Population structure of swordfish across the ICCAT/IOTC management boundary

Wendy West, Charlene da Silva and Sven Kerwath

Department of Forestry, Fisheries and the Environment, South Africa

Abstract

South Africa is a member of the Indian Ocean Tuna Commission (IOTC) and International Commission for the Conservation of Atlantic Tuna (ICCAT), the two regional fisheries management organisations that are responsible for the management of large pelagic fishes in the Indian and the Atlantic oceans, respectively. The 20°E longitudinal line represents the artificial reporting and management boundary between these two organisations, but it remains uncertain if the artificial indeed reflects a biological meaningful separation of populations of large pelagic fishes. The broadbill swordfish Xiphias gladius is a circumglobally distributed apex predator in temperate pelagic waters and an important target of longline fisheries in all major oceans. Previous studies confirmed genetic differentiation between the Atlantic and Indian Ocean stocks but there is no agreement on the direction of gene flow and where, or indeed if, a population boundary exists. Eleven microsatellite loci were included in this study of the fine scale population structure of swordfish caught in South African waters. Despite the poor quality of old DNA samples, muscle material of 267 swordfish around the entire range of South Africa's coastline was utilised. A map of admixture proportions indicated a potential admixture zone between 14°E and 27°E. Gene flow and migration seem to occur in both directions, but weak differentiation suggests that the Indian Ocean and Atlantic Ocean contain separate stocks which return to their ocean of origin to reproduce. Due to passive drift of larvae and active dispersal of adults, swordfish would be prone to admixture and genetic homogenisation. The swordfish represents one of several species that occur in stocks not only straddling the 20°E reporting boundary, but with significant annual fluctuations in catch reporting resulting from slight changes in distribution across this boundary. Further studies need to be undertaken to ensure this phenomenon does not affect stock assessments of these species on either side of this boundary.

Introduction

Swordfish stock structure has been inferred by catch rates, catch-at-age and catch-at-length data within ocean basins such as the Atlantic (Neilson *et al*., 2007) and Pacific (Hinton and Alvarado Bremer, 2007); and by tag recapture studies (García-Cortés *et al*., 2003; Holdsworth *et al*., 2007), growth parameters (Tserpes and Tsimenides, 1995; Arocha and Lee, 1996) and spawning areas (Amorim and Arfelli, 1980; Arocha and Lee, 1996).

Various studies, mostly on broader geographical scales, have included investigations into the population structure of swordfish between the Indian and Atlantic oceans.. The early study by Chow *et al*. (1997) reported that Indian Ocean samples were not significantly different from the samples of South Atlantic and Pacific oceans. Subsequent studies detailed genetic differentiation between the Indian and South Atlantic oceans (Alvarado Bremer *et al*., 1999; Chow and Takeyama, 2000; Kotoulas *et al*., 2006; Alvarado Bremer *et al*., 2007). According to Chow and Takeyama (2000), the penetration by Indo-Pacific swordfish into the Atlantic seemed negligible and the spawning grounds of the Atlantic and Indo-Pacific were separated. Similarly, Alvarado Bremer *et al*. (2007) concluded that there was likely historical gene flow from the Indo-Pacific to the Atlantic, though currently the gene flow was restricted.

A more recent study by Muths *et al*. (2013) conducted on a relatively regional geographical scale compared 177 samples collected at the Cape of Good Hope and Namibia (*i.e.* Atlantic) with 812 samples from the SWIO region using mitochondrial DNA (ND2) and 19 microsatellite loci. The SWIO samples with the closest proximity to the Atlantic samples were from Mozambique (n=115) and South Madagascar (n=228). The overall value of F-statistics for ND2 sequences confirmed that the Atlantic and Indian Ocean swordfish represented two distinct genetic stocks. Indo-Pacific differentiation was also significant but lower than that observed between the Atlantic and Indian Ocean. The co-occurrence of two genetic clades, previously only one in the Atlantic, could be explained by unidirectional gene flow from the Indo-Pacific into the South Atlantic. The fact that a second clade was now observed in the Indian Ocean (at the low frequency of 2% but in all the Indian Ocean areas) seems to suggest that a flux of Atlantic swordfish into the Indian Ocean could also occur. The results of their study had management implications for the tRFMOs to consider. Our study utilised the same microsatellite markers and extends on findings through a large sampling effort on a finer sampling scale along the entire South African coastline.

. This work attempts to address whether genetic stock differentiation, based on microsatellite data, exists for swordfish caught off the South African coastline straddling the management border (20°E) of the Indian and Atlantic oceans.

Materials and Methods

For swordfish 63 microsatellite markers exist (Benson *et al.,* 2009), 51 of which have been characterised in publications (Reeb *et al*., 2003; Kasapidis *et al*., 2009; Bradman *et al*., 2011). Since swordfish microsatellite makers have been established and used in previous studies, this type of marker was deemed suitable for this study.

Sample design

Observers onboard pelagic longline vessels collected 602 swordfish muscle samples throughout 2005. Samples were collected around South Africa's coastline from -24° to -38° S and 10° to 35°E, sampling the Indian and Atlantic oceans; and were stored in 70% ethanol. The catch location was recorded, and the sex identified in all samples (Figure 1).

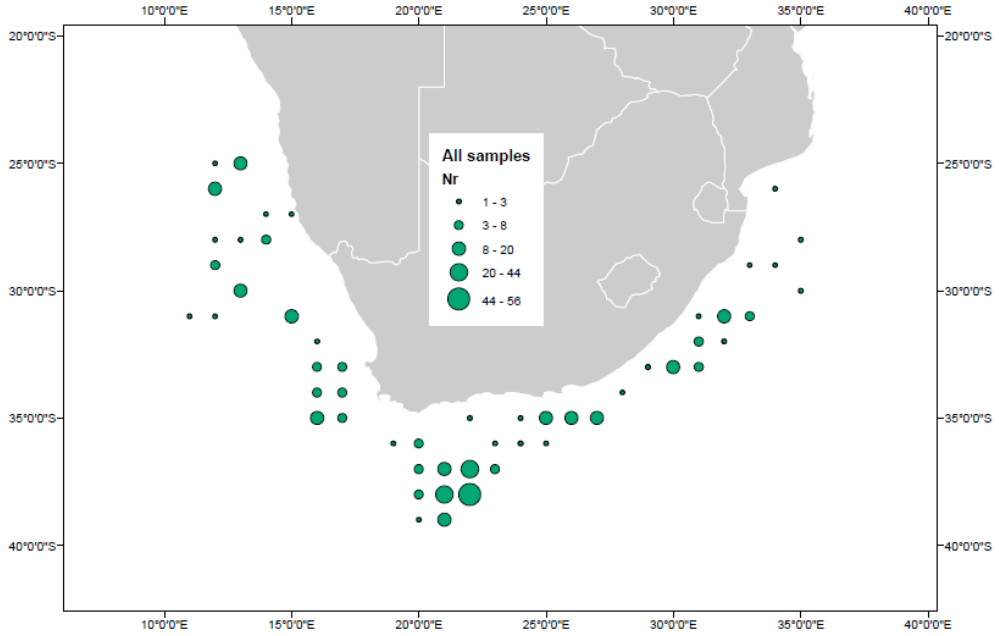


Figure 1. Catch location of all samples collected by onboard observers in 2005.

The management boundary at 20°E that separates the Indian Ocean and Atlantic Ocean stocks was initially used to assign samples to localities, referred to as Scenario 1 (Figure 2, i). Scenario 1 was used for marker performance and genetic diversity analyses. Samples caught west and east of the 20°E boundary are classified West (<20°E) and East (>20°E), respectively.

Putative population scenarios

The individuals were subsequently grouped into 3 additional population scenarios based on the longitude geographic parameter to vary the potential stock boundaries and compare the results of population differentiation and assignment tests (Figure 2, ii-iv).

Scenario 2. West (<17°E), South (17°E -30°E), East (>30°E)

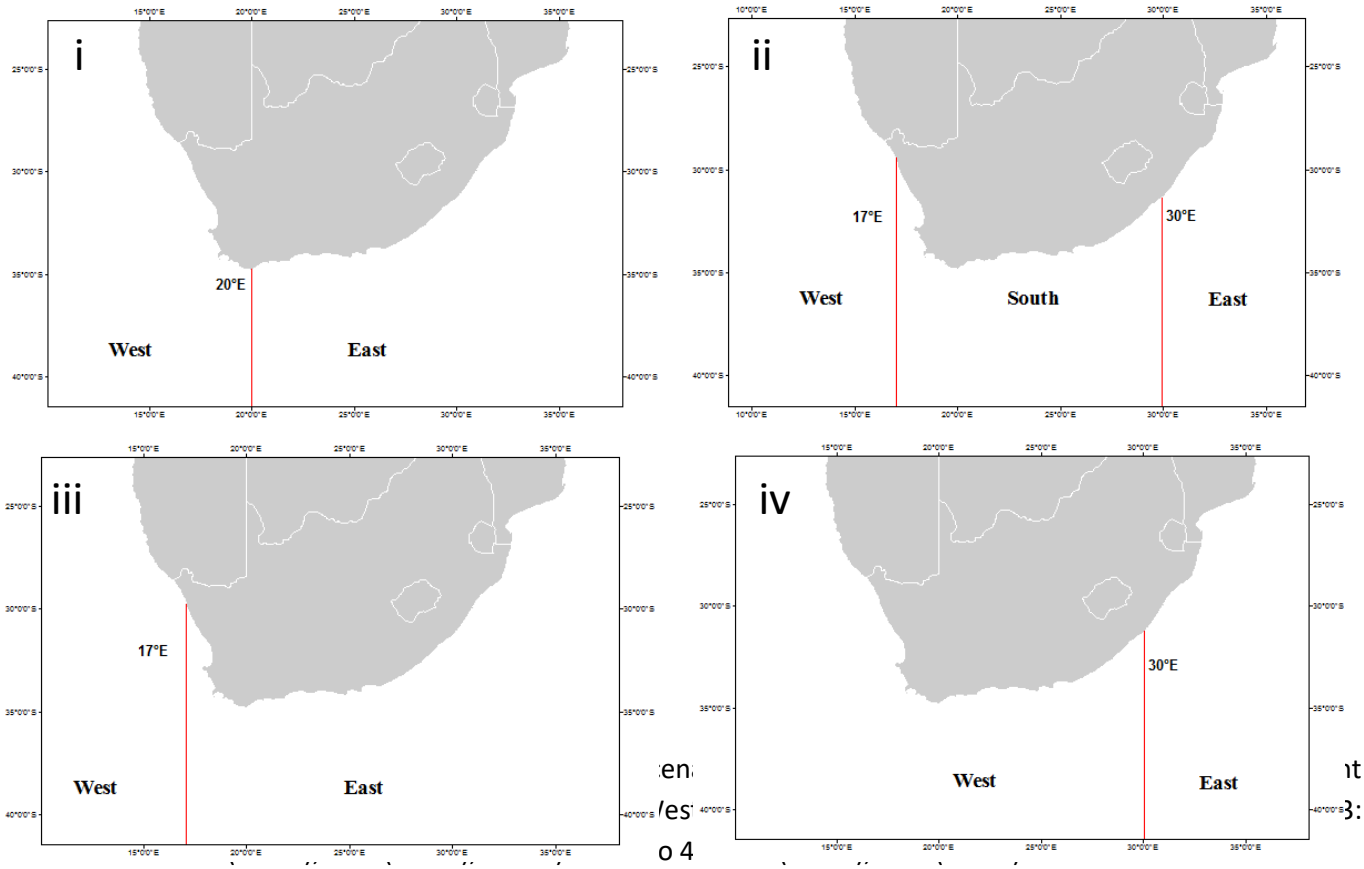
Scenario 3. West (<17°E), East (>17°E)

Scenario 4. West (<30°E), East (>30°E)

The boundary lines were chosen to reflect the westerly extent of the Agulhas Current Retroflection at 17°E and the westerly extent of the Mozambique Channel at 30°E. The number of samples per scenario is included in Table 2. Sample filtering has been subsequently applied based on the quality of the sample for genetics analyses. The final number of individuals is indicated in the Results.

Table 2. Number of individuals per scenario included in analyses.

Scenario		Scenario code	Number of individuals
1	West (<20°E)	W20	73
	East (>20°E)	E20	194
2	West (<17°E)	W17	62
	South (17-30°E)	South	180
	East (>30°E)	E30	25
3	West (<17°E)	W17	62
	East (>17°E)	E17	205
4	West (<30°E)	W30	242
	East (>30°E)	E30	25



Comparison of DNA extraction methods

Due to the age of the samples, the ethanol in the sample vials evaporated over time and all samples were desiccated. Three commercial extraction kits, SureFood® PREP, Qiagen DNeasy® mericon Food Kit, Qiagen DNeasy® Blood & Tissue Kit, and a modified Cetyltrimethyl ammonium bromide (CTAB) extraction protocol (Saghai-Maroof *et al*., 1984) were used to test the efficiency of extracting DNA from desiccated tissue samples. Efficiency was based on DNA quantity (ng/µl) and DNA purity (260/280 nm ratio and 260/230 nm ratio of absorbance). Ratios of 1.8 and 2.0-2.2, respectively, are generally accepted as values for "pure" DNA (T042 Technical Bulletin). The DNA quantity and purity were measured using the Nanodrop® ND-1000 spectrophotometer.

The four extraction methods were tested on 10 samples (A to J). Since there was limited time in which to process 602 samples, a kit rather than the CTAB method was the preferred option for extractions. Three of the 10 samples, of varying DNA yield, that were extracted with the DNeasy® Blood and Tissue Kit were tested for PCR success and to determine the minimum DNA yield threshold for PCR success.

Markers A3 and A8, two markers of varying size range (bp), were chosen to test for PCR success of desiccated samples.

DNA extraction

After testing and found to be the most optimal, DNA extractions for all the 602 samples were conducted with the DNeasy® Blood and Tissue Kit. The Quick-Start Protocol can be found at www.qiagen.com/handbooks. Samples of muscle (25 mg) were incubated for one hour at 56°C with 180 µl Buffer ATL and 20 µl proteinase K. In separate repeated steps of spinning in the centrifuge for 1-3 minutes at 8,000 – 14,000 rpm, 200 µl Buffer AL, 200 µl ethanol, 500 µl Buffer AW1 and 500 µl Buffer AW2 were added to the sample with DNeasy Mini spin columns. The DNA was eluted in 100 µl Buffer AE. The DNA product was stored at -20°C until further use.

Preparation of primers

Initially, 3 microsatellites markers (Xg66, Xg144, Xg166) were chosen from Reeb *et al*. (2003) and 16 (A3, A4, A7, A8, A10, A113, A115, B6, B108, B112, C4, C7, C8, C10, D2B, D11) from Bradman *et al*. (2011), for this study. The potential multiplex groups of markers were arranged by considering the annealing temperature and size (bp) of the marker, and the fluorescent dye for the forward primer selected accordingly when ordering the primers (Blue- FAM, Yellow- NED, Red- PET or Green- VIC). The primers were resuspended in MilliQ water to create a 100 mM stock solution. Primers were placed on a shaker for 1-2 hours before storing at -20°C. The stock solutions were diluted into 10 mM working solutions.

