999; Pita et al., 2014). Interestingly, the only previous study based on SNP markers (Milano et al., 2014) failed to detect population structure within the Northeast Atlantic when using neutral markers, which could be due to a lack of evolutionarily informative signal in the few (299) markers used. Yet, when we base our inferences in 300 randomly selected SNPs, we still observe population structure, but only in half of the ten subsets. This suggests that using a few selected hundreds SNPs as in previous studies (Milano et al., 2014) are not enough for a reliable inference of hake population structure. Using a small subset of outlier SNPs ($n=7$ or $n=6$) both Milano et al. (2014) and Westgaard et al. (2017) showed differentiation between the Bay of Biscay and the North Sea and/or Norwegian Sea samples and argued that the differences obtained using neutral and outlier loci are due to adaptation to local conditions. Although it is not unusual to find adaptive divergences in fish even when neutral markers suggest panmixia at local level (Hemmer-Hansen et al., 2007; Freamo et al., 2011), this does not seem to be the case for European hake. The analysis of a consensus panel of 116 outlier SNPs potentially under selective divergence confirmed the genetic structure observed with the total dataset with a

stronger signal and failed in finding additional substructure, as observed before (Moore et al., 2014), suggesting that the genetic divergence observed in the European hake is not only due to selection. This contradicts the views of Milano et al. (2014) and Westgaard, et al. (2017), and further highlights the importance of using genome-wide based markers in order to reliably detect genetic differentiation and local adaptation.

European hake within the Bay of Biscay constitutes a seasonally stable homogeneous population

The lack of differentiation observed within the northwestern Iberian Peninsula and the eastern Bay of Biscay, with very low and not significant F_{ST} , suggests that all the specimens from the area are representative of a unique genetic population, partially solving the dubious genetic homogeneity present within the Bay of Biscay (Castillo et al., 2005; Pita et al., 2014; Pita et al., 2016). Additionally, in our study we have tested the seasonal stability of the European hake connectivity within the southern stock (northwestern Iberian Peninsula). Our analyses, based either on neutral or outlier loci do not show differentiation among samples collected in summer and winter within the northwestern Iberian Peninsula, supporting previous views (Lundy et al., 2000; Pita et al., 2016) and most likely explained by the fact that hake can spawn several times within the reproductive season (Murua et al., 2006; Murua, 2010). Yet, further studies with bigger and seasonal representative datasets and including only spawning individuals are necessary to better investigate any potential spatio-temporal sub-structuring within the Bay of Biscay.

Management implications

In the northeast Atlantic, assessment and management of the European hake is done independently for two stocks, the southern and the northern, separated by the Capbreton Canyon. The biomass of both stocks was in historical minimum and below precautionary biological reference points in the last years of the previous century (ICES, 2018). At the beginning of the new century the stocks started recovering and now the biomass of both are above precautionary levels. In the case of the northern stock the recovery has been extraordinary and the current biomass level doubles the historical

maximum estimated by the stock assessment model at the beginning of the 1980s. Furthermore, the fishing mortality is well below the maximum sustainable yield target. However, the recovery of the southern stock has not been sufficiently strong and although the biomass is above the reference levels the fishing mortality is still too high. This study suggest that the delimitation of the assessment and management areas should be revised. In terms of the assessment, the southern stock and the southern part of the northern stock should be assessed jointly, and a separate stock should be considered in the northern part of the species distribution which includes the Norwegian Sea and possibly the North Sea, although this has yet to be confirmed with additional analyses. Since 2006 the biomass of the northern stock has increased tenfold (ICES, 2018), resulting in an increased abundance of the stock in the North Sea during spring and summer (Baudron and Fernandes, 2015). Additionally, recent explicit stock assessment models (Vigier et al., 2018) predict significant movement between the North Sea and the Celtic Sea and the Bay of Biscay suggesting that, under the hypothesis of a separate population in the northern part of the distribution, the North Sea could be a mixing area of both populations. In sum, our results support the need for defining new assessment and management units for European hake. Yet, despite covering a wide range of the species distribution, our study does not provide information on where the new stock boundary should be located; therefore, future studies including samples from additional locations between the southern Bay of Biscay and the Norwegian Sea are necessary. Importantly, genetic analyses should also be complemented with additional evidence based on alternative techniques (morphometrics, vital rates, etc...) that would help providing biologically meaningful information for adequately defined stock boundaries.

Authors Contribution

PA, FSR and NRE designed the study. AL and NRE analysed the data. AL, NRE and DG interpreted the data and wrote the first draft of the paper. All authors performed critical reading of the first draft and contributed to the revisions.

Supplementary data

Supplementary figures and tables are available at X

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For Review Only

Tables

Table 1: Observed and expected heterozygosities per group of samples when using all or only outlier SNPs. MED: Mediterranean Sea; EBOB: Eastern Bay of Biscay; NWIPs: Northwestern Iberian Peninsula (summer); NWIPw: Northwestern Iberian Peninsula (winter); NOR: Norwegian Sea

POP	n	14120 total SNPs		116 outlier SNPs	
		Ho	He	Ho	He
MED	14	0.25732	0.26521	0.29418	0.30206
EBOB	18	0.24744	0.24918	0.27476	0.27110
NWIPs	30	0.23193	0.24219	0.27043	0.27541
NWIPw	30	0.2348	0.24144	0.25000	0.24945
NOR	18	0.25252	0.25221	0.31828	0.31676

Table 2: Pairwise FST value matrix when using all or only outlier SNPs (below and above diagonal respectively). Values in bold are significant after Bonferroni correction for multiple testing.

	MED	EBOB	NWIPs	NWIPw	NOR
MED	/	0.464	0.443	0.480	0.470
EBOB	0.052	/	0.008	0.005	0.496
NWIPs	0.048	0	/	0.005	0.453
NWIPw	0.049	0	0	/	0.498
NOR	0.058	0.025	0.023	0.025	/

Figures

Figure 1: Sampling sites of European hake and ICES delimitation for the Northern (top) and Southern (bottom) stock management units. The border between Northern and Southern stocks approximate the Capbreton Canyon.

Figure 2: Graphical representation of the Bayesian clustering approach implemented in STRUCTURE when using all (top) or only outlier SNPs (bottom) and all (left) or only the Eastern Bay of Biscay and Northwestern Iberian Peninsula samples (right) for the best K value according to the Evanno method, which is K=2 for all dataset except for the all SNPs all samples dataset, for which it is K=4. Refer to Table 1 for location codes.

Figure 3: Principal Component Analysis (PCA) when using all (top) or only outlier SNPs (bottom) and all (left) or only the Eastern Bay of Biscay and Northwestern Iberian Peninsula samples (right). The variance explained by each axis of the PCA reflects the amount of variation observed when using the total or the outlier SNPs dataset.