Primer optimisation

The annealing temperatures of the markers as detailed by Reeb *et al*. (2003) and Bradman *et al*. (2011) were used to test the success of the PCR protocol on the available thermal cycler machines. One sample of relatively good DNA quantity (84 ng/µl) was used to test PCR amplification success through singleplex reactions. PCR amplification was performed in a volume of 10 µL with 50 ng of template DNA, 10 µM of each primer, 1 x Go*Taq* Flexi Buffer (Promega), 200 µM of dNTPs; 1 mM $MgCl_2$ and 0.5 units of *Taq* polymerase. The PCR was attained by a denaturation step of 5 minutes at 95°C, and continued with 35 cycles containing a 30 second denaturation segment at 95°C, a 45 second annealing segment at the optimum temperature, and a 30 second elongation segment at 72°C. The final elongation step was 10 minutes at 72°C.

Gel electrophoresis

Success of single reaction PCR amplification at the aforementioned annealing temperatures was tested through agarose gel electrophoresis. A gel was made with 1x TBE buffer, 2% agarose powder and 1-2 µl ethidium bromide (for DNA staining) and placed in an electrophoresis bath containing 1x TBE buffer. Four microliters of PCR product and 2 µl of 5x DNA loading buffer were loaded onto the gel, with one lane dedicated to the 500 bp size standard, and run for 1 hour at ~120V until the dye front reached ¾ of the total length of the gel. The gel was visualised by the ethidium bromide staining and UV-light exposure.

Capillary electrophoresis

The PCR products were amplified separately and electrophoresed in four multiplex panels to test the success of electrophoresis on pooled PCR products. Capillary electrophoresis of PCR products with a GeneScan 600 LIZ® size standard (Applied Biosystems) was conducted on an ABI 3730xl DNA Analyzer at the Central Analytical Facilities, Stellenbosch University.

Allele scoring

Allele scoring was conducted manually with the aid of Peak Scanner Software 2 (Applied Biosystems). Automatic binning of the alleles was performed with the program FLEXIBIN (Amos *et al*., 2007).

DNA quantity thresholds for singleplex and multiplex PCR

To determine the DNA quantity threshold for PCR success in multiplex reactions, groups of multiplex PCR reactions were tested with varying numbers of markers per reaction over a range of DNA quantities. The multiplex reactions were tested on samples of DNA quantity >20 ng/µl. The KAPA2G Fast Multiplex PCR Kit was used for multiplex reactions in a 10 µl reaction volume with 50 ng template DNA, 0.2 µM of each primer and 1X 2X KAPA2G Fast Multiplex Mix (containing 3 mM $MgCl_2$ at 1X).

Four multiplex reactions of 4- and 5-markers per reaction (three reactions with four markers each and one reaction with five markers = 19 markers) were tested on five samples ranging in DNA quantity of between 27 and 112 ng/µl (27, 37, 75, 84 and 112 ng/µl). Six multiplex groups of 2- and 3-markers per

reaction (two reactions with two markers each and five reactions with three markers each = 19 markers) per reaction were tested on three samples ranging in DNA quantity of between 20 and 35 ng/µl (20, 26 and 35 ng/µl). The characteristics of the markers included in this study are listed in Table 3.

Table 3. Characteristics of the 19 microsatellite loci used in this study. Markers A10, A115, C4 were excluded from the study due to poor amplification success.

Locus	Motif sequence	Ta°C	Size range (bp)	Primers (5' - 3')
A3	(GACA)6	58	95-115	F: CAGTCGGGCGTCATCAAGTGAACCATCAGCGGCTCCT† R: GTTTCATCCTTGACTGGCACCTCCG
A4	(GACA)6	62	240-288	F: CAGTCGGGCGTCATCAGGGCAAGTAGATAACAGAATTA† R: GTTTCTTAGCCCATCACCCAATCCATCGT
A7	(GACA)6	62	271-283	F: CAGTCGGGCGTCATCAAGCAGACTCTGAGCCAAGTGCAA† R: GTTTCTTCATCACCAATCAGCCACC
A8	(GTCT)7	58	222-238	F: GTTTCTTGCCCTTGCCTGGAG† R: CAGTCGGGCGTCATCAGTGTTGGCAGGTGGTCTGGAG
A10	(CAGA)10	58	349-369	F: CAGTCGGGCGTCATCAGATTAAGGCAGCGGAGTCGAG† R: GTTTCTTCGCTGGCAAGGCATTAGTTCAG
A113	(TCTG)6	54	212-226	F: GTTTCTTTCGCTGACAGACTTTACGACA† R: CAGTCGGGCGTCATCAATCAGCTTCCAGGACAACACA
A115	(ACAG)8	58	379-495	F: CAGTCGGGCGTCATCAGCAAATGTGTTTAGCCGGAGA† R: GTTTCTTTCCTGAATGGCAGTAATTGTG
B6	(GGAT)6	52	244-258	F: GTTTCTTGTGTACAGGATAACCGTCTTT† R: CAGTCGGGCGTCATCAAGGGCAGTCAATTAGGTAGGC
B108	(CCAT)13	58	168-226	F: CAGTCGGGCGTCATCATTCAGTTTGTTGGCAGTTATT † R: GTTTCTTCCATCCAGCCCTCCACTTATT
B112	(GATG)15	50	206-254	F: CAGTCGGGCGTCATCAGTTTATGTCAGCACAAGCACC† R: GTTTCTTCTGCAAGTTTCACCGTTTCTA
C4	(TAGA)12	56	448-500	F: GTTTCTTATCCGTCTCAGAGCAACTGGC† R: CAGTCGGGCGTCATCACTCTTAGTGACCCACGGGAAT
C7	(GATA)18	56	216-260	F: GTTTCGGAACGCACATGCAGAGCTTA† R: CAGTCGGGCGTCATCATTGGTCAAAGCTGCTCATATC
C8	(CTAT)22	58	152-236	F: CAGTCGGGCGTCATCACCTTCAATGTAGAGATGGCAGG† R: GTTTCAAATGTCGGTGGAGCTGTGGACAGA
C10	(CATA)14	52	194-270	F: CAGTCGGGCGTCATCAAATGGAGACTGCGATTAAGAT† R: GTTTCTTCAGTCTTTCTGCCATAACTCA
D2B	(CAGT)8	58	157-185	F: CAGTCGGGCGTCATCAAAGCAACAACATTGTCTTCTG† R: GTTTCTGGCGTGAACGTGGCTCAATCC
D11	(TCAG)7	54	233-245	F: AGTCGGGCGTCATCAATGCAGGATTCCGCTGACCAGT† R: GTTTCTTTGGATGTGGATATACGGCACC
Xg66	(CA)11	52	140-150	F: TTTTCACCTTGTCAGTGTGCG† R: ACAGACGTATACAACCACCTG
Xg166	(CAA)7	52	140-150	F: GTGAGTCATGTGTCAGTGTGG† R: CCTCTGCCTGAAATACTTCAG
Xg144	(GGA)7	52	130	F: TTCCACTCATACCTCTGTCATC† R: ACCACATCCATTATAGCATGTTG

†Indicates fluorescent primer

Sample filtering criteria

A series of criteria were applied to remove individuals and markers that had poor amplification success. A threshold of 60% amplification success per individual was applied. All samples with 10 or more markers (from total of 16) that had amplified were kept in the dataset. Of these remaining samples, a threshold of 80% amplification success per marker was applied. All markers with more than 80% amplification success were kept in the dataset for the analyses.

Genetic diversity

Data were organised in Microsoft Excel and prepared for input into the analysis software packages. Departures from neutrality of loci were tested using LOSITAN (Antão *et al*., 2008) with 50,000 simulations, ''neutral'' mean F_{ST}, confidence intervals of 95% and a false discovery rate (FDR) of 0.1 with the infinite alleles model (IAM). LOSITAN detects loci under selection through an F_{ST}-outlier detection method when compared to calculated global F_{ST} values expected under neutrality.

Indices of genetic diversity were calculated in Arlequin 3.5.2.1. (Excoffier and Lischer, 2010), and included average number of alleles per locus (A), observed heterozygosity (H_O) and expected heterozygosity (H_E). The allele frequencies were calculated per locus and are included in Appendix B. To compare levels of polymorphism across loci, the polymorphic information content (PIC) of each marker for each of the two populations was estimated from observed allele frequencies (Botstein *et al*., 1980). The PIC statistic is a function of both allele number and frequency and, therefore, is a better estimator of discriminatory power than is the number of alleles alone (Anderson and Karel, 2014). The PIC was calculated in CERVUS 3.0.7 (Kalinowski *et al*., 2007). The allelic richness (R_s), which is a standardized index of the mean number of alleles per locus irrespective of sample size, was calculated with Fstat 2.9.3.2 (Goudet, 2001).

The coefficient of genetic differentiation (F_{ST}, among population variation), the inbreeding coefficient (F_{IS}, within population variation) and the overall fixation index (F_{IT}, total population variation) were estimated through the estimator of Weir and Cockerham (1984) using Genepop 4.2 (Raymond and Rousset, 1995) with numerical resampling by bootstrapping (1,000 times) and jack-knife procedures in order to estimate confidence intervals and the significance of the values. Positive F_{IS} values

demonstrate an excess of homozygotes (positive correlation between homologous allele) or conversely, a deficiency of heterozygotes, relative to the Hardy-Weinberg model. This could be due to inbreeding, hence the label. F_{ST} values in the range of 0.0 to 0.05 may indicate little genetic differentiation, whereas values of F_{ST} above 0.25 indicate large genetic differentiation (Wright, 1978; Hartl and Clark, 1997).

Departures from HWE and linkage disequilibrium (LD) were tested for each site at each locus using Genepop 4.2 (Raymond and Rousset, 1995) with 10,000 burn-in steps, and 500 batches of 5,000 Monte Carlo Markov Chain (MCMC) steps per batch. Statistical significance was assessed at each locus before and after sequential Bonferroni correction (Rice, 1989). In cases where observed genotype frequencies deviated significantly from HWE expectations, the program MICRO-CHECKER 2.2.3 (Van Oosterhout *et al*., 2004) was used to infer the most probable cause of the HWE departures: null alleles (Pemberton *et al*., 1995), stuttering during the PCR amplification and large allele dropout (Wattier *et al*., 1998). When a locus revealed evidence of null alleles, the frequency of null alleles (p_n) was estimated by averaging estimations derived from three independent methods (Chakraborty *et al*., 1992; Brookfield, 1996; Van Oosterhout *et al*., 2006).The following population differentiation and population assignment tests were conducted on the four putative population scenarios (Figure 2).

Population differentiation

Population differentiation was evaluated for each population scenario (i to iv) with pairwise F_{st} tests executed in Arlequin 3.5.2.1 (Excoffier and Lischer, 2010) with 1,000 permutations and a significance level of 0.05. Fisher's exact test of genic (allele distribution) and genotypic (genotype distribution) distributions between pairs of populations in each scenario (i-iv) was conducted with Genepop 4.2 (Raymond and Rousset 1995; Rousset 2008) as an indication of population differentiation, with 1, 000 burn-in steps, and 100 batches of 1, 000 MCMC steps per batch. To assess variation within and among populations, a locus-by-locus and population specific analysis of molecular variance (AMOVA, Excoffier *et al*., 1992) was conducted in Arlequin 3.5.2.1 (Excoffier and Lischer, 2010). The test was run among populations (F_{ST}) and among individuals within populations (F_{IS}) for each population scenario (i-iv). For all calculations, significance was assessed by 1,000 random permutations.

Population assignment

Genepop 4.2 (Raymond and Rousset, 1995) was used to test for isolation-by-distance (IBD) in Scenario II, the only scenario where levels of geographic distance is applicable with the East and West separated by the South, by plotting $F_{ST} / (1 - F_{ST})$ (*i.e.* genetic distance) against the geographic distance between sampling sites, with a Mantel test (1,000 permutations of the data). This provided a one-tailed p-value for significance of the matrix correlation and a corresponding R^2. The Mantel test was carried out to examine whether the genetic distances between population pairs were linearly related to their geographical distances. The geographic position of each sampling block was specified as the coordinates at the mid-point of each sampling block, and F_{ST} values previously calculated in Genepop 4.2 were used.

First generation migrants, *i.e.* individuals born in a population other than the one in which they were sampled, were identified with GeneClass 2.0 (Paetkau *et al*., 2004; Piry *et al*., 2004) with a likelihood-based test statistic, L_{home}/L_{max}. L_{home}**,** the likelihood of finding a given individual in the population in which it was sampled, and L_{home}/L_{max}**,** the ratio of L_{home} to the greatest likelihood among all sampled populations. The Bayesian criterion of Rannala and Mountain (1997) in combination with the resampling method of Paetkau *et al*. (2004) was used to determine the critical value of the test statistic beyond which individuals were assumed to be migrants. Paetkau *et al*. (2004)'s probability computation of individual genotypes coming from each locality was calculated with a Markov chain (MC) resampling procedure by comparing individual genotypes to 1,000 simulated individuals per locality, and if the value was below $P < 0.05$, the individual was 'rejected' from that population. The frequency-based simulation method introduced by Paetkau *et al*. (2004) was selected as it is more representative of real population processes than other methods (*e.g.* Rannala and Mountain, 1997; Cornuet *et al,*. 1999) which have been shown to produce an inflated rate of type I errors (Paetkau *et al*., 2004; Piry *et al*., 2004). Nei's (1972) standard genetic distance (*Ds*) for each population scenario was calculated in Genetix 4.05 (Belkhir *et al*., 2004).

Three-dimensional factorial correspondence analysis (3D-FCA) was performed with Genetix 4.05 (Belkhir *et al*., 2004) to explore population divisions and relationships of swordfish, independent from a prior knowledge of their relationships. This analysis places all individuals in a hyperspace which has as many dimensions as there are alleles at different loci. The algorithm looks for independent (orthogonal) directions or eigenvectors in this hyperspace along which the inertia is maximum. The eigenvectors determine a series of axes, and, by convention, the first axis is the one that has the highest contribution to the total inertia (Belkhir *et al*., 2004).

A model-based Bayesian clustering algorithm was undertaken using STRUCTURE 2.3.4 (Pritchard *et al*., 2000) to determine the number of genetic discrete populations (*K*) with the highest posterior probability based on the microsatellite genotypes. The simulated *K* values ranged from 1 to 10. Twenty independent runs were implemented for each specific *K*-value in order to verify the consistency of the results. The simulations were conducted assuming an admixture model with correlated allele frequencies, which is considered as the superior model for detecting structure among closely related populations (Falush *et al*., 2003; Hubisz *et al*., 2009). MCMC consisted of 100,000 burn-in iterations followed by 100,000 iterations. STRUCTURE was selected because it performs well at low levels of population differentiation (Latch *et al*., 2006). Structure Harvester (Earl and von Holdt, 2012) was used to determine the optimal number of clusters (*K*), independent of the prior population allocation per individual, by obtaining the mean posterior probability of the data (L(K)) and the *ΔK* approach of Evanno *et al*. (2005). CLUMPP (Jakobsson and Rosenberg, 2007) aligned and averaged cluster assignments across replicate analyses from STRUCTURE utilising the greedy algorithm, while DISTRUCT 1.1 (Rosenberg, 2007) provided a visual representation of the aligned cluster assignments. Results from STRUCTURE were compared with the results from TESS 2.3.1. (Durand *et al*., 2009).

TESS 2.3.1. (Durand *et al*., 2009) implements Bayesian clustering for spatial population genetic studies, including simultaneous analysis from geographical data (Chen *et al*., 2007). The method is based on a hierarchical mixture model where the prior distribution on admixture proportions (admixture model) is defined as a Hidden Gaussian Random Field (HGRF, admixture model) on a spatial individual network (tessellation). The program seeks population structure from individual multilocus genotypes sampled at distinct geographical locations without assuming predefined populations (Durand *et al*., 2009). The conditional autoregressive (CAR) Gaussian admixture model was run in TESS, with the suggested burn-in period of 10,000 replicates and 50,000 MCMC iterations (Durand *et al*., 2009) was run 20 times for each number of clusters from *K*max = 2 to *K*max = 10. For each value of *K*max, the deviance information criterion (DIC) was computed. The ideal cluster number was chosen according to when the DIC values reached a plateau. The estimated admixture coefficients was averaged for the runs from the chosen *K*max using the software CLUMPP. The resulting averaged Q matrix was visualized in DISTRUCT 1.1 (Rosenberg, 2007).

The TESS estimations of admixture proportions were displayed spatially with the tessplot function from "plot.admixture.r" (http://membres-timc.imag.fr/Olivier.Francois/admix_display.html) with the R packages *maps* and *fields* that interpolates expected admixture proportions on every point on a grid and displays it as a probability surface, known as universal kriging (Durand *et al*., 2009).

Effective population size

The effective population size (N_e) is an essential parameter that informs about the sustainable management and conservation of exploited species (O'Leary *et al.,* 2013). It determines how vulnerable populations are to losing genetic diversity due to genetic drift, and consequently, it assesses their responsiveness and adaptation capabilities (Laconcha *et al.,* 2015). The software NEESTIMATOR 2.01 (Do *et al.,* 2014) was used to determine N_e with the Linkage Disequilibrium and the Heterozygote excess methods.

Marker power

Finally, WHICHLOCI 1.0 was used to test the power of the markers to assign individuals to their current assigned populations by using allelic frequencies with 95% accuracy. An allele frequency differential was used following methods described in Shriver *et al*. (1997) to rank loci. The Whichloci (Banks *et al*., 2003) method is a resampling technique that generates simulated populations from observed allele frequencies in experimental samples, and then assigns experimental individuals to populations on the basis of the likelihood of an individual's genotype in each population. The software employs an empirical method for determining which combination of loci most likely provides a predefined population assignment power for individuals as well as statistical bounds on the performance of any particular group of loci. The log odds (LOD) level of assignment stringency was set at LOD = 1.

Results

Comparison of DNA extraction methods

The results of the four extraction methods tested on 10 samples (A to J) indicated that the 260/230 nm ratio was <0.7 for all extraction methods, possibly due to ethanol contamination in the samples. The CTAB extraction method had the highest DNA yield (average 247.9 ng/µl) and best average 260/280 nm ratio (1.97). The DNeasy® Blood and Tissue Kit achieved an average DNA yield of 26.6 ng/µl (± 20.77 ng/µl s.d.) and an average 260/280 nm ratio of 1.47. The SureFood® PREP kit had a higher average yield than the DNeasy® Blood and Tissue Kit (56.6 ng/µl) but with large deviation around the mean (115.2 ng/µl). The DNeasy® mericon Food Kit had the lowest DNA yield of 4.4 ng/µl.

The PCR success of markers A3 and A8 tested on three samples (F, G, J) extracted with the DNeasy Blood and Tissue kit are indicated in Figure 3. Sample J had the lowest DNA yield (18.1 ng/µl) and neither of the markers amplified. Sample F (24.06 ng/µl) and Sample G (84.38 ng/µl) amplified successfully. At this stage the minimum threshold for singleplex PCR success was assumed to be at around 20 ng/µl, and the DNeasy® Blood and Tissue Kit was deemed suitable for the project.

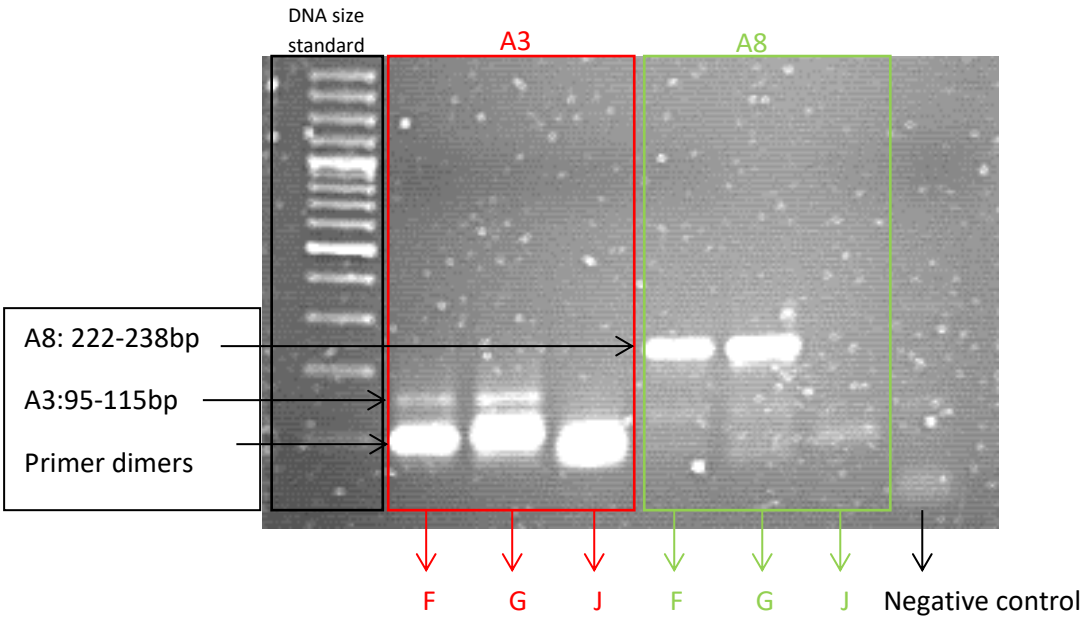


Figure 3. PCR of markers A3 and A8 on samples F, G and J extracted using the DNeasy® Blood and Tissue Kit. The 500bp DNA size standard is indicated.

DNA quantity thresholds for singleplex and multiplex PCR

The average DNA quantity of all samples was 35.73 ng/µl, average 260/280 nm ratio was 1.58 and the average 260/230 nm ratio was 0.53. The singleplex reactions had a success threshold of 20 ng/µl, which removed 164 samples (27.7%) from the project that were <20 ng/µl. Samples with >37 ng/µl had the greatest 4- and 5-marker multiplex PCR amplification success based on genotyping results. A DNA threshold of multiplex reactions with 4- and 5-markers per reaction was set at DNA quantity >70 ng/µl. The sample with >35 ng/µl DNA amplified and genotyped successfully with 2- and 3-marker multiplex groups. Therefore, samples were divided into three sets of PCR reactions; 1) singleplex reactions with samples 20-34 ng/µl (180 samples, 41% of the remaining samples), 2) multiplex reactions of 2- or 3- markers per reaction on samples 35-69 ng/µl (205, 47% of the remaining samples), and 3) multiplex reactions of 4- or 5- markers per reaction on samples >70 ng/µl (53, 12% of the remaining samples).

Through the process of optimising primers and the singleplex and multiplex PCR reactions, it was realised that the microsatellites A10, A115 and C4 (the three longest markers) consistently struggled to amplify with inconsistent amplification success with samples of highest DNA quantity. For this reason it was therefore decided to remove these three markers from the study. In general, the desiccated state of the muscle samples adversely affected the template DNA quantity and quality and overall amplification and genotyping success. Amplification was therefore successfully conducted on only 438 of the original 602 samples.

Sample filtering criteria

Once the filtering criteria had been applied per individual and per marker the final dataset consisted of 267 individuals (194 Indian Ocean (>20°E) and 73 Atlantic Ocean (<20°E)) (Figure 4) and 11 markers. The sex ratio was skewed with 181 females and 86 males in the dataset. The markers that were removed due to poor amplification success were A3, A4, C7, D11 and Xg66.

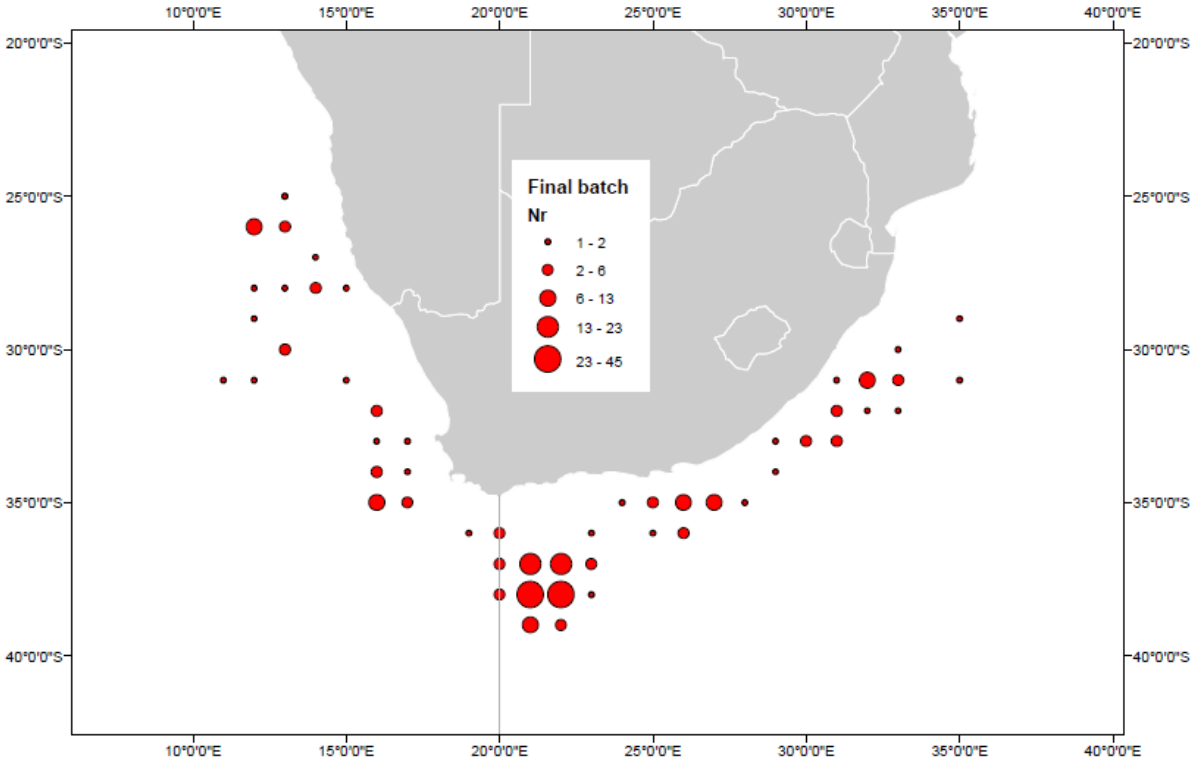


Figure 4. Geographic catch locations of individuals used in the final dataset.

Statistical analyses

Genetic diversity

F_{ST}-outlier analysis of the 11 loci on all individuals indicated that none of the loci were under selection (Figure 5). The 11 microsatellite loci varied in the number of alleles (5 – 25, mean = 11.4), observed heterozygosity (0. 13 – 0.89, mean = 0.62) and expected heterozygosity (0. 22 – 0.94, mean = 0.69) among populations (Table 4). The number of individuals genotyped in E20 ranged from 155 to 189 (median 176) and in W20 ranged from 57 to 69 (median 64). This was due, in part, to variation in the number of individuals collected from the two areas (Table 2) but may also be related to tissue degradation that prevented successful PCR amplification of both alleles in many individuals. Both populations had the same PIC value of 0.66. Allelic richness was 8.57 in the East and 8.31 in the West. Allelic frequencies of the 11 loci for E20 and W20 can be seen in Appendix B. The F_{ST} measure of -0.00001 is an indication of minimal to no detectable genetic differentiation between the two populations. The positive F_{IS} and F_{IT} values for all loci combined indicate heterozygosity deficiency in the total population (Table 5).

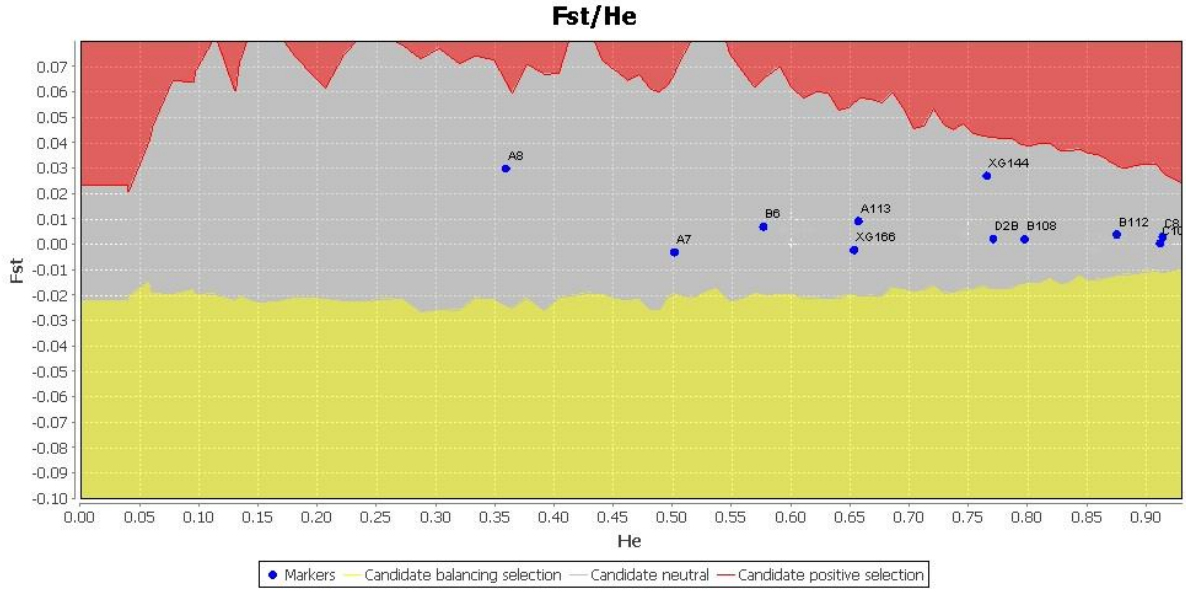


Figure 5. Identification of candidate loci under selection inferred from F_{ST} outlier analysis (P < 0.05) of 11 microsatellite markers.

Table 4. Summary statistics for microsatellite DNA variability in swordfish. Number of individuals (*N*), number of alleles (*A*), observed heterozygosity (H_O), expected heterozygosity (H_E), the Polymorphic Information Content (*PIC*) and allelic richness (R_S) was calculated for each locus per population (East (>20°E) and West (<20°E)).