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1 **Genome-wide SNP based population structure in European hake reveals the need for**
2 **harmonizing biological and management units**

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Abstract (<200 words)

Despite its economic importance, the population structure of the European hake, *Merluccius merluccius*, is unresolved, and the species is assessed based on two stocks (northern and southern) separated by the Capbreton Canyon. In order to shed light into the European hake population structure, ~~here we have discovered~~ we used Restriction site Associated DNA sequencing (RAD-seq) to discover and ~~genotyped~~ genotype thousands of genome-wide single nucleotide polymorphisms in more than a hundred samples. Our population genetic inferences confirm differentiation of Mediterranean and northeast Atlantic locations and reveal management relevant information within the latter. First, ~~the~~ hake in the Norwegian Sea is genetically different from that of the rest of the locations under study and, second, samples from the eastern Bay of Biscay and the northwestern Iberian Peninsula are not genetically different. These results imply that samples from the northern stock belong to different genetic populations, and that samples belonging to locations included in the northern and southern stocks are part of a single genetically homogeneous population. Although the definition of the boundary between the northern and southern stocks and the potential need for additional stocks still requires further analyses, the mismatch between biological and management units should already be considered in further assessments of European hake.

Keywords: RAD-seq; SNPs; stock delimitation; genetic connectivity; European Hake; fisheries management

Introduction

Determining spatiotemporal genetic variation is important for sustainable fisheries management (Hauser and Carvalho, 2008). Yet, intrinsic characteristics of marine fish such as large population sizes, high dispersal capacity and ~~large numbers of offspring~~ high fecundity often translate into low genetic ~~variation~~ differentiation even at interoceanic distances (Nielsen and Kenchington, 2001; Nielsen et al., 2009). This often results in a mismatch between biological and management units (Reiss et al., 2009), which can ~~lead~~ lead to reduced productivity, local reduction of populations and in extreme cases, to local population collapse (Bonfil, 2005). An example of potential mismatch between biological and management units is the European hake (*Merluccius merluccius*) (Reiss et al., 2009), an important commercial fish that inhabits the Northeast Atlantic Ocean and adjacent seas (Korta et al., 2015; ICES, 2018). Within the Northeast Atlantic Ocean, the European hake is managed by the International Council for the Exploration of the Sea (ICES) assuming two stocks separated by the Capbreton Canyon, the northern stock ~~going~~ extending northward to Norway (ICES Division 3a, Sub-areas 2, 4, 6 and 7, and Divisions 8a,b,d) and the southern stock, southward to Gibraltar Strait (ICES Divisions 8c and 9a) (ICES, 2011; ICES, 2018). Yet, this separation is not based on clear biological evidences other than the assumption of the barrier effect of the Capbreton Canyon (Casey and Pereiro, 1995). Additionally, in locations from the northern stock (e.g. Bay of Biscay), the main spawning season ranges from January to March, whereas in locations from the southern stock (Galicia and Portugal), spawning occurs all year round with the presence of several other peaks in summer and autumn (Korta et al., 2015; Dominguez-Petit, 2017).

There is still no consensus on the population structure of the European hake (Roldán et al., 1998; Castillo et al., 2005; Pita et al., 2014). Studies based on otolith chemistry, which reflects where fish has been born and grown, have detected differences between samples from southern (represented by northern Portugal) and northern (represented by the Norwegian Sea and West of Scotland) stocks (Swan et al., 2006), whereas others (Tanner et al., 2012) found no significant differences between samples from the northern (represented by the Celtic Sea and the Armorican Shelf) and the southern stock

(represented by samples from the Portugal and Galician Shelf). Early studies based on microsatellite genetic markers resulted in conflicting results. For example Lundy et al. (1999) found differentiation within ~~samples from~~ the northern (e.g. Celtic Sea and Norwegian Sea) and southern (e.g. Cantabrian Sea and southern Portugal) stocks but no differentiation between ~~samples belonging to two different~~ the two stocks (e.g Celtic Sea and Bay of Biscay). ~~On the other hand,~~ Castillo et al. (2005) confirmed ~~no~~ the lack of differentiation between samples from the Bay of Biscay (ICES divisions 8c vs. 8a,b,d) belonging to the two stocks but found no differentiation between the Cantabrian Sea and Portuguese coast samples (ICES divisions 8 and 9). More recent studies using microsatellites have confirmed the lack of differentiation between Bay of Biscay samples belonging to northern and southern stocks and suggested a possible differentiation of North Sea samples (Pita et al., 2011; Pita et al., 2014).

Despite the advent of High Throughput Sequencing technologies and their demonstrated potential to study population structure in marine fish (Bernatchez et al., 2017), so far only one study has applied this technique to study population connectivity in the European hake (Milano et al., 2014), and is only based on 381 single nucleotide polymorphisms (SNPs), a very low number compared to the tens of thousands used in other studies aimed at resolving population structure of marine fish (Rodríguez-Ezpeleta et al., 2016; Rodríguez-Ezpeleta et al., 2017). ~~In their work,~~ Milano et al. (2014) did not find differentiation between northern and southern stocks when using neutral markers but found differentiation within the southern (between Galicia and North Portugal) and within the northern (between North Sea and the other locations) stocks when using 7 outlier loci. This result was further confirmed by another study based on a subset of this dataset (Westgaard et al., 2017); yet, neutral markers did not show the differentiation observed with outlier loci, even when using a bigger sample size, meaning that additional and more powerful analyses are needed to better ~~understand~~ resolve the European hake population structure.

Here, we have discovered and genotyped genome-wide SNP markers in European hake to (i) define the population structure of this commercially important species along the Mediterranean-Norwegian gradient, (ii) investigate the possibility of seasonal genetic

changes within southern stock samples, and (iii) test if the current assessment and management units and population structure of European hake coincide. In order to reach these goals, we generated Restriction-site-Associated DNA sequencing (RAD-seq) data from 111 European hake individuals that span four geographic areas where this species inhabits: the Norwegian Sea, the eastern Bay of Biscay, the northwestern Iberian Peninsula (including samples from both spawning seasons) and the Mediterranean Sea (Balearic Sea), enabling the opportunity to explore population genetic structure and scan for selective divergences in this species. Our study reveals a mismatch between biological populations and management units in the European hake, which should be considered in the assessment of this commercially important species.

Materials and methods

Sampling, DNA extraction and RAD-seq library preparation

European hake samples from the Mediterranean Sea, the northwestern Iberian Peninsula (southern stock), the eastern Bay of Biscay (northern stock) and the Norwegian Sea (northern stock) were obtained from scientific surveys and commercial fisheries (**Figure 1; Table S1**). Samples include fish in spawning and pre-spawning condition, assumed to be at or close to where they will spawn, and juveniles of age 0 (less than 20 cm), assumed to be close to where they were born (de Pontual et al., 2013).