	N		*A*		*Ho*		*He*		*PIC*		*Rs*	
	E20	W20	E20	W20	E20	W20	E20	W20	E20	W20	E20	W20
A8	189	67	7	5	0.21	0.13	0.26	0.22	0.25	0.21	4.01	2.63
A7	176	62	7	5	0.43	0.39	0.48	0.43	0.45	0.40	4.68	4.75
A113	176	65	7	5	0.55	0.58	0.65	0.66	0.62	0.61	5.58	4.98
B6	155	62	7	6	0.56	0.65	0.55	0.60	0.50	0.54	5.94	5.00
B108	180	67	16	12	0.65	0.67	0.79	0.81	0.77	0.79	10.61	9.40
B112	177	61	13	12	0.77	0.79	0.87	0.88	0.86	0.85	9. 15	9.60
C8	168	64	25	23	0.85	0.89	0.93	0.94	0.93	0.93	17.21	17.11
C10	163	61	20	20	0.82	0.84	0.91	0.92	0.90	0.91	14.35	18.24
D2B	181	69	11	7	0.71	0.68	0.76	0.76	0.72	0.72	7. 10	5.00
Xg166	181	65	11	7	0.62	0.69	0.63	0.67	0.60	0.63	6.88	4.84
Xg144	158	57	15	10	0.59	0.63	0.74	0.77	0.70	0.73	8.80	9.89
Average			12.6	10.18	0.61	0.63	0.69	0.70			8.58	8.31

Table 5. Average Weir and Cockerman's F-statistics (F_{IS}, F_{IT}, F_{ST}) per locus among populations, E20 and W20.

Locus	F_{IS}	F_{ST}	F_{IT}
A8	0. 2461	0.0008	0. 2467
A7	0. 1108	-0.0026	0. 1085
A113	0. 1445	-0.0001	0. 1444
B6	-0.0401	0.0014	-0.0386
B108	0. 1806	-0.0002	0. 1805
B112	0. 1156	-0.0029	0. 113
C8	0.0782	-0.0025	0.0759
C10	0.0976	-0.0007	0.097
D2B	0.0754	-0.0043	0.0714
Xg166	0.0085	0.0067	0.0151
Xg144	0. 1972	0.0058	0. 2019
All	0. 1055	-0.0001	0. 1054

Significant departures from HWE were observed in 14 of 22 single locus exact tests (Table 6) and all deviations except one (B6) were towards heterozygote deficiencies in either one or both of the populations (Table 4). Loci B6, B112, C8 and C10 were in HWE for the West and D2B and Xg166 for the East and West. Five (A8, A113, B108, C10, Xg144) of the nine loci that departed from HWE displayed null alleles and homozygote excess, and of those all except one locus (Xg144) underwent stuttering during PCR amplification (Table 7). These results indicate that true null alleles (*i.e.* alleles that failed to amplify because of base substitutions or deletions in PCR priming sites flanking microsatellite arrays) (O'Reilly *et al*., 2004) could have been the cause of departures from HWE in only one of the nine loci. There was evidence of 16 of the 55 loci pairs with significant (Initial $\alpha = 0.05$) LD before and after Bonferroni correction (Table 8). Of the loci pairs with significant LD, 13 included loci with null alleles (A8, A113, B108, C10, Xg144).

Table 6. Statistical significant departures from HWE per locus, p-value (initial α=0.05) was assessed before (*) and after (**) sequential Bonferroni correction.

Locus	E20	W20
A8	0.000**	0.000**
A7	0.000**	0.040*
A113	0.000**	0.013*
B6	0.001**	-
B108	0.000**	0.017*
B112	0.004*	-
C8	0.027*	-
C10	0.002*	-
D2B	-	-
Xg166	-	-
Xg144	0.000**	0.000**

Table 7. The results of MICRO-CHECKER testing for the presence of homozygote excess, stuttering during PCR amplification, large allele dropout and null alleles for the 11 loci from East (>20°E) and West (<20°E). na = not applicable.

Locus	Homozygote excess	Stuttering	Large allele dropout	Null alleles	Null frequency
A7	N	N	N	N	na
A8	Y	Y	N	Y	0.30
A113	Y	Y	N	Y	0.18
B6	N	N	N	N	na
B108	Y	Y	N	Y	0.22
B112	N	N	N	N	na
C8	N	N	N	N	na
C10	Y	Y	N	Y	0.20
D2B	N	N	N	N	na
XG144	Y	N	N	Y	0.21
XG166	N	N	N	N	na

Table 8. Loci pairs with significant linkage disequilibrium (LD), p-value (initial α=0.05) was assessed before (*) and after (**) sequential Bonferroni correction. Loci with null alleles are indicated with ^. S.E. = standard error.

Population	Locus #1	Locus #2	P-Value	S.E.
East (>20°E)	A8^	B6	0.000**	0.00
	A113^	B108^	0.021*	0.01
	A113^	C8	0.041*	0.02
	B6	C8	0.0165*	0.01
	B108^	C8	0.005*	0.01
	B108^	C10^	0.021*	0.01
	B112	C10^	0.049*	0.02
	C8	D2B	0.000**	0.00
	A113^	Xg166	0.035*	0.02
	C8	Xg166	0.037*	0.02
	Xg166	Xg144^	0.000**	0.00
West (<20°E)	A7	A113^	0.042*	0.01
	B108^	C8	0.027*	0.02
	B112	C10^	0.008*	0.00
	A7	Xg144^	0.046*	0.01
	C10^	Xg144^	0.024*	0.01

Population differentiation

The joint null hypothesis of no heterogeneity between population pairs for any locus was rejected in 4 of 6 tests of genic differentiation (Table 9). Exact tests of genic differentiation between population pairs showed that significant heterogeneity was detected of the W17 and W20 populations with other locations. Pairwise F_{ST} indicated significant population structuring for scenario 2 between W17 and E30, and for scenario 4, between W30 and E30 (Table 10). AMOVA results indicated negligible heterogeneity between populations in every scenario with F_{ST} values between 0 and 0.007 (P between 0.45 and 0.94), likewise for genetic differentiation among individuals (F_{IS} between -0.04 and 0.018 (Table 11). Most of the time, negative variance component values indicate an absence of genetic structure (Schneider *et al.,* 2000). This result is concordant with the genotypic differentiation tests.

Table 9. Probability values of genic differentiation (lower diagonal) and genotypic differentiation (upper diagonal) tests for each population scenario. Significant p-values (<0.05) is indicated by an asterisk *.

		Scenario 1		Scenario 2			Scenario 3		Scenario 4	
		East (>20°E)	West (<20°E)	East (>30°E)	South (17-30°E)	West (<17°E)	East (>17°E)	West (<17°E)	East (>30°E)	West (<30°E)
Scenario 1	East (>20°E)	-	0. 13							
	West (<20°E)	0.01*	-							
Scenario 2	East (>30°E)			-	0. 21	0.05				
	South (17-30°E)			0. 17	-	0. 18				
	West (<17°E)			0.02*	0.04*	-				
Scenario 3	East (>17°E)						-	0. 15		
	West (<17°E)						0.02*	-		
Scenario 4	East (>30°E)								-	0.09
	West (<30°E)								0.08	-

Table 10. Pairwise F_{ST} (lower diagonal) and corresponding significance values (upper diagonal) (*p<0.05) for the four population scenarios.

		Scenario 1		Scenario 2			Scenario 3		Scenario 4	
		East (>20°E)	West (<20°E)	East (>30°E)	South (17-30°E)	West (<17°E)	East (>17°E)	West (<17°E)	East (>30°E)	West (<30°E)
Scenario 1	East (>20°E)	-	0.51							
	West (<20°E)	-0.0003	-							
Scenario 2	East (>30°E)			-	0. 12	0.04*				
	South (17-30°E)			0.00373	-	0.54				
	West (<17°E)			0.0067	-0.0004	-				
Scenario 3	East (>17°E)						-	0.49		
	West (<17°E)						0.00004	-		
Scenario 4	East (>30°E)								-	0.03*
	West (<30°E)								0.00558	-

Table 11. Analysis of molecular variance (AMOVA) among populations for the four population scenarios.

	Genetic Structure	Variance component	% of total	Fixation index
Scenario 1	Among populations	-0.00074	-0.02	Fst = 0.000 (P = 0.98)
	Among individuals within populations	-0.03429	-1. 16	Fis = 0.018 (P = 0.85)
	Within individuals	2.99813	101. 18	Fit = 0.008 (P = 0.86)
Scenario 2	Among populations	0.00442	0. 15	Fst = 0.00149 (P = 0.94)
	Among individuals within populations	-0.03675	-1. 24	Fis = -0.01241 (P = 0.87)
	Within individuals	2.99813	101.09	Fit = -0.01090 (P = 0.85)
Scenario 3	Among populations	0.0003	0.01	Fst = 0.00010 (P = 0.95)
	Among individuals within populations	-0.03469	-1. 17	Fis = 0.00010 (P = 0.88)
	Within individuals	2.99813	101. 16	Fit = -0.01160 (P = 0.86)
Scenario 4	Among populations	0.01703	0.57	Fst = 0.00572 (P = 0.45)
	Among individuals within populations	-0.03759	-1. 26	Fis = -0.01270 (P = 0.89)
	Within individuals	2.99813	100.69	Fit = -0.00691 (P = 0.87)

Population assignment

Nei's (1972) Standard Distance (*Ds*) was calculated per scenario and resulted in the largest genetic distance between the populations of scenario 3, W17 and E17, and scenario 2, W17 and E30 (Table 12). The Mantel regression test indicated a lack of correlation between genetic divergence and geographic distance (R^2 = 0. 2969, P = 0.54) across populations in scenario 2 (Figure 6). The rate at which individuals correctly assign to their sampled locality can also be used as an assessment of population genetic structure (Manel *et al*., 2005). Scenarios 1 and 3 had the highest percentage of

migrants (8.23% and 6.37%, respectively), and the misassignments (*i.e.* migrants) were individuals that had originated in neighbouring East (>17°E and >20°E) populations and were caught in the West (<17°E) (Table 13). A summary across population scenarios indicates that on average 5.8% of migrants originating from the South and East were caught in the West, 1.5% of migrants originating from the South were caught in the West and East, and 1.62% of migrants from the West and South were caught in the East.

Three-dimensional factorial correspondence analyses (3D-FCA) explained 100.00% of the overall variation in scenarios 1, 3 and 4, and 54.45% of the variation in scenario 2, with additional separation noticeable on the second axis. Every scenario displayed considerable overlap of individuals from each population, and the boundary between proposed populations was not clear, with admixture zones present (Figure 7).

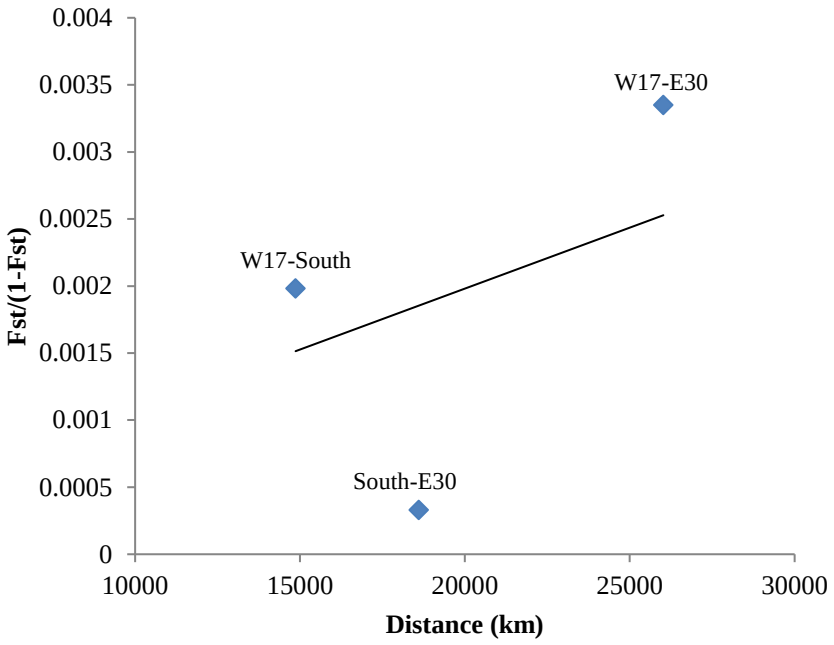


Figure 6. Isolation-by-distance (IBD) plotting the genetic distance (F_{ST} / (1 − F_{ST})) against the geographic distance (km) for E30, South and W17.

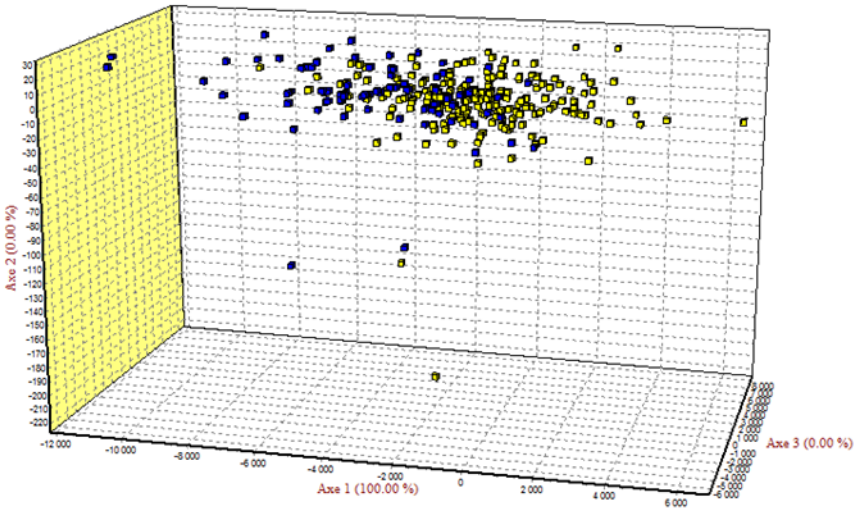
Table 12. Nei's (1972) genetic distance *Ds* for scenarios 1 to 4.

		Scenario 1	Scenario 2		Scenario 3	Scenario 4
		East (>20°E)	South (17-30°E)	East (>30°E)	East (>17°E)	East (>30°E)
Scenario 1	West (<20°E)	0.013				
Scenario 2	East (>30°E)		0.033			
	West (<17°E)		0.016	0.044		
Scenario 3	West (<17°E)				0.044	
Scenario 4	West (<30°E)					0.036

Table 13. The results of the detection of migrants tests conducted in GeneClass 2.0, for each population scenario.

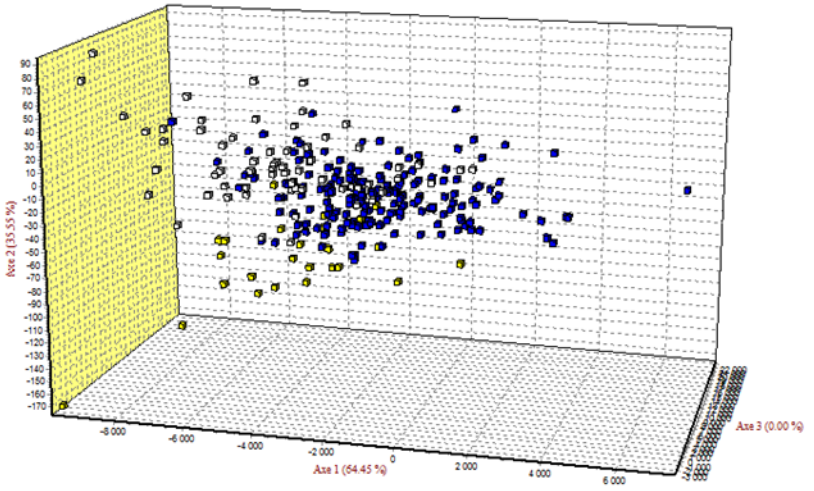
		Correct population		
Scenario 1	**Assigned population**	West (<20°E)	East (>20°E)	
	West (<20°E)		17	
	East (>20°E)	5		
	Total number of migrants:	22		
	Percentage migrants:	8.23%		
Scenario 2	**Assigned population**	West (<17°E)	South (17-30°E)	East (>30°E)
	West (<17°E)		2	0
	South (17-30°E)	1		0
	East (>30°E)	1	6	
	Total number of migrants:	10		
	Percentage migrants:	3.75%		
Scenario 3	**Assigned population**	West (<17°E)	East (>17°E)	
	West (<17°E)		14	
	East (>17°E)	3		
	Total number of migrants:	17		
	Percentage migrants:	6.37%		
Scenario 4	**Assigned population**	West (<30°E)	East (>30°E)	
	West (<30°E)		0	
	East (>30°E)	12		
	Total number of migrants:	12		
	Percentage migrants:	4.49%		

i)



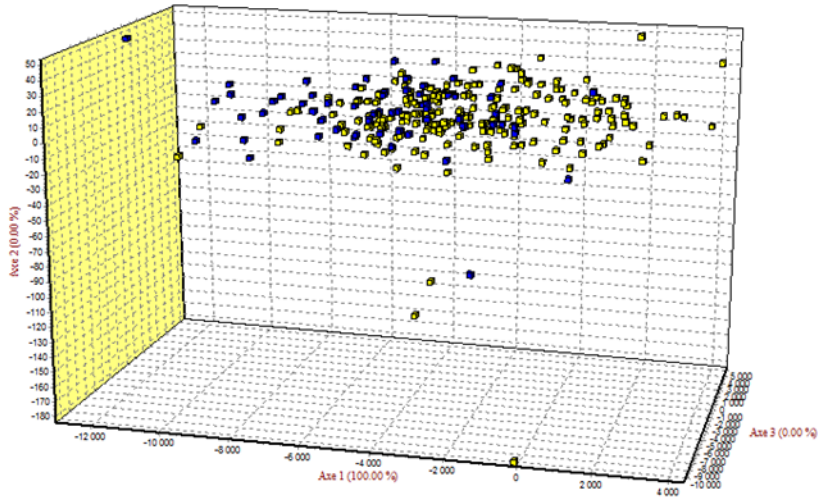
Blue: West (<20°E) Yellow: East (>20°E)

ii)



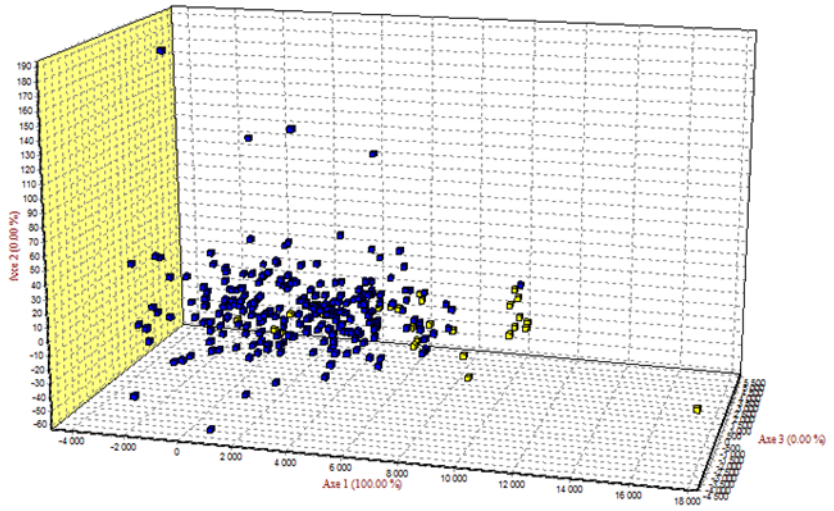
White: West (<20°E) Blue: South (17 – 30°E) Yellow: East (>20°E)

iii)



Blue: West (<17°E) Yellow: East (>17°E)

iv)



Blue: West (<30°E) Yellow: East (>30°E)

STRUCTURE performs well at low levels of population differentiation (Latch *et al*. 2006) and may be able to detect structure in this data if it is present. Calculation of *ΔK*, a measure of the second order rate of change in the likelihood of *K* (Evanno *et al*., 2005), from the STRUCTURE output produced a modal value of the statistic at *K* = 6 (Figure 8a). While the largest value of *ΔK* was at *K* = 4, a second mode was present at *K* = 6. In cases where STRUCTURE finds clustering solutions with similar probabilities at different values of *K*, the lowest value is typically the most accurate (Pritchard *et al*., 2000; Pritchard and Wen, 2004). The expectation was two clusters of swordfish since there are two recognised stocks, the South Atlantic and the Indian Ocean.

While Evanno's *ΔK* method seeks to detect the uppermost hierarchical level of population structure, the method is less reliable at lower levels of genetic differentiation and may incorrectly estimate *K* (Waples and Gaggiotti*,* 2006). Therefore, the estimation of *K* using the *ad hoc* evaluation of mean posterior probabilities from multiple analyses of *K*, *i.e.* L(*K*) (Pritchard *et al.,* 2000), appears to be more appropriate in this instance than the *ΔK* approach (Evanno *et al.,* 2005). The mean posterior probabilities of L(*K*) indicated *K* = 4 (Figure 8b).

Genetic clustering between W20 and E20 was not visually detected in the Bayesian clustering analysis averaged in CLUMPP and displayed by DISTRUCT for *K* = 2, 3 or 4 (Figure 9). The proportion clustering membership (Q) did not produce patterns concordant with the geographic boundary. In *K* = 2, individuals with majority cluster 1 (yellow) *Q* are found across the geographic locations.

The model-based clustering results in TESS indicated the existence of four clusters based on the lowest DIC value (Figure 10). Average membership coefficients of each individual to either *K*=2, 3 or 4 from TESS analyses is displayed in Figure 11. As with the results from STRUCTURE, there are no clear memberships of individuals to either of the two populations. A posterior predictive map of the admixture proportions for *K*max = 4 (Figure 12) was generated from the spatial interpolation (kriging) procedure. The *K*max = 4 may indicate sublevels of structuring. When considering *K*max = 4 the red and green clusters (or admixture proportions) distribute together, and the blue and yellow clusters are neighbouring and distribute together. Therefore, a weak differentiation boundary may be present in the region of 27°E. A posterior predictive map of the admixture proportions for *K*max = 2 was included to visualise the clustering without the potential sublevels of structuring mentioned by Evanno *et al*. (2005) (Figure 13). At this level of clustering an admixture zone between 14°E and 27°E may exist. A graphical summary of the population differentiation and population assignments tests are indicated in Figure 14.

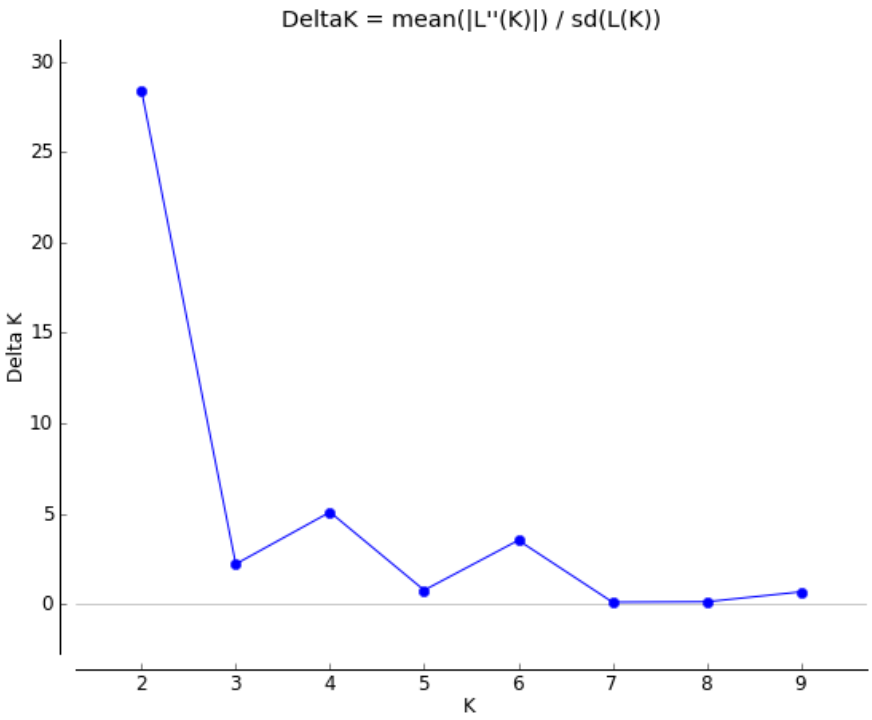
Effective population size

The results from the Linkage Disequilibrium method of determining N_e (NEESTIMATOR) produced an alarmingly small value of N_e = 337 in the East (>20°E), with wide 95% confidence intervals of between 48.9 and infinity, and N_e = 1821.1 in the West (<20°E), with wide 95% confidence intervals of between 438.3 and infinity. Similarly, the Heterozygote Excess method determined the effective population size to equal infinity in both populations. These results indicate that the data is not informative enough to determine N_e.

Marker performance

The results from WHICHLOCI indicated that with a stringency of LOD = 1, neither the 11 loci combined nor 6 loci (markers with null alleles removed) combined were sufficient to assign the individuals to the current population assignment of W20 and E20 with 95% confidence.

a)



b)

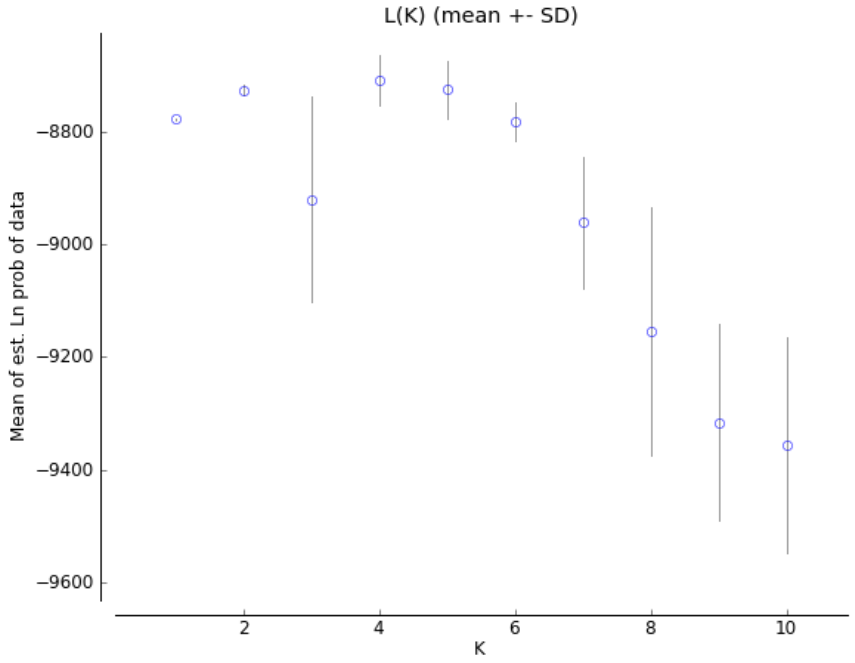
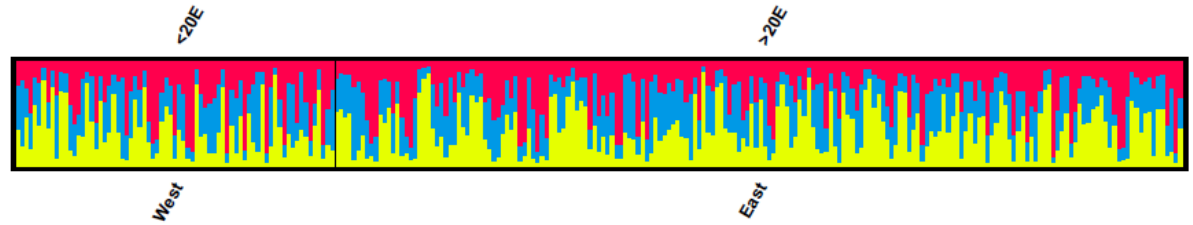


Figure 8. Assignment of swordfish to populations by the STRUCTURE program. The peak of (a) Evanno's delta k (*ΔK*) and (b) the mean log likelihood of the data [L(*K*)] represents the most likely number of subpopulations.

i)

ii)



iii)

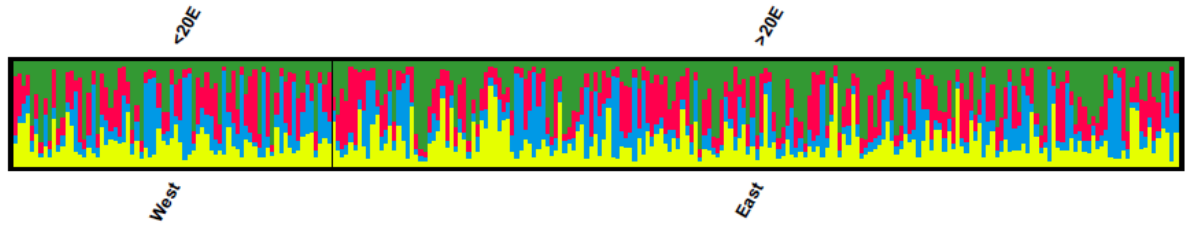
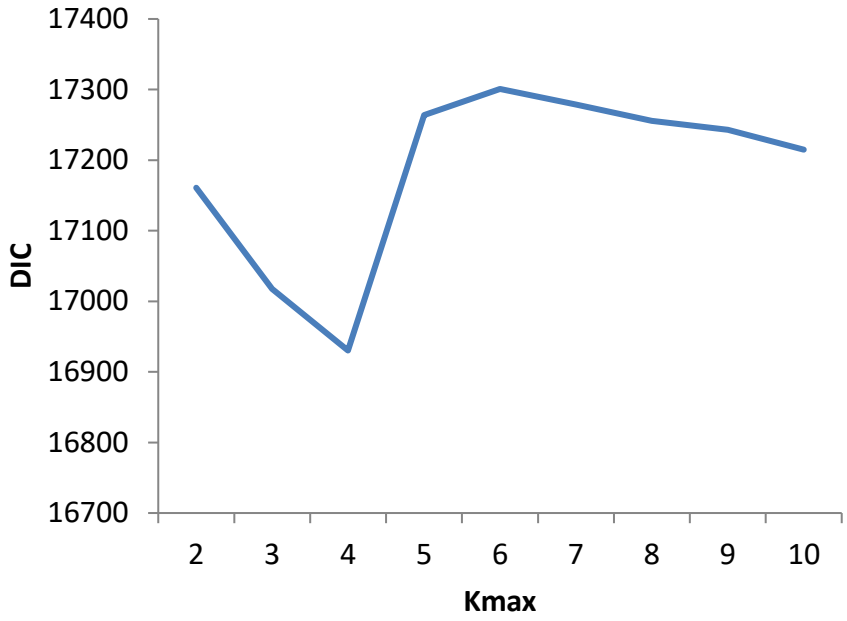