From each specimen, a ~1 cm³ muscle sample was excised and stored in 96% molecular grade ethanol at -20 °C prior to DNA extraction. Genomic DNA was extracted from about 20 mg of muscle tissue using the Wizard® Genomic DNA Purification kit (Promega, WI, USA) following manufacturer's instructions ~~for "Isolating Genomic DNA from Tissue Culture Cells and Animal Tissue".~~ Extracted DNA was suspended in Milli-Q water and concentration was determined with the Quant-iT dsDNA HS assay kit using a Qubit® 2.0 Fluorometer (Life Technologies). DNA integrity was assessed by electrophoresis, ~~migrating about 100 ng of GelRed™-stained DNA on an agarose 1.0% gel.~~ RAD-seq libraries were prepared following the methods of Etter et al. (2011). Between 300 and 400 ng of genomic DNA per sample was digested using the *SbfI* restriction enzyme and

then ligated to modified Illumina P1 adapters containing 5bp ~~uniquesample-specific~~ barcodes. Pools of 32 DNA samples were sheared using the Covaris® M220 Focused-ultrasonicator™ Instrument (Life Technologies) and size-selected to 300-500 pb by cutting DNA fragments migrated through an agarose gel. Following the ligation of the Illumina P2 adaptors, each genomic library was amplified using 12 PCR cycles. Finally, the pools were paired-end sequenced (100 bp) on an Illumina HiSeq2000. ~~Raw sequences are available at NCBI SRA Bioproject # X [to be added upon acceptance].~~

RAD-tag assembly and SNP calling

Generated RAD-tags were analyzed using *Stacks* version 2.1 (Catchen et al., 2013). Quality filtering and demultiplexing was performed using the *Stacks* module *process_radtags*, truncating all reads to 90 nucleotides after sequence quality verification in FastQC (Andrews, 2010) in order to avoid lower quality bases at the end of the read. The module *clone_filter* was applied to reads whose forward and reverse pairs passed quality filtering to remove PCR duplicates, and only samples with at least 400,000 clone and quality-filtered reads remaining were kept. The module *ustacks* was then used to assemble orthologous tags (stacks) per individual, with a minimum coverage depth required to create a stack (parameter -m) of 5, and a maximum nucleotide mismatches allowed between stacks (parameter -M) of 2. RAD loci were then assembled using the module *cstacks* with a maximum of 2 mismatches allowed between sample tags when generating the catalog (parameter -n). Matches to the catalog for each sample were searched using *sstacks*. The module *populations* was used to export from the catalog the SNPs present in RAD loci found in at least 75% of the individuals. The software *PLINK* version 1.07 (Purcell et al., 2007) was used to further filter the data. The initial filtering steps were based on a maximum of 10% of missing values per locus, and a maximum of 25% of missing values per individual. Subsequently, SNPs with a minimum allele frequency (MAF) smaller than 0.05 and which failed the Hardy Weinberg equilibrium (HWE) test at $p < 0.05$ in at least two areas of study were excluded from downstream analyses. Only the first filtered SNP of each RAD-tag was kept. Additionally, an alternative dataset with $M=4$ and $n=6$ (keeping the rest of the parameters unchanged) and another with MAF threshold of 0.1 (keeping the rest of the parameters

unchanged) were also explored for consistency as suggested ~~(Díaz-Arce and Rodríguez-Ezpeleta, 2019)~~(Díaz-Arce and Rodríguez-Ezpeleta, 2019). The genotype files are available at X [to be added upon acceptance].

Identification of outlier loci

Outlier loci, potentially under selection, were identified using the independent approaches used by *Bayescan* version 2.0 (Foll and Gaggiotti, 2008) and *Arlequin* version 3.5.2.2 (Excoffier and Lischer, 2010) using the whole dataset and only the Bay of Biscay (Western and Eastern) samples. In *Bayescan*, 20 pilot runs of 5,000 iterations each were run, with a burn-in of 50,000 iterations, 5,000 samples with a thinning interval of 10 and a prior odd increased to 100 in order to minimize potentially false positives in a dataset with > 10,000 loci. Using these parameters, loci with an α -value significantly > 0 ~~(indicative of diversifying selection)~~ and q-values < 0.05 were considered "outliers". In addition, in the hierarchical Fdist model implemented in *Arlequin*, loci with significantly higher F_{ST} values ($p < 0.001$) were considered "outliers". These stringent parameters were used to account for potential false positive outliers discovery (Narum and Hess, 2011; Ahrens et al., 2018). Outputs from *Bayescan* and *Arlequin* were intersected in order to create a consensus panel of shared loci potentially under selection-~~which were annotated by~~ which were annotated by matching the SNP flanking regions against non-redundant database of Genbank (www.ncbi.nlm.nih.gov/genbank/) using BLAST (Altschul et al., 1990). In addition, a multivariate approach to outlier loci discovery was also applied using the package *pcadapt* (Luu et al., 2016) in R version 3.5.0 (R Core Team, 2018).

Genetic diversity and structure analyses

Analyses were performed on the whole SNP dataset, on the neutral SNP dataset and on the outlier SNP dataset. Expected and observed heterozygosity per group (H_e and H_o respectively), pairwise average F_{ST} among groups and distributions of F_{ST} and F_{IS} values were computed with *Arlequin* version 3.5.2.2 (Excoffier and Lischer, 2010). Principal component analyses (PCA) and a three-cluster based discriminant analyses of principal

components (DAPC) (Jombart et al., 2010) were performed without any *a priori* assignment of individuals to populations using the package *adegenet* (Jombart and Ahmed, 2011) in R version 3.5.0 (R Core Team, 2018). The best number of PCs to retain for the DAPC analysis was inferred using the cross-validation test. The genetic ancestry of each individual was estimated using the admixture model as implemented in the Bayesian clustering approach in STRUCTURE version 2.3.4 (Pritchard et al., 2000) without any prior population assignment. Estimations were obtained from the 300,000 iterations that followed a burn-in period of 100,000 iterations. Each value of potential ancestral population (K from 1 to 5) was examined with CLUMPP (Jakobsson and Rosenberg, 2007) to identify common modes, and results were plotted using DISTRUCT (Rosenberg, 2004). The value of K that better fits the data was identified according to the Evanno method (Evanno et al., 2005) as implemented in StructureHarvester (Earl and vonHoldt, 2012). Additionally, ten subsets of 300 randomly selected SNPs were analyzed with K=3 in order to investigate the effect of using a smaller dataset of genome-wide representative SNPs.