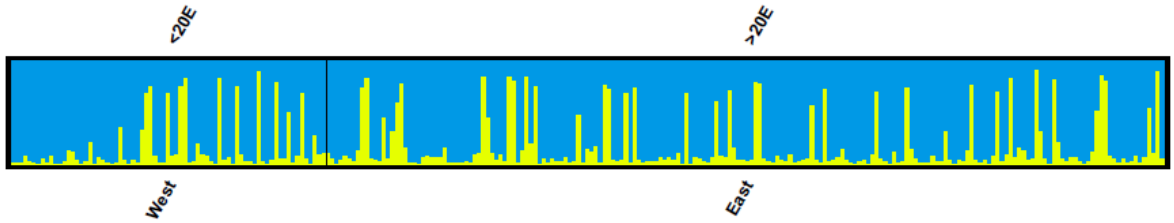
Figure 9. Bayesian clustering results inferred by STRUCTURE for three scenarios of *K*, i) *K* = 2, ii) *K* = 3, iii) *K* = 4



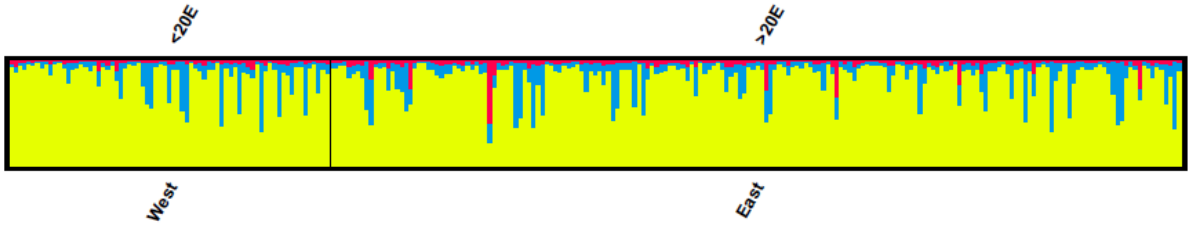
i)

Figure 10. The deviance information criterion (DIC) for 20 TESS runs with *K*max ranging from 2 to 10.

i)



ii)



IIi)

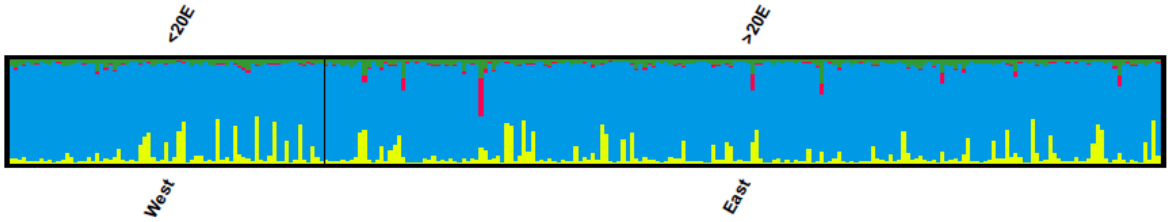


Figure 11. Bar plots representing admixture proportions for swordfish from a spatial assignment test performed in TESS 2.3.1. for i) *K* = 2, ii) *K* = 3, and iii) *K* = 4.

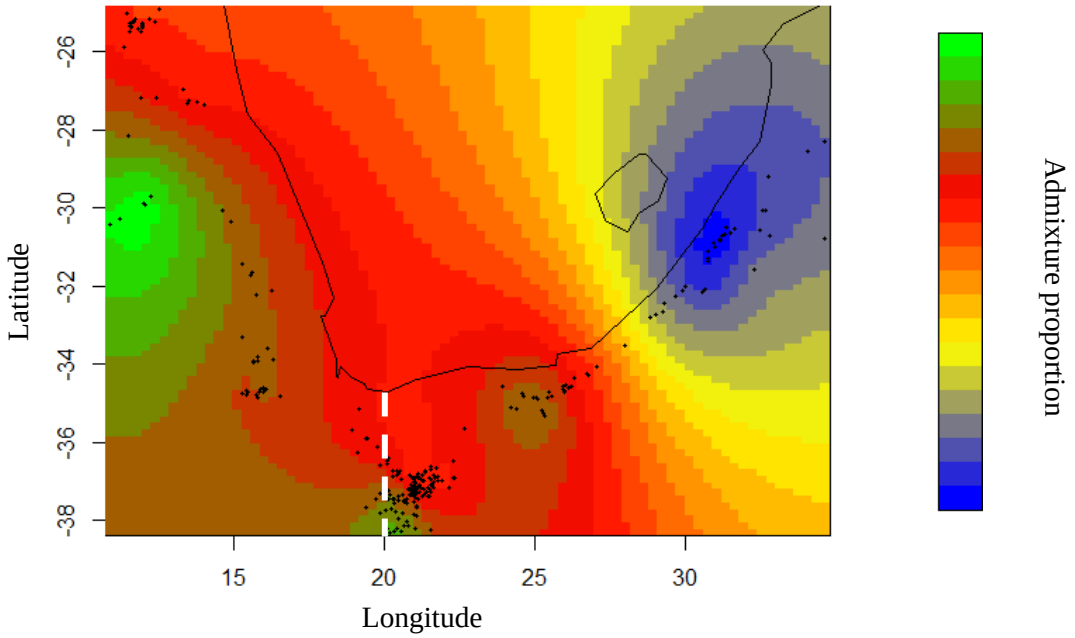


Figure 12. Posterior predictive map of the admixture proportions for *K*max = 4 clusters generated from the spatial interpolation (kriging) procedure implemented in TESS 2.3.1. Each colour (green, red, yellow, blue) indicates one of the four clusters. The current management boundary at 20°E is indicated.

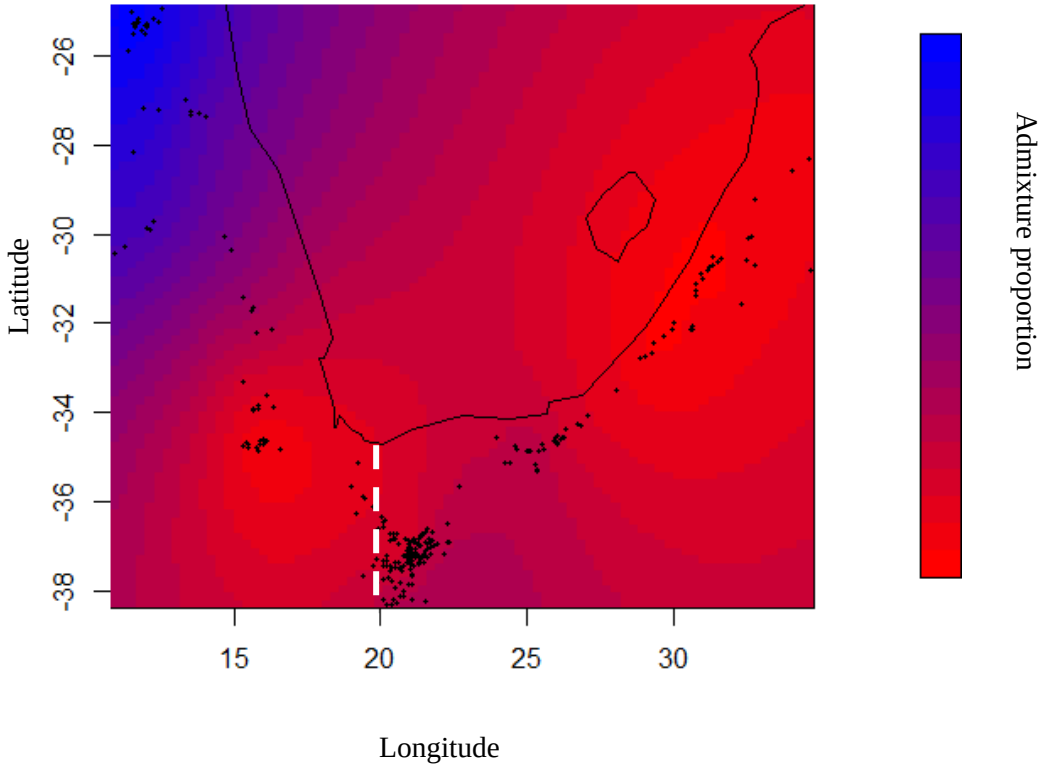


Figure 13. Posterior predictive map of the admixture proportions for *K*max = 2 clusters generated from the spatial interpolation (kriging) procedure implemented in TESS 2.3.1. Each colour (blue and red) indicates one of the two clusters. The current management boundary at 20°E is indicated.

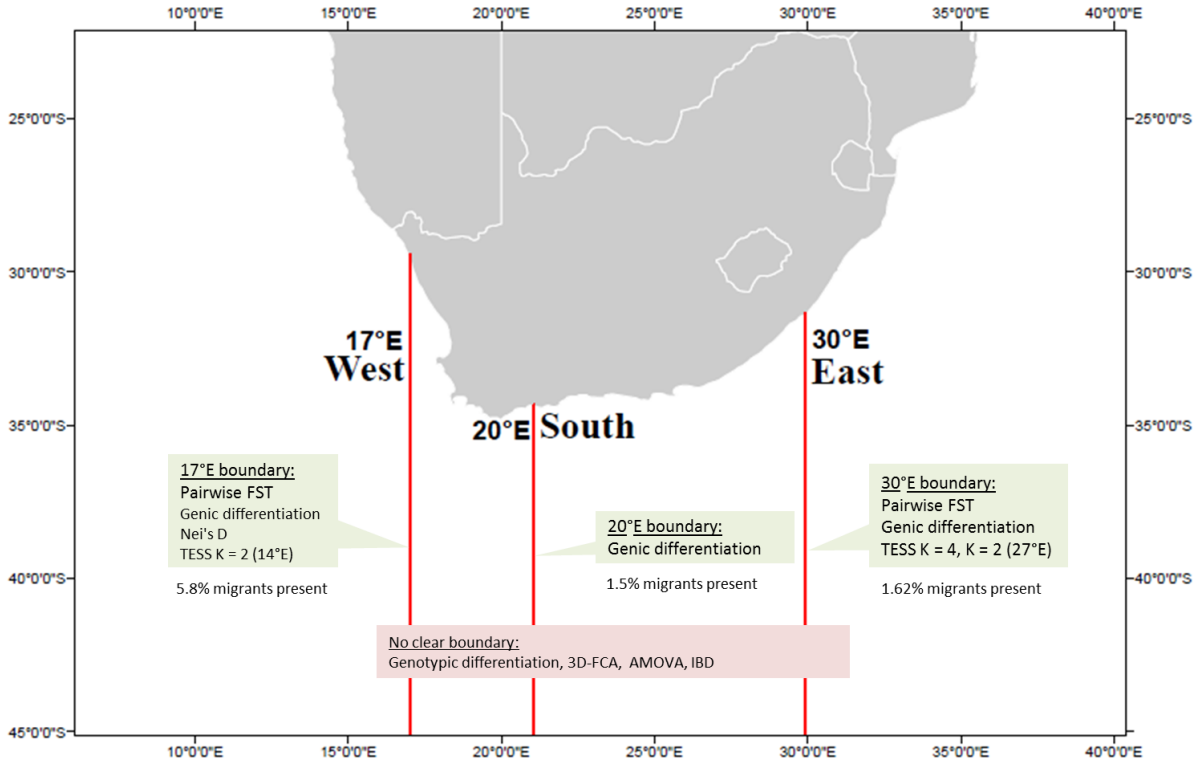


Figure 14. A graphic summary of the tests conducted on swordfish in this study.

Discussion

In the marine environment many studies have failed to detect statistically significant population structuring because of low differentiation, especially over small geographical distances (*e.g.* in cod, Árnason *et al*., 1992; Gjøsæter *et al*., 1992). Low levels of differentiation in marine organisms are most likely due to extensive gene flow (Ward *et al*., 1994; Waples, 1998; Avise, 2000) and do not necessarily imply that structuring does not exist, but that more powerful means are required to detect them (Knutsen *et al*., 2003). Marine organisms, even if weakly differentiated on a small geographical scale, often show evidence of differentiation over larger distances, probably because the long distance acts as an isolation mechanism (Knutsen *et al*., 2003).

Weak structure was detected between the two swordfish populations (West <20°E and East >20°E) sampled in a regional geographic scale around South Africa. This is not surprising since active dispersal of adult swordfish of the two recognised stocks results in mixing in this area, in addition to genetic homogenisation due to passive drift of larvae.

Despite the weak structure of swordfish in this study, the results corroborate previous findings of population differentiation in swordfish between the Indian and Atlantic oceans that were sampled at larger geographical scales than this study (Chow *et al*., 1997; Alvarado Bremer *et al*., 1999; Chow and Takeyama, 2000; Kotoulas *et al*., 2006; Alvarado Bremer *et al*., 2007; Muths *et al*., 2013). Previous evidence of weak differentiation between the Indian and Atlantic oceans with microsatellites includes studies by Ward *et al*. (1997), Alvarado Bremer *et al*. (1998); Chow *et al*. (2000), Graves and McDowell (2003); Durand *et al*. (2005), Chiang *et al*. (2008), Albaina *et al*. (2013) and Laconcha *et al*. (2015).

The low numbers of individuals successfully genotyped per area, 94 from W20 and 173 from E20, may have contributed to some loss of power. Nevertheless, Ruzzante (1998) showed that samples of 50 or greater are sufficient to produce relatively precise estimates of F_{ST} with highly variable microsatellites (O'Reilly *et al*., 2004). Reeb *et al*. (2003) indicated that the markers Xg66, Xg144 and Xg166 displayed significant divergence between two populations (Ecuador and Mediterranean), and observed heterozygosity of between 0.15 and 0.960, deeming them suitable for population genetics studies. These three markers were also utilised in the study by Ward *et al*. (2001) on the population structure of Australian swordfish, with two markers (Xg66 and Xg144) providing the highest gene differentiation values. However, a study by Kasapidis *et al*. (2008) on the stock structure of swordfish in the Pacific revealed Xg144 to be least polymorphic. The 16 markers chosen from Bradman *et al*. (2011) (A3, A4,

A7, A8, A10, A113, A115, B6, B108, B112, C4, C7, C8, C10, D2B, D11) were deemed suitable for population structure studies on swordfish; however, four of the markers (B6, B108, B112, C4) did not meet the HWE, similarly to the current study.

O'Reilly *et al*. (2004) suggested that null alleles may be common in large marine populations that have increased sequence heterogeneity, attributed to increased effective population sizes and reduced loss of variation due to drift. However, the use of degraded tissue samples provided low quality DNA which may have resulted in unsuccessful amplification and had a direct impact on the degree of null allele markers. This has impacted the departures from HWE and LD since five of the nine markers not in HWE had null alleles and 13 of the 16 pairs of markers in LD were markers with null alleles. In addition to the presence of null alleles, the departure from HWE, common in marine fish (O'Connell and Wright, 1997; Karlsson and Mork, 2005), can be explained by factors such as inbreeding, the Wahlund effect, or selection (Wittke-Thompson *et al*., 2005).

The positive F_{IS} and F_{IT} values reflect homozygote excess and/or null alleles in the markers. Of the extraction methods tested, the CTAB protocol provided the highest DNA yield. It is recommended that for future studies on degraded tissue samples, the time is dedicated to the CTAB extraction protocol to maximise the success of the amplification and genotyping procedures that follow.

On this regional geographical scale, the pairwise F_{ST} values were less than 0.05, the level that indicates little genetic differentiation. This level of weak differentiation is similar in magnitude to those reported for other marine fish species with potentially high levels of gene flow (*e.g.* Elliot and Ward, 1992 (orange roughy *Hoplostethus atlanticus*); Gold *et al*., 1994 (red drum *Sciaenops ocellatus*); Bentzen *et al*., 1996 (Atlantic cod *Gadus morhua*); Borsa *et al*., 1997 (flounders); Ruzzante *et al*., 1999 (cod *Gadus* spp.); Shaw *et al*., 1999 (Atlantic herring *Clupea harengus*); Lundy *et al*., 2000 (European hake *Merluccius merluccius*); Nesbø *et al*., 2000 (Atlantic mackerel *Scomber scombrus*); De Innocentiis *et al*., 2001 (Dusky grouper *Epinephelus marginatus*); McPherson *et al*., 2001 (Atlantic herring *Clupea harengus*); Wirth and Bernatchez, 2001 (European eel *Anguilla anguilla*); Withler *et al*., 2001 (Pacific Ocean perch *Sebastes alutus*); Knutsen *et al*., 2003 (Atlantic cod *Gadus morhua*)).

Despite the low pairwise F_{ST} values, significant differentiation was present between W17/E30 (Scenario 2) and between W30/E30 (Scenario 4) (Table 10). The genic differentiation tests built on this result and included significant differentiation of W17/S (Scenario 2), W17/E30 (Scenario 2), W17/E17 (Scenario 3), W20/E20 (Scenario 1) (Table 9). These results indicate variation in the interactions

between the populations, supporting scenarios of individuals moving from the Atlantic stock (<20°E) into the Indian Ocean area (>20°E) and *vice versa*; a result in concordance with Muths *et al*. (2013). Because the level of differentiation was quite low, it may at first be dismissed as not being biologically meaningful (*c.f.* Waples 1998; but see Wirth and Bernatchez, 2001). Knutsen *et al*. (2003) concludes that this is unwarranted; any statistically significant difference in allele frequency, no matter how small, indicates that the samples are from separate statistical populations.

There was additional evidence for swordfish distributing across the management boundary through the presence of first-generation migrants that originated from E17 and South caught in the W17 region, and migrants that originated from W17 and South caught in the E17 region (Figure 14). The assignment tests, however, indicated that the proportion of migrants between the two populations was very low. When the largest Nei's genetic distance (*D*) values are considered (0.044 and 0.036) then an indication of population differentiation exists, and the Indian Ocean stock distribution extends over the management boundary at 20°E.

The 3D-FCA for all scenarios revealed extensive overlap of individuals between the populations for all scenarios. There was no clear boundary between populations, indicating a large degree of admixture. It was not surprising then that the population structure did not follow an isolation-by-distance pattern since the West, South and East regions are relatively in proximity. As the species is wide ranging, migratory and potentially admixed, the geographical range may be too restricted to detect a relationship of genetic distance with geographic distance. Other studies have reported an IBD distance pattern of geographical distances between samplings sites in excess of 1,000 km (*e.g.* Mork *et al*., 1985; Pogson *et al*., 1995, 2001).

The estimated number of clusters (*K*max = 4) generated by STRUTCURE and TESS does not indicate structure in the bar plots of individual admixture proportions across the geographic range. Although results from STRUCTURE and TESS analysis suggest that swordfish form four genetic clusters, it can be argued that two clusters are more likely (Barker *et al*., 2015). Hubisz *et al*. (2009) cautioned that STRUCTURE can overestimate the number of clusters, for example when there is inbreeding or relatedness among some individuals. Moreover, the number of clusters is not well-defined in settings where the allele frequencies vary smoothly across the landscape (Wasser *et al*., 2004). The additional *K* = 2 bar plots differ between STRUCTURE and TESS outputs yet both indicate the possibility of admixture between the populations, since the proportions of individuals that have dominant cluster 1 (yellow) or cluster 2 (blue) are distributed across the geographic range. The boundaries suggested

from the posterior predictive maps of admixture proportions for *K* = 4 (Figure 12) and *K* = 2 (Figure 13) are independent of any suggested boundaries created per scenario (17°E, 20°E, 30°E) yet the map indicates degrees of admixture in the South with a separation between the Atlantic and Indian oceans in the West and East between 14°E and 27°E.

The insufficiency of the 11 loci to allocate individuals into the current W20 and E20 populations, according to WHICHLOCI, could be attributed to few markers utilised once the markers with null alleles were removed.

A large effective population size that limits genetic drift can be one of the causes of HWE and genetic homogeneity. The effective population size is the number of individuals in a population who contribute offspring to the next generation. In this study the data was insufficient to determine the effective population size, and its relation to the census population size (*i.e*. estimated total biomass) of the two stocks. The effective population size may, however, be one of the causes of weak differentiation in this area.

The southern African region, separating the Indian and Atlantic oceans, is in the 30-40°S latitudinal range, an area suitable for tropical and temperate tunas and for billfish to inhabit. Weak differentiation at regional geographical scales in this area is therefore not surprising as these species have wide environmental parameter limits that extend across this area.

Ocean currents are a predominant environmental factor influencing contemporary levels of gene flow between populations, especially in species with pelagic eggs or larvae, or in species with highly migratory adults (Magoulas *et al*., 2006; Tzeng, 2007; Zhan *et al*., 2010). The presence of the Benguela Current and the Agulhas Current, may be a type of geographical barrier that influences swordfish distribution patterns directly (*e.g.* the Agulhas Current and Agulhas Current Retroflection directing the swimming behaviour) or indirectly (*e.g.* influencing the availability of food and creating suitable water temperatures that will attract swordfish). The Agulhas Current is part of the subtropical Indian Ocean gyre (STIOG) and transports about 70–78 Sv (1 Sv = 10^6 m^3 s^{-1}) of tropical and subtropical waters along the eastern margin of southern Africa (Lutjeharms, 2007). At the southern tip of Africa, between 16°E and 20°E, the current retroflects with the majority of its waters flowing back into the Indian Ocean as the Agulhas Return Current (Feron *et al*., 1992). Only a relatively small proportion of the Agulhas Current's warm and salty waters, approximately 2–15 Sv, are transported into the South Atlantic through the Indian-Atlantic Ocean Gateway *via* the Agulhas leakage (de Ruijter *et al*., 1999;

Richardson, 2007). Backeberg *et al*. (2012) suggested that intensified Indian Ocean winds cause enhanced mesoscale variability of the Agulhas Current system, potentially resulting in an increase in Agulhas leakage. Simon *et al*. (2013) concluded that variability in the upstream Agulhas Current hydrography is strongly linked to the dynamics of the Agulhas Return Current and strength of the Southwest Indian Ocean subtropical gyre (SWIOSG) and that downstream variability in the leakage area (Atlantic sector) at least partly reflects regional variations of the Agulhas Current as a whole. Surface speeds of the Agulhas Current (AC) may be in excess of 2 m/s (Lutjeharms, 2007). Donohue *et al*. (2000) showed that on occasion the current extends to the bottom whereas on other occasions its vertical penetration was only to a depth of 2300 m. Despite the intense jet-like features of the Agulhas Current, the swordfish have been able to traverse this current from the Atlantic into the Indian Ocean.

In a study by West *et al.* (2012), the horizontal movement patterns of swordfish tagged in 2011 supports the notion that movement is independent of the current and demonstrate that the apparent boundary between the Atlantic and Indian oceans is not insurmountable by this species. The study observed one particular individual that was tagged at 18°E and crossed the 20°E longitude twice, returning to the Atlantic Ocean after swimming into the Indian Ocean as far as 33°E.

Muths *et al*. (2013), with samples of swordfish collected in 2009, concluded that based on the results of mtDNA and microsatellite markers, "the boundary between Atlantic and Indian swordfish populations was not so strict and might be more considered as a transition zone between 17°E and 23°E east that is spatio-temporarily driven by the Agulhas Current activity. The results of this study and of Muths *et al*. (2013) indicate admixture or a transition zone in the south in two time periods that are four years apart.

Evidence of mixed stocks of swordfish has been alluded to by Viñas *et al*. (2007) in the North Atlantic-Mediterranean Sea region, concluding that the Mediterranean stock extends beyond the Strait of Gibraltar to at least 10°W and mixes with the North Atlantic stock. The possibility that the Atlantic-Mediterranean Sea transition is a phylogeographic break (Patarenello *et al*., 2007) does not beg consideration in the Indian-Atlantic Ocean region. Similarly, the North Atlantic-South Atlantic stocks are two oceanic gyres separated only by the equator. Chow *et al*. (2002) concluded a boundary zone between the two stocks at around 10°N to 2°N and Chow *et al*. (2007) conducted further studies confirming that the boundary between the North Atlantic and South Atlantic at 5°N should be reconsidered. More recently, Smith *et al*. (2015) sampled the North Atlantic, South Atlantic and Mediterranean Sea extensively to study genetic differentiation of 774 individuals, indicating

admixture zones in the North Atlantic and suggesting too that the 5°N management boundary extend to 20°N-25°N from 45°W. It seems that swordfish stock structure between oceans is more complex than the management boundaries indicate.

The weak differentiation observed in the current study may also reflect their very recent divergence along with presumably large effective population sizes (Benestan *et al*., 2015). The amount of time since divergence may be insufficient for a significant genetic structure to be revealed by microsatellites. Significant levels of differentiation at neutral loci may be realized only at the largest geographical scales (O'Reilly *et al*., 2004).

Despite the poor quality of the degraded DNA samples, with resultant smaller sampling size, and the fact that the sampling is now somewhat dated, the results of this study provided evidence for admixture of swordfish between the boundary of the Indian and Atlantic oceans. There is evidence of gene flow and migration in this area in both directions, though the evidence for weak differentiation suggests that the Indian Ocean and Atlantic Ocean contain separate stocks and that swordfish stocks coexist around South Africa but return to their ocean of origin to reproduce. Although the results of this study indicate weak differentiation, it provides preliminary rather than conclusive evidence of admixed stocks around South Africa between, but not exclusive to, 14°E to 27°E. It is, however, sufficient to motivate for the two tuna Regional Fisheries Management Organisations (tRFMOs), ICCAT and IOTC, to begin to reconsider how this stock is managed between the two oceans.

Further investigations into temporal and sex-biased genetic differentiation are needed, including the ecological determinants that drive this differentiation (*e.g.* sea temperature, currents, salinity, food availability) (Banks *et al*., 2007; Selkoe *et al*., 2010; White *et al*., 2010; Benestan *et al*., 2015). The use of modern markers and more comprehensive sampling across this inter-oceanic boundary integrated with local ecological investigations could help to shed more light on the exact ecological process in shaping genetic population structure of swordfish (Benestan *et al*., 2015). Utilising large numbers of SNP markers could improve regional population structure delineation and population assignment success in a context of weak genetic structure (Benestan *et al*., 2015), and this marker could therefore also be considered in future studies in this region.

References

Adkinson, M. D. (1996). Population differentiation in Pacific salmon: local adaptation, genetic drift, or the environment? *Canadian Journal of Fisheries and Aquatic Sciences*, 52, pp. 2762 – 2777.

Aguila, R. D., Perez, S. K. L., Catacutan, B. J. N., Lopez, G. V., Barut, N. C. and Santos, M. D. (2015) Distinct yellowfin tuna (*Thunnus albacares*) stocks detected in Western and Central Pacific Ocean (WCPO) using DNA microsatellites. *PLoS ONE*, 10(9), e0138292.

Albaina A., Iriondo M., Velado I., Laconcha U., Zarraonaindia I., Arrizabalaga H., Pardo M. A., Lutcavage, M., Grant, W.S. and Estonba, A. (2013). Single nucleotide polymorphism discovery in albacore and Atlantic bluefin tuna provides insights into worldwide population structure. *Animal Genetics*, 44, pp. 678 – 692.

Alvarado Bremer, J. R., Baker, A. J. and Mejuto, J. (1995). Mitochondrial DNA control region sequences indicate extensive mixing of swordfish (*Xiphias gladius*) populations in the Atlantic Ocean. *Canadian Journal of Fisheries and Aquatic Sciences*, 52, pp. 1720 – 1732.

Alvarado Bremer, J. R., Mejuto, J., Greig, T. W. and Ely, B. (1996). Global population structure of the swordfish (*Xiphias gladius* L.) as revealed by analysis of the mitochondrial DNA control region. *Journal of Experimental Marine Biology and Ecology*, 197, pp. 295 – 310.

Alvarado Bremer, J. R., Stequert, B., Robertson, N. W. and Ely, B. (1998). Genetic evidence for inter-oceanic subdivision of bigeye tuna (*Thunnus obesus*) populations. *Marine Biology*, 132, pp. 547 - 557.

Alvarado Bremer, J. R., Mejuto, J., Gómez-Márquez, J., Viñas, J. and Boán, F. (1999). Genetic analysis of bigeye tuna population subdivision. *Collective Volume of Scientific Papers ICCAT*, 49, pp. 370 - 373.

Alvarado Bremer, J. R., Mejuto, J., Gómez-Márquez, J., Boán, F., Carpintero, P., Rodríguez, J. M., Viñas, J., Greig, T. W. and Ely, B. (2005). Hierarchical analyses of genetic variation of samples from breeding and feeding grounds confirm the genetic partitioning of Northwest Atlantic and South Atlantic populations of swordfish (*Xiphias gladius* L.). *Journal of Experimental Marine Biology and Ecology*, 327, pp. 167 – 182.

Alvarado Bremer, J. R., Hinton, M. G. and Greig, T. W. (2006). Evidence of spatial genetic heterogeneity in Pacific swordfish (*Xiphias gladius*) revealed by the analysis of *LDH*-A sequences. *Bulletin of Marine Science*, 79, pp. 493 – 503.

Alvarado Bremer, J., Mejuto, J., Gómez-Márquez, J., Pla-Zanuy, C., Viñas, J., Marques, C., Hazín, F., Griffiths, M., Ely, B., García-Cortés, B. and Greig, T. W. (2007). Genetic population structure of Atlantic swordfish: current status and future directions. *Collective Volume of Scientific Papers ICCAT*, 61, pp. 107 – 118.

Amorim, A. F. and Arfelli, C. A. (1980). Reproduccion del pez espada (*Xiphias gladius* L. 1758) en el sudeste y sur del brasil. *Collective Volume of Scientific Papers ICCAT*, 9, pp. 624 - 626.

Amos, W., Hoffman, J. I., Frodsham, A., Zhang, L., Best, S. and Hill, A.V.S. (2007). Automated binning of microsatellite alleles: problems and solutions. *Molecular Ecology Notes*, 7, pp. 10 – 14.

Anderson, J. D. and Karel, W. J. (2014) Limited genetic structure of Gulf Menhaden (*Brevoortia patronus*), as revealed by microsatellite markers developed for the genus *Brevoortia* (Clupeidae). *Fishery Bulletin*, 112, pp. 71 - 81.

Antão, T., Lopes, A., Lopes, R., Beja-Pereira, A. and Luikart, G. (2008). LOSITAN: a workbench to detect molecular adaptation based on a Fst-outlier method. *BMC Bioinformatics*, 9: pp. 323.

Appleyard, S. A., Grewe, P. M., Innes, B. H. and Ward, R. D. (2001). Population structure of yellowfin tuna (*Thunnus albacares*) in the western Pacific Ocean, inferred from microsatellite loci. *Marine Biology*, 139, pp. 383 – 393.