Results

SNP datasets

Of the 111 analyzed samples, only one was removed for having too few reads. Considering the remaining samples, between 402 599 and 3 053 042 quality-filtered RAD-tags per individual were obtained (average of 1 365 352). Ninety percent of the reads were used to build the stacks, with a range per individual between 81% and 95%, and the average effective per-sample coverage is 34.1x (stdev=12.8x, min=11.3x, max=72.9x). The number of RAD-loci present in at least 75% of the individuals was 29 090, totaling 452 702 SNPs. After filtering for missing data, minor allele frequency and Hardy-Weinberg equilibrium and keeping a single SNP per tag, the number of RAD-loci and SNPs remaining for analysis was 14 120 (**Figure S1**). The amount of missing data ~~in~~ was less than 5% overall, and ~~no significant patterns (p-value=datasets with 0.01) and 1% of missingness between groups~~ missing data, albeit composed of individuals were found. From fewer SNPs produced equivalent results (not shown). Of the remaining

SNPs, 147 (1% of the total) and 217 SNPs (1.5% of the total) were considered outliers according to the Bayesian approach implemented in *Bayescan* and the hierarchical Fdist model implemented in *Arlequin* when using the whole dataset (**Figure S2**), and 116 of them were common to both approaches. No outlier loci were found when using only the northwestern Iberian Peninsula and the Eastern Bay of Biscay samples. Additionally, 347 SNPs (2.4% of the total) were considered outliers according to the multivariate approach implemented in *pcadapt*. Of these loci, 108 were included in the 116 shared outlier datasets by *Bayescan* and *Arlequin*. Annotation of each of the outlier SNPs is available in **Table S2**.

Genetic diversity and population structure

Ranges of observed and expected heterozygosity per groups were similar, both when using the whole or the only outlier SNP dataset, indicating no deficits of heterozygous genotypes in all samples (**Table 1**). STRUCTURE analyses using the best K values according to the Evanno method for the whole or the outlier only datasets (**Table S3**) resulted in three well differentiated clusters: i) the Mediterranean Sea samples, ii) the northwestern Iberian Peninsula and the eastern Bay of Biscay samples and iii) the Norwegian Sea samples (**Figure 2**), with no further structure observed when including only the eastern Bay of Biscay and the northwestern Iberian Peninsula samples. Analyses based on higher values of K resulted in no further differentiation (**Figure S3**) and analyses based on ten subsets of 300 randomly selected SNPs datasets resulted in the provided varying results (**Figures S4**): same differentiation pattern (5 of the five subsets), in no differentiation within the Atlantic (4 of the four subsets) or in differentiation between the Mediterranean plus Norwegian Sea samples and the northwestern Iberian Peninsula plus the eastern Bay of Biscay samples (**Figures S4 one subset**). The Analysis of Principal Components (PCA) on the whole dataset were congruent with the STRUCTURE analyses, revealing the same three well differentiated groups (**Figure 3**), even with alternative RAD-tag assembly parameter and MAF threshold values (**Figure S5**), and with the Discriminant Analysis of Principal Component (DAPC) when using three clusters (**Figure S6**).

The locus-by-locus F_{ST} and F_{IS} were distributed around zero (**Figure S7**) suggesting a balanced amount of heterozygosity and homozygosity along the loci, which is congruent with the low number of outliers detected. Pairwise F_{ST} values among the three differentiated areas were significant ranging from 0.023 to 0.058, from 0.443 to 0.498 and from 0.014 to 0.042 (**Table 2, S4 and S4S5**) when using all, only the outlier SNPs and only the neutral SNPs respectively. Low and not significant differentiation was detected within samples from southern Europe, even when using only adult samples or only outlier SNPs suggesting that all samples from the Northern Spanish coast are part of a single population with no seasonal demographic changes.

Discussion

Genome-wide population structure of European hake does not support current stock delineation

Our genome-wide marker-based population structure inference of European hake supports differentiation between Mediterranean and Northeast Atlantic hake. Within the Northeast Atlantic, our study showed a clear differentiation between the Norwegian sea and southern Europe, but did not find differences between samples of the Bay of Biscay and northwestern Iberian Peninsula, belonging to the northern and southern stocks. These results confirm previous findings using microsatellites that found no differentiation between samples within the Bay of Biscay and Celtic sea, but found differentiation between these regions and the Norwegian Sea (Lundy et al., 1999; Pita et al., 2014). Interestingly, the only previous study based on SNP markers (Milano et al., 2014) failed to detect population structure within the Northeast Atlantic when using neutral markers, which could be due to a lack of evolutionarily informative signal in the few (299) markers used. Yet, when we base our inferences in 300 randomly selected SNPs, we still observe population structure, although not all but only in half of the ten subsets are able to distinguish the three groups (Figures S4). This suggests that using thousands of genome-wide distributed SNPs rather than a few selected hundreds SNPs as in previous studies (Milano et al., 2014) are required not enough for a reliable

inference of hake population structure. Using a small subset of outlier SNPs ($n=7$ or $n=6$) both Milano et al. (2014) and Westgaard et al. (2017) showed differentiation between the Bay of Biscay and the North Sea and/or Norwegian Sea samples and argued that the differences obtained using neutral and outlier loci are due to adaptation to local conditions. Although it is not unusual to find adaptive divergences in fish even when neutral markers suggest panmixia at local level (Hemmer-Hansen et al., 2007; Freemo et al., 2011), this does not seem to be the case for European hake. The analysis of a consensus panel of 116 outlier SNPs potentially under selective divergence confirmed the genetic structure observed with the total dataset with a stronger signal and failed in finding additional substructure, as observed before (Moore et al., 2014), suggesting that the genetic divergence observed in the European hake is not ~~due to the direct effect of fitness only (Holderegger et al., 2006)~~ only due to selection. This contradicts the views of Milano et al. (2014) and Westgaard, et al. (2017), and further highlights the importance of using genome-wide based markers in order to reliably detect genetic differentiation and local adaptation.

European hake within the Bay of Biscay constitutes a seasonally stable homogeneous population

The lack of differentiation observed within the northwestern Iberian Peninsula and the eastern Bay of Biscay, with very low and not significant F_{ST} , ~~supportssuggests~~ that all the specimens from the area are representative of a unique genetic population, partially solving the dubious genetic homogeneity present within the Bay of Biscay (Castillo et al., 2005; Pita et al., 2014; Pita et al., 2016). Additionally, in our study we have tested the seasonal stability of the European hake connectivity within the southern stock (northwestern Iberian Peninsula). Our analyses, based either on neutral or outlier loci do not show differentiation among samples collected in summer and winter within the northwestern Iberian Peninsula, supporting previous views (Lundy et al., 2000; Pita et al., 2016) and most likely explained by the fact that hake can spawn several times within the reproductive season (Murua et al., 2006; Murua, 2010). Yet, further studies with bigger and seasonal representative datasets and including only spawning individuals are

necessary to better investigate any potential spatio-temporal sub-structuring within the Bay of Biscay.

Management implications

In the northeast Atlantic, assessment and management of the European hake is done independently for two stocks, the southern and the northern, separated by the Capbreton Canyon. The biomass of both stocks was in historical minimum and below precautionary biological reference points in the last years of the previous century (ICES, 2018). At the beginning of the new century the stocks started recovering and now the biomass of both are above precautionary levels. In the case of the northern stock the recovery has been extraordinary and the current biomass level doubles the historical maximum estimated by the stock assessment model at the beginning of the 1980s. Furthermore, the fishing mortality is well below the maximum sustainable yield target. However, the recovery of the southern stock has not been sufficiently strong and although the biomass is above the reference levels the fishing mortality is still too high.