Appleyard, S. A., Ward, R. D. and Grewe, P. M. (2002). Genetic stock structure of bigeye tuna in the Indian Ocean using mitochondrial DNA and microsatellites. *Journal of fish Biology*, 60, pp. 767 – 770.

Árnason, E., Pálsson, S. and Arason, A. (1992). Gene flow and lack of population differentiation in Atlantic cod, *Gadus morhua* L., from Island, and comparison of cod from Norway and Newfoundland. *Journal of Fish Biology*, 40, pp. 751 – 770.

Arocha, F. and Lee, D. W. (1996). Maturity at size, reproductive seasonality, spawning frequency, fecundity and sex ratio in swordfish from the Northwest Atlantic. *Collective Volume of Scientific Papers ICCAT*, 45, pp. 350 - 357.

Avise, J. C. (2000). *Phylogeography: The History and Formation of Species*. Cambridge, MA: Harvard University Press, pp. 447.

Backeberg, B. C., Penven, P. and Rouault, M. (2012). Impact of intensified Indian Ocean winds on mesoscale variability in the Agulhas system. *Nature Climate Change*, 2, pp. 608 – 612.

Banks, M. A., Eichert, W. and Olsen, J. B. (2003). Which genetic loci have greater population assignment power? *Bioinformatics*, 19, pp. 1436 – 1438.

Banks, S. C., Piggott, M. P., Williamson, J. E., Bové, U., Holbrook, N. J. and Beheregaray, L. B. (2007). Oceanic variability and coastal topography shape genetic structure in a long-dispersing sea urchin. *Ecology*, 88(12), pp. 3055–3064.

Barker, A. M., Nosal, A. P., Lewallen, E. A. and Burton, R. S. (2015). Genetic structure of leopard shark (*Triakis semifasciata*) populations along the Pacific coast of North America. *Journal of Experimental Marine Biology and Ecology*, 472, pp. 151 – 157.

Baverstock, P. R. and Moritz, C. (1996). Project design. In: Hillis, D. M., Moritz, C. and Mable, B. K., ed., *Molecular systematics*. 2nd ed. Cambridge: Sinauer Associates, pp. 17 – 27.

Beacham, T. D., Pollard, S. and Le, K. D. (1999). Population structure and stock identification of steelhead in southern British Columbia, Washington, and the Columbia River based on microsatellite DNA variation. *Transactions of the American Fisheries Society*, 128(6), pp. 1068 – 1084.

Beacham, T. D., Brattey, J., Millar, K. M., Withler, R. E. (2002). Multiple stock structure of Atlantic cod (*Gadus morhua*) off Newfoundland and Labrador determined from genetic variation. *ICES Journal of Marine Science*, 54, pp. 650 – 655.

Begg, G. A. and Waldman, J. R. (1999). An holistic approach to fish stock identification. *Fisheries Research*, 43, pp. 35-44.

Belkhir, K., Borsa, P., Chikhi, L., Raufaste N. and Bonhomme, F. (2004). GENETIX 4.02, logiciel sous Windows TM pour la génétique des populations, Laboratoire Génome, Populations, Interactions; CNRS UMR 5000; Université Montpellier II, Montpellier (France).

Benestan, L., Gosselin, T., Perrier, C., Sainte-Marie, B., Rochette, R. and Bernatchez, L. (2015). RAD-genotyping reveals fine-scale genetic structuring and provides powerful population assignment in a widely distributed marine species. *Molecular Ecology*, 24, pp. 3299 – 3315.

Benson, D.A., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J. and Sayers, E.W. (2009). GenBank. *Nucleic Acids Research*, 37, D26 – 31.

Bentzen, P., Taggart, C. T., Ruzzante, D. E. and Cook, D. (1996). Microsatellite polymorphism and the population structure of Atlantic cod (*Gadus morhua*) in the Northwest Atlantic. *Canadian Journal of Fisheries and Aquatic Sciences*, 53, pp. 2706 – 2721.

Bernard, A. M, Shivji, M. S., Prince, E. D., Hazin, F. H. V., Arocha, F., Domingo, A. and Feldheim, K. A. (2014). Comparative population genetics and evolutionary history of two commonly misidentified billfishes of management and conservation concern. *BMC Genetics,* 15, pp. 141.

Billington, N. (2003). Mitochondrial DNA. In: Hallerman, E. M., ed., *Population Genetics- Principles and Applications for Fisheries Scientists*. Bethesda, MD: American Fisheries Society, pp. 59 – 100.

Borsa, P., Blanquer, A. and Berrebi, P. (1997.) Genetic structure of the flounders *Platichthys flesus* and *P. stellatus* at different geographic scales. *Marine Biology*, 129, pp. 233 – 246.

Botstein, D., White, R. L., Skolnick, M. and Davis, R. W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *The American Journal of Human Genetics,* 32, pp. 314 – 331.

Bourjea, J., Lapègue, S., Gagnevin, L., Broderick, D., Mortimer, J.A., Ciccione, S., Roos, D., Taquet, C. and Grizel, H. (2007). Phylogeography of the green turtle, *Chelonia mydas* in the Southwest Indian Ocean. *Molecular Ecology*, 16, pp. 175 – 186.

Boustany, A. M., Reeb, C. A. and Block, B. A. (2008). Mitochondrial DNA and electronic tracking reveal population structure of Atlantic bluefin tuna (*Thunnus thynnus*). *Marine Biology*, 156, pp. 13 – 24.

Bradman, H. M., Grewe, P. M. and Appleton, B. (2011). Direct comparison of mitochondrial markers for the analysis of swordfish stock structure. *Fisheries Research*, 109, pp. 95 – 99.

Brookfield, J. F. Y. (1996). A simple new method for estimating null allele frequency from heterozygote deficiency. *Molecular Ecology*, 5, pp. 4534 – 4555.

Carlsson, J., McDowell, J. R., Díaz-Jaimes, P., Carlsson, J. E. L., Boles, S.B., Gold, J. R. and Graves, J. E. (2004). Microsatellite and mitochondrial DNA analyses of Atlantic bluefin tuna (*Thunnus thynnus thynnus*) population structure in the Mediterranean Sea. *Molecular Ecology,* 13, pp. 3345 – 3356.

Carvalho, G. R., and Hauser, L. (1994). Molecular genetics and the stock concept in fisheries. *Reviews in Fish Biology and Fisheries*, 4, pp. 326 – 350.

Castro, J.A., Picornell, A. and Ramon, M. (1998). Mitochondrial DNA: a tool for populational genetics studies. *International Microbiology*, 1, pp. 327 – 332.

Chakraborty, R., De Andrade, M., Daiger, S. P. and Budowle, B. (1992) Apparent heterozygote deficiencies observed in DNA typing data and their implications in forensic applications. *Annals of Human Genetics*, 56, pp. 45 – 57.

Chen, C., Durand, E., Forbes, F. and François, O. (2007) Bayesian clustering algorithms ascertaining spatial population structure: a new computer program and a comparison study. *Molecular Ecology Notes*, 7, pp. 747 – 756.

Cheng, J., Yanagimoto, T., Song, N. and Gao, T. (2015). Population genetic structure of chub mackerel *Scomber japonicas* in the Northwestern Pacific inferred from microsatellite analysis. *Molecular Biology Reports*, 42, pp. 373 – 382.

Chiang, H. C., Hsu, C. C., Lin, H. D., Ma, G. C., Chiang, T. Y. and His, Y. Y. (2006). Population structure of bigeye tuna (*Thunnus obesus*) in the South China Sea, Philippine Sea and Western Pacific Ocean inferred from mitochondrial DNA. *Fisheries Research*, 79, pp. 219 – 225.

Chiang, H. C., Hsu, C. C., Wu, G. C. C., Chang, S. K. and Yang, H. Y. (2008). Population structure of bigeye tuna (*Thunnus obesus*) in the Indian Ocean inferred from mitochondrial DNA. *Fisheries Research*, 90, pp. 305 – 312.

Chow, S., Okamoto, H., Uozumi, Y., Takeuchi, Y. and Takeyama, H. (1997). Genetic stock structure of the swordfish (*Xiphias gladius*) inferred by PCR-RFLP analysis of the mitochondrial DNA control region. *Marine Biology*, 127, pp. 359 – 367.

Chow, S., Okamoto, H., Miyabe, N., Hiramatsu, K. and Barut, N. (2000). Genetic divergence between Atlantic and Indo-Pacific stocks of bigeye tuna (*Thunnus obesus*) and admixture around South Africa. *Molecular Ecology*, 9, pp. 221 – 227.

Chow, S. and Takeyama, H., (2000). Nuclear and mitochondrial DNA analyses reveal four genetically separated breeding units of the swordfish. *Journal of Fish Biology*, 56(5), pp. 1087 – 1098.

Chow, S., Nohara, K., and Takeuchi, Y. (2002). Boundary between North and South Atlantic stocks of swordfish (*Xiphias gladius*): an implication from nuclear DNA analysis. *Collective Volume of Scientific Papers ICCAT*, 54, pp. 1544 – 1546.

Chow, S., Clarke, S., Nakadate, M., and Okazaki, M. (2007). Boundary between the North and South Atlantic populations of the swordfish (*Xiphias gladius*) inferred by a single nucleotide polymorphism at calmodulin gene intron. *Marine Biology*, 152, pp. 87 - 93.

Çiftci, Y. and Okumuş, I. (2002). Fish population genetics and applications of molecular markers to fisheries and aquaculture: I- Basic Principles of Fish Population Genetics. *Turkish Journal of Fisheries and Aquatic Sciences*, 2, pp. 145 – 155.

Clark, T. B., Ma, L., Saillant, E. and Gold, J. R. (2004). Microsatellite DNA markers for population-genetic studies of Atlantic bluefin tuna (*Thunnus thynnus thynnus*) and other species of genus *Thunnus*. *Molecular Ecology Notes*, 4, pp. 70 – 73.

Coates, A. G., Jackson, J. B. C., Collins, L. S., Cronin, T. M., Dowsett, H .J., Bybell, L. M., Jung, P. and Obando, J. A. (1992). Closure of the Isthmus of Panama: the near-shore marine record of Costa Rica and western Panama. *Geological Society of America Bulletin,* 104, pp. 814 – 828.

Cornuet, J. M., Piry, S., Luikart, G., Estoup, A. and Solignac, M. (1999). New methods employing multilocus genotypes to select or exclude populations as origins of individuals. *Genetics*, 153, pp. 1989 – 2000.

Dammannagoda, S. T., Hurwood, D. A., and Mather, P. B. (2008). Evidence for fine geographical scale heterogeneity in gene frequencies in yellowfin tuna (*Thunnus albacares*) from the north Indian Ocean around Sri Lanka. *Fisheries Research*, 90, pp. 147 – 157.

Dammannagoda, S. T., Hurwood, D. A. and Mather, P. B. (2011). Genetic analysis reveals two stocks of skipjack tuna (*Katsuwonus pelamis*) in the north western Indian Ocean. *Canadian Journal of Fisheries and Aquatic Sciences*, 68(2), pp. 210 – 223.

Davies, C. A., Gosling, E. M., Was, A., Brophy, D. and Tysklind, N. (2011). Microsatellite analysis of albacore tuna (*Thunnus alalunga*): population genetic structure in the North-East Atlantic Ocean and Mediterranean Sea. *Marine Biology*, 158, pp. 2727 – 2740.

De Innocentiis, S., Sola, L., Cataudella, S. and Bentzen, P. (2001). Allozyme and microsatellite loci provide discordant estimates of population differentiation in the endangered dusky grouper (*Epinephelus marginatus*) within the Mediterranean Sea. *Molecular Ecology*, 10, pp. 2163 – 2175.

de Ruijter, W. P. M., Biastoch, A., Drijfhout, S. S., Lutjeharms, J. R. E., Matano, R. P., Pichevin, T., van Leeuwen, P. J., and Weijer, W. (1999). Indian-atlantic interocean exchange: Dynamics, estimation and impact. *Journal of Geophysical Research*, 104, pp. 20 885–20 910.

De Woody, J. A. and Avise, J. C. (2000). Microsatellite variation in marine, freshwater, and anadromous fishes compared with other animals. *Journal of Fish Biology*, 56, pp. 461 – 473.

Diaz-Jaimes, P., Uribe-Alcocer, M., Ortega-García, S. and Durand, J.D. (2006). Spatial and temporal mitochondrial DNA genetic homogenetiy in dolphinfish populations (*Coryphaena hippurus*) in the eastern central Pacific. *Fisheries Research*, 80, pp. 333 – 338.

Do, C., Waples, R. S., Peel, D., Macbeth, G. M., Tillett, B. J. and Ovenden, J. R. (2014). NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (N_E) from genetic data. *Molecular Ecology Resources*, 14, pp. 209 – 214.

Donohue, K. A., Firing, E. and Beal, L. (2000). Comparison of three velocity sections of the Agulhas Current and Agulhas Undercurrent. *Journal of Geophysical Research*, 105(12), pp. 28 585 – 28 593.

Durand, J. D., Collet, A., Chow, S., Guinand, B. and Borsa, P. (2005). Nuclear and mitochondrial DNA markers indicated unidirectional gene flow of Indo-Pacific to Atlantic bigeye tuna (*Thunnus obesus*) populations, and their admixture off southern Africa. *Marine Biology*, 147, pp. 313 – 322.

Durand, E., Jay, F., Gaggiotti, O. E. and François, O. (2009). Spatial inference of admixture proportions and secondary contact zones, *Molecular Biology and Evolution,* 26, 1963 – 1973.

Duncan, K. M., Martin, A. P., Bowen, B. W. and De Couet, H. G. (2006). Global phylogeography of the scalloped hammerhead shark (*Sphyrna lewini*). *Molecular Ecology*, 15, pp. 2239 – 2251.

Earl, D. A. and von Holdt, B. M. (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*. 4, pp. 359 – 361.

Edwards, A. W. F. (2008). G. H. Hardy (1908) and Hardy-Weinberg Equilibrium. *Genetics*, 179, pp. 1743 – 1150.

Ellegren, H. (2004). *Microsatellites: simple sequences with complex evolution. Nature Reviews Genetics*, 5, pp. 435 – 445.

Elliott, N. G. and Ward, R. D. (1992). Enzyme variation in orange roughy (*Hoplostethus atlanticus*) samples from southern Australian and New Zealand waters. *Australian Journal of Marine and Freshwater Research*, 45, pp. 51 – 67.

Ely, B., Viñas, J., Alvarado Bremer, J., Jaime, R., Black, D., Lucas, L., Covello, K., Labrie, A. and Thelen, E. (2005). Consequences of the historical demography on the global population structure of two highly migratory cosmopolitan marine fishes: the yellowfin tuna (*Thunnus albacares*) and the skipjack tuna (*Katsuwonus pelamis*). *BMC Evolutionary Biology*, 5, p. 19.

Evanno, G., Regnaut, S. and Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, 14, pp. 2611 – 2620.

Excoffier, L., Smouse, P. E., Quattro, J. M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, 131, pp. 479–491.

Excoffier, L. and Lischer, H. E. L. (2010). Arlequin suite ver 3.5.2.1: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, 10, pp. 564 – 567.

Fabbri, E., Caniglia, R., Mucci, N., Thomsen, H. P., Krag, K., Pertoldi, C., Loeschcke, V. and Randi, E. (2012). Comparison of single nucleotide polymorphisms and microsatellites in non-invasive genetic monitoring of a wolf population. *Archives of Biological Science Belgrade*, 