This study suggest that the delimitation of the assessment and management areas should be revised. In terms of the assessment, the southern stock and the southern part of the northern stock should be assessed jointly, and a separate stock should be considered in the northern part of the species distribution which includes the Norwegian Sea and possibly the North Sea, although this has yet to be confirmed with additional analyses. Since 2006 the biomass of the northern stock has increased tenfold (ICES, 2018), resulting in an increased abundance of the stock in the North Sea during spring and summer (Baudron and Fernandes, 2015). Additionally, recent explicit stock assessment models (Vigier et al., 2018) predict significant movement between the North Sea and the Celtic Sea and the Bay of Biscay suggesting that, under the hypothesis of a separate population in the northern part of the distribution, the North Sea could be a mixing area of both populations.

In sum, our results support the need for defining new assessment and management units for European hake. Yet, despite covering a wide range of the species distribution, our study does not provide information on where the new stock boundary should be located; therefore, future studies including samples from additional locations between the southern Bay of Biscay and the Norwegian Sea are necessary. Importantly, genetic

analyses should also be complemented with additional evidence based on alternative techniques (morphometrics, vital rates, etc...) that would help providing biologically meaningful information for adequately defined stock boundaries.

Authors Contribution

PA, FSR and NRE designed the study. AL and NRE analysed the data. AL, NRE and DG interpreted the data and wrote the first draft of the paper. All authors performed critical reading of the first draft and contributed to the revisions.

Supplementary data

Supplementary figures and tables are available at X

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Tables

Table 1: Observed and expected heterozygosities per group of samples when using all or only outlier SNPs. MED: Mediterranean Sea; EBOB: Eastern Bay of Biscay; NWIPs: Northwestern Iberian Peninsula (summer); NWIPw: Northwestern Iberian Peninsula (winter); NOR: Norwegian Sea

POP	n	14120 total SNPs		116 outlier SNPs	
		Ho	He	Ho	He
MED	14	0.25732	0.26521	0.29418	0.30206
EBOB	18	0.24744	0.24918	0.27476	0.27110
NWIPs	30	0.23193	0.24219	0.27043	0.27541
NWIPw	30	0.2348	0.24144	0.25000	0.24945
NOR	18	0.25252	0.25221	0.31828	0.31676

Table 2: Pairwise FST value matrix when using all or only outlier SNPs (below and above diagonal respectively). Values in bold are significant after Bonferroni correction for multiple testing.

	MED	EBOB	NWIPs	NWIPw	NOR
MED	/	0.464	0.443	0.480	0.470
EBOB	0.052	/	0.008	0.005	0.496
NWIPs	0.048	0	/	0.005	0.453
NWIPw	0.049	0	0	/	0.498
NOR	0.058	0.025	0.023	0.025	/

Figures

Figure 1: Sampling sites of European hake and ICES delimitation for the Northern (top) and Southern (bottom) stock management units. The border between Northern and Southern stocks approximate the Capbreton Canyon.

Figure 2: Graphical representation of the Bayesian clustering approach implemented in STRUCTURE when using all (top) or only outlier SNPs (bottom) and all (left) or only the Eastern Bay of Biscay and Northwestern Iberian Peninsula samples (right) for the best K value according to the Evanno method, which is K=2 for all dataset except for the all SNPs all samples dataset, for which it is K=4. Refer to Table 1 for location codes.

Figure 3: Principal Component Analysis (PCA) when using all (top) or only outlier SNPs (bottom) and all (left) or only the Eastern Bay of Biscay and Northwestern Iberian Peninsula samples (right). The variance explained by each axis of the PCA reflects the amount of variation observed when using the total or the outlier SNPs dataset.

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Supplementary Tables

Table S1: Samples used for this study and their associated metadata. In Sex column, 1 indicates male and 2, female; maturity stages are in Walsh scale

SampleID	Area	Season	Stage	Lat	Long	Size (mm)	Weight (g)	Sex	Maturity stage	Catch Date
CAN0016	Eastern Bay of Biscay	Autumn	Adult	43.57	-1.90	724	2720.6	2	3	29/11/2016
CAN0017	Eastern Bay of Biscay	Spring	Adult	43.57	-1.90	725	2700	2	3	25/05/2016
CAN0049	Eastern Bay of Biscay	Spring	Adult	43.71	-1.69	610	1435	2	3	25/04/2016
CAN0054	Eastern Bay of Biscay	Spring	Adult	43.57	-1.90	680	2616	2	3	25/05/2016
CAN0018	Eastern Bay of Biscay	Winter	Adult	43.57	-1.90	620	1590.8	2	3	25/01/2017
CAN0019	Eastern Bay of Biscay	Winter	Adult	43.57	-1.90	668	2171	2	3	31/01/2017
CAN0020	Eastern Bay of Biscay	Winter	Adult	43.57	-1.90	670	2057	2	3	31/01/2017
CAN0031	Eastern Bay of Biscay	Winter	Adult	43.57	-1.90	623	1885.2	2	3	02/02/2017
CAN0061	Eastern Bay of Biscay	Winter	Adult	43.57	-1.90	698	2378	2	3	31/01/2017
CAN0062	Eastern Bay of Biscay	Winter	Adult	43.57	-1.90	621	1888	2	3	01/12/2016
CAN0063	Eastern Bay of Biscay	Winter	Adult	43.57	-1.90	694	2402	2	3	25/01/2017
CAN0064	Eastern Bay of Biscay	Winter	Adult	43.57	-1.90	608	1447.8	2	3	31/01/2017
CAN0087	Eastern Bay of Biscay	Winter	Adult	43.57	-1.90	660	1866	2	3	01/02/2017
CAN0090	Eastern Bay of Biscay	Winter	Adult	43.57	-1.90	648	2028.1	2	3	02/02/2017
CAN0104	Eastern Bay of Biscay	Winter	Adult	43.57	-1.90	592	1465	2	3	31/01/2017
CAN0105	Eastern Bay of Biscay	Winter	Adult	46.83	-3.00	910	5800	2	3	20/03/2016
CAN0107	Eastern Bay of Biscay	Winter	Adult	43.57	-1.90	641	1889.3	2	3	31/01/2017
CAN0109	Eastern Bay of Biscay	Winter	Adult	43.57	-1.90	800	3486	2	3	31/01/2017
GaV0029	Northwestern Iberian Peninsula	Summer	Adult	43.25	-9.10	534	1090	2	2	10/07/2017
GaV0032	Northwestern Iberian Peninsula	Summer	Adult	43.25	-9.10	523	1138	2	4	10/07/2017
GaV0034	Northwestern Iberian Peninsula	Summer	Adult	43.25	-9.10	559	1386	2	3	10/07/2017
GaV0039	Northwestern Iberian Peninsula	Summer	Adult	43.25	-9.10	532	1196	2	4	06/06/2017
GaV0041	Northwestern Iberian Peninsula	Summer	Adult	43.25	-9.10	553	1284	2	2	10/07/2017
GaV0046	Northwestern Iberian Peninsula	Summer	Adult	43.25	-9.10	525	1146	2	2	10/07/2017
GaV0053	Northwestern Iberian Peninsula	Summer	Adult	43.25	-9.10	558	1252	2	4	04/07/2017
GaV0058	Northwestern Iberian Peninsula	Summer	Adult	43.25	-9.10	535	1180	2	2	06/06/2017
GaV0066	Northwestern Iberian Peninsula	Summer	Adult	43.25	-9.10	515	988	2	2	06/06/2017
GaV0077	Northwestern Iberian Peninsula	Summer	Adult	43.25	-9.10	544	1248	2	2	06/06/2017
GaV0085	Northwestern Iberian Peninsula	Summer	Adult	43.25	-9.10	500	1172	2	4	10/07/2017
GaV0094	Northwestern Iberian Peninsula	Summer	Adult	43.25	-9.10	556	1354	2	2	06/06/2017
GaV0106	Northwestern Iberian Peninsula	Summer	Adult	43.25	-9.10	531	1266	2	2	10/07/2017
GaV0108	Northwestern Iberian Peninsula	Summer	Adult	43.25	-9.10	514	1050	2	2	06/06/2017
GaV0110	Northwestern Iberian Peninsula	Summer	Adult	43.25	-9.10	553	1290	2	2	06/06/2017
GaV0021	Northwestern Iberian Peninsula	Summer	Juvenile	43.79	-7.52	174	N/A	N/A	N/A	27/06/2017
GaV0022	Northwestern Iberian Peninsula	Summer	Juvenile	43.79	-7.51	157	N/A	N/A	N/A	27/06/2017
GaV0023	Northwestern Iberian Peninsula	Summer	Juvenile	43.90	-7.67	160	N/A	N/A	N/A	27/06/2017
GaV0025	Northwestern Iberian Peninsula	Summer	Juvenile	43.79	-7.52	176	N/A	N/A	N/A	27/06/2017
GaV0027	Northwestern Iberian Peninsula	Summer	Juvenile	43.79	-7.52	162	N/A	N/A	N/A	27/06/2017
GaV0028	Northwestern Iberian Peninsula	Summer	Juvenile	43.89	-7.81	160	N/A	N/A	N/A	27/06/2017
GaV0074	Northwestern Iberian Peninsula	Summer	Juvenile	43.89	-7.81	167	N/A	N/A	N/A	27/06/2017

1											
2											
3											
4	GaV0083	Northwestern Iberian Peninsula	Summer	Juvenile	43.48	-8.65	198	N/A	N/A	N/A	06/06/2017
5	GaV0084	Northwestern Iberian Peninsula	Summer	Juvenile	43.48	-8.65	188	N/A	N/A	N/A	06/06/2017
6	GaV0091	Northwestern Iberian Peninsula	Summer	Juvenile	43.53	-8.76	194	N/A	N/A	N/A	06/06/2017
7	GaV0096	Northwestern Iberian Peninsula	Summer	Juvenile	43.90	-7.67	165	N/A	N/A	N/A	27/06/2017
8	GaV0098	Northwestern Iberian Peninsula	Summer	Juvenile	43.89	-7.81	152	N/A	N/A	N/A	27/06/2017
9	GaV0099	Northwestern Iberian Peninsula	Summer	Juvenile	43.79	-7.51	177	N/A	N/A	N/A	27/06/2017
10	GaV0100	Northwestern Iberian Peninsula	Summer	Juvenile	43.89	-7.81	193	N/A	N/A	N/A	27/06/2017
11	GaV0101	Northwestern Iberian Peninsula	Summer	Juvenile	43.52	-8.53	185	N/A	N/A	N/A	28/06/2017
12	Gal0030	Northwestern Iberian Peninsula	Winter	Adult	43.25	-9.10	570	1430	2	2	01/03/2017
13	Gal0033	Northwestern Iberian Peninsula	Winter	Adult	43.25	-9.10	590	2230	2	3	08/02/2017
14	Gal0038	Northwestern Iberian Peninsula	Winter	Adult	43.25	-9.10	580	1626	2	4	20/02/2017
15	Gal0042	Northwestern Iberian Peninsula	Winter	Adult	43.25	-9.10	540	1294	2	2	20/02/2017
16	Gal0057	Northwestern Iberian Peninsula	Winter	Adult	43.25	-9.10	578	1604	2	4	20/02/2017
17	Gal0059	Northwestern Iberian Peninsula	Winter	Adult	43.25	-9.10	580	1594	2	3	08/02/2017
18	Gal0065	Northwestern Iberian Peninsula	Winter	Adult	43.25	-9.10	525	1182	2	2	08/02/2017
19	Gal0067	Northwestern Iberian Peninsula	Winter	Adult	43.25	-9.10	565	1202	2	2	01/03/2017
20	Gal0068	Northwestern Iberian Peninsula	Winter	Adult	43.25	-9.10	585	1574	2	2	01/03/2017
21	Gal0073	Northwestern Iberian Peninsula	Winter	Adult	43.25	-9.10	563	1418	2	4	20/02/2017
22	Gal0076	Northwestern Iberian Peninsula	Winter	Adult	43.25	-9.10	540	1346	2	2	01/03/2017
23	Gal0081	Northwestern Iberian Peninsula	Winter	Adult	43.25	-9.10	501	1034	2	2	08/02/2017
24	Gal0088	Northwestern Iberian Peninsula	Winter	Adult	43.25	-9.10	545	1232	2	2	08/02/2017
25	Gal0103	Northwestern Iberian Peninsula	Winter	Adult	43.25	-9.10	550	1574	2	4	20/02/2017
26	Gal0111	Northwestern Iberian Peninsula	Winter	Adult	43.25	-9.10	574	1512	2	2	20/02/2017
27	Gal0024	Northwestern Iberian Peninsula	Winter	Juvenile	43.78	-7.51	162	N/A	N/A	N/A	25/03/2017
28	Gal0026	Northwestern Iberian Peninsula	Winter	Juvenile	43.78	-7.51	160	N/A	N/A	N/A	25/03/2017
29	Gal0035	Northwestern Iberian Peninsula	Winter	Juvenile	44.04	-8.07	154	N/A	2	N/A	25/03/2017
30	Gal0037	Northwestern Iberian Peninsula	Winter	Juvenile	43.67	-8.60	176	N/A	N/A	N/A	24/03/2017
31	Gal0047	Northwestern Iberian Peninsula	Winter	Juvenile	43.60	-8.52	164	N/A	N/A	N/A	26/03/2017
32	Gal0056	Northwestern Iberian Peninsula	Winter	Juvenile	42.64	-9.26	191	N/A	N/A	N/A	28/03/2017
33	Gal0071	Northwestern Iberian Peninsula	Winter	Juvenile	43.67	-8.60	187	N/A	N/A	N/A	24/03/2017
34	Gal0078	Northwestern Iberian Peninsula	Winter	Juvenile	43.48	-8.54	174	N/A	2	N/A	26/03/2017
35	Gal0079	Northwestern Iberian Peninsula	Winter	Juvenile	43.67	-8.60	157	N/A	N/A	N/A	24/03/2017
36	Gal0080	Northwestern Iberian Peninsula	Winter	Juvenile	43.54	-8.53	162	N/A	N/A	N/A	26/03/2017
37	Gal0082	Northwestern Iberian Peninsula	Winter	Juvenile	43.47	-8.70	190	N/A	2	N/A	26/03/2017
38	Gal0089	Northwestern Iberian Peninsula	Winter	Juvenile	43.69	-8.57	155	N/A	N/A	N/A	26/03/2017
39	Gal0092	Northwestern Iberian Peninsula	Winter	Juvenile	43.48	-8.54	195	N/A	2	N/A	26/03/2017
40	Gal0093	Northwestern Iberian Peninsula	Winter	Juvenile	43.69	-8.57	181	N/A	N/A	N/A	26/03/2017
41	Gal0097	Northwestern Iberian Peninsula	Winter	Juvenile	43.48	-8.54	171	N/A	N/A	N/A	26/03/2017
42	MED0001	Mediterranean Sea	Autumn	Adult	39.22	3.00	250	51.03	N/A	N/A	18/11/2016
43	MED0002	Mediterranean Sea	Autumn	Adult	39.22	3.00	204	52.2	N/A	N/A	18/11/2016
44	MED0003	Mediterranean Sea	Autumn	Adult	39.22	3.00	192	49.4	N/A	N/A	18/11/2016
45	MED0004	Mediterranean Sea	Autumn	Adult	39.22	3.00	209	62.52	N/A	N/A	18/11/2016
46	MED0005	Mediterranean Sea	Autumn	Adult	39.22	3.00	197	51.13	N/A	N/A	18/11/2016
47	MED0006	Mediterranean Sea	Autumn	Adult	39.22	3.00	200	51.45	N/A	N/A	18/11/2016
48	MED0007	Mediterranean Sea	Autumn	Adult	39.22	3.00	206	64.64	N/A	N/A	18/11/2016
49	MED0008	Mediterranean Sea	Autumn	Adult	39.22	3.00	204	53.05	N/A	N/A	18/11/2016

MED0009	Mediterranean Sea	Autumn	Adult	39.22	3.00	197	51.54	N/A	N/A	18/11/2016
MED0010	Mediterranean Sea	Autumn	Adult	39.22	3.00	208	55.38	N/A	N/A	18/11/2016
MED0011	Mediterranean Sea	Autumn	Adult	39.22	3.00	197	46.34	N/A	N/A	18/11/2016
MED0012	Mediterranean Sea	Autumn	Adult	39.22	3.00	207	60.8	N/A	N/A	18/11/2016
MED0013	Mediterranean Sea	Autumn	Adult	39.22	3.00	196	62.67	N/A	N/A	18/11/2016
MED0014	Mediterranean Sea	Autumn	Adult	39.22	3.00	192	44.92	N/A	N/A	18/11/2016
MED0015	Mediterranean Sea	Autumn	Adult	39.22	3.00	196	50.66	N/A	N/A	18/11/2016
NOR0036	Norwegian Sea	Autumn	Adult	62.60	7.11	1050	530	2	3	02/11/2017
NOR0040	Norwegian Sea	Autumn	Adult	62.60	7.11	1140	560	2	3	02/11/2017
NOR0043	Norwegian Sea	Autumn	Adult	63.97	11.20	1480	580	2	3	30/10/2017
NOR0044	Norwegian Sea	Autumn	Adult	68.12	14.41	4094	800	1	4	02/11/2017
NOR0045	Norwegian Sea	Autumn	Adult	63.46	10.86	1915	660	2	4	31/10/2017
NOR0048	Norwegian Sea	Autumn	Adult	68.12	14.41	6115	900	1	4	02/11/2017
NOR0050	Norwegian Sea	Autumn	Adult	62.60	7.11	1875	630	2	3	02/11/2017
NOR0051	Norwegian Sea	Autumn	Adult	62.60	7.11	2335	690	2	3	02/11/2017
NOR0052	Norwegian Sea	Autumn	Adult	62.60	7.11	2085	680	1	4	02/11/2017
NOR0055	Norwegian Sea	Autumn	Adult	63.78	11.32	5535	930	1	4	30/10/2017
NOR0060	Norwegian Sea	Autumn	Adult	63.97	11.20	2450	680	2	3	30/10/2017
NOR0069	Norwegian Sea	Autumn	Adult	63.79	11.37	2186	680	2	3	31/10/2017
NOR0070	Norwegian Sea	Autumn	Adult	63.46	10.86	4615	850	1	4	31/10/2017
NOR0072	Norwegian Sea	Autumn	Adult	62.60	7.11	2030	670	2	4	02/11/2017
NOR0075	Norwegian Sea	Autumn	Adult	62.60	7.11	3085	730	2	4	02/11/2017
NOR0086	Norwegian Sea	Autumn	Adult	63.46	10.86	5255	870	1	4	31/10/2017
NOR0095	Norwegian Sea	Autumn	Adult	63.46	10.86	2070	640	2	4	31/10/2017
NOR0102	Norwegian Sea	Autumn	Adult	63.97	11.20	1465	580	2	3	30/10/2017

Table S2: Results from mapping the flanking region of the outlier SNPs against the non-redundant database. Only the match with the lowest E-value is shown.

SNP	Accession	Definition	E value
4525	XM_022196154.1	PREDICTED: Acanthochromis polyacanthus gap junction alpha-3 protein-like (LOC110952557), mRNA	9.02e-119
4926	XM_013030874.1	PREDICTED: Dipodomys ordii cytoplasmic FMR1 interacting protein 2 (Cyfip2), mRNA	2.19e-35
6046	XM_022760061.1	PREDICTED: Seriola dumerili protocadherin-11 X-linked-like (LOC111232503), mRNA	8.53e-159
6190	XM_021583880.1	PREDICTED: Oncorhynchus mykiss disks large homolog 3-like (LOC110504964), mRNA	1.73e-21
7282	LR584051.1	Echeneis naucrates genome assembly, chromosome: 10	1.76e-06
8086	XM_028589501.1	PREDICTED: Perca flavescens family with sequence similarity 193 member B (fam193b), transcript variant X2, mRNA	1.41e-72
8358	FJ356236.1	Gadus morhua interferon gamma gene, complete cds	2.49e-35
8380	AC145509.2	Gasterosteus aculeatus clone CH213-128O17, complete sequence	6.96e-31
8730	CP032600.1	Lateolabrax maculatus linkage group 3 sequence	1.53e-17
12175	CP032601.1	Lateolabrax maculatus linkage group 4 sequence	2.52e-25
13653	XM_028584606.1	PREDICTED: Perca flavescens myosin heavy chain, fast skeletal muscle-like (LOC114559754), mRNA	1.12e-53
15080	XM_028587403.1	PREDICTED: Perca flavescens DET1 and DDB1 associated 1 (dda1), mRNA	2.60e-89
16396	LR131939.1	Cottoperca gobio genome assembly, chromosome: 9	1.34e-12
16634	XM_020653185.1	PREDICTED: Labrus bergylta adenosylhomocysteinase (ahcy), mRNA	7.48e-65
18187	LR535848.1	Mastacembelus armatus genome assembly, chromosome: 16	2.77e-44

18439	CP032588.1	Lateolabrax maculatus linkage group 14 sequence	5.99e-41
18447	XM_021315672.1	PREDICTED: Fundulus heteroclitus deoxyribodipyrimidine photo-lyase-like (LOC105925179), mRNA	2.85e-24
19723	AM482911.2	Vitis vinifera contig VV78X039251.4, whole genome shotgun sequence	8.14e-05
19876	XR_003281901.1	PREDICTED: Carassius auratus uncharacterized LOC113080568 (LOC113080568), ncRNA	5.82e-61
20488	LR584244.1	Takifugu rubripes genome assembly, chromosome: 14	3.71e-18
20717	XM_023395945.1	PREDICTED: Seriola lalandi dorsalis glypican-4-like (LOC111646457), mRNA	2.23e-15
21822	LR584244.1	Takifugu rubripes genome assembly, chromosome: 14	1.71e-21
21958	LR535850.1	Mastacembelus armatus genome assembly, chromosome: 18	7.61e-40
21962	LR131921.1	Cottoperca gobio genome assembly, chromosome: 14	1.29e-27
23239	LR131918.1	Cottoperca gobio genome assembly, chromosome: 11	4.75e-12
24825	XM_027031460.1	PREDICTED: Electrophorus electricus monocarboxylate transporter 8-like (LOC113591097), mRNA	1.33e-07

Table S3: Evanno statistics for the whole and outlier SNP only datasets when using all or only the eastern Bay of Biscay and the northwestern Iberian Peninsula samples; values corresponding best K value are underlined

	K	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	Ln''(K)	DeltaK
All SNPs, all samples	1	-1163684	13.2	NA	NA	NA
	2	-1156679	141.65	7005.32	2783.34	19.65
	3	-1152457	122.94	4221.98	6810.52	55.39
	4	-1155045	567.79	-2588.54	37513.34	66.07
	5	-1195147	50925.22	-40101.88	69276.86	1.36
	6	-1165972	9190.08	29174.98	NA	NA
Outlier SNPs, all samples	1	-13232	0.2588	NA	NA	NA
	2	-10659	0.6301	2572.3	1519	2410.8
	3	-9606	0.6797	1053.3	965.84	1420.97
	4	-9519	1.5287	87.46	56.24	36.79
	5	-9487	8.3415	31.22	57.34	6.87
	6	-9514	13.0903	-26.12	NA	NA
All SNPs, southern samples	1	-866473	12.04	NA	NA	NA
	2	-868999	896.29	-2525.1	11028.5	12.3
	3	-882552	3369.37	-13553.6	20239	6
	4	-875867	2780.82	6685.4	NA	NA
Outlier SNPs, southern samples	1	-6727.78	0.21	NA	NA	NA
	2	-6496.5	0.98	231.27	276.47	280.71
	3	-6541.7	15.45	-45.2	56.37	3.65
	4	-6530.53	51.99	11.16	NA	NA

Table S4: Pairwise FST value matrix when using neutral SNPs dataset without the 116 shared outliers and the total SNPs dataset (below and above diagonal respectively). Values in bold are significant after Bonferroni correction for multiple testing.

	MED	EBOB	NWIPs	NWIPw	NOR
MED	/	0.052	0.048	0.049	0.058
EBOB	0.037	/	0.008	0.005	0.025
NWIPs	0.033	0	/	0.005	0.023
NWIPw	0.033	0	0	/	0.025
NOR	0.042	0.015	0.014	0.014	/

Table S5: Pairwise FST value matrix when using all SNPs from only adult specimens. Values in bold are significant after Bonferroni correction for multiple testing.

	<u>MED</u>	<u>EBOB</u>	<u>NWIPs</u>	<u>NWIPw</u>	<u>NOR</u>
<u>MED</u>	/				
<u>EBOB</u>	0.054	/			
<u>NWIPs</u>	0.056	<u>0</u>	/		
<u>NWIPw</u>	0.056	<u>0</u>	<u>0</u>	/	
<u>NOR</u>	0.061	0.026	0.028	0.026	/

Supplementary Figures

Figure S1: Number of RAD-loci and SNPs in the catalog (TOTAL) and those retained at each filtering step. Note that because a single SNP per loci was selected for downstream analyses, the number of SNPs retained at the end of the process is equal to the number of RAD loci remaining after the HWWE filtering step.

Figure S2: Results from the Bayesian scan for selection implemented in *Bayescan*, and the hierarchical model test for selection implemented in *Arlequin*. *Bayescan* outliers are defined as loci with q-value lower than 0.05 (in red), while *Arlequin* outliers are defined as loci that fall above the 99% quantile of the FST vs Observed Heterozygosity (in red).

Figure S3: Graphical representation of the Bayesian clustering approach implemented in STRUCTURE when using all (top) or only outlier SNPs (bottom) and all (left) or only the Eastern Bay of Biscay and Northwestern Iberian Peninsula samples (right) for the second best K value according to the Evanno method, which is K=3 for all cases. Refer to Table 1 for location codes.

Figure S4: Graphical representation of the Bayesian clustering approach implemented in STRUCTURE when using 10 random subsets of 300 SNPs for K=3. Refer to Table 1 for location codes.

Figure S5: Principal Component Analysis (PCA) when using alternative RAD-tag assembly parameter values ($m=5$, $M=4$, $n=6$), and an alternative Minimum Allele Frequency (MAF) value threshold (0.1).

Figure S6: Discriminant Analysis of Principal Component (DAPC) for $K=3$ using all 14120 SNPs (A), and the 116 outlier SNPs only (B).

Figure S7: Distribution of FST and FIS values per SNP.

Figure S8: Principal Component Analysis (PCA), left, and Discriminant Analysis of Principal Component (DAPC), right, for K = 3 using 347 outlier SNPs selected by the multivariate approach implemented in *pcadapt*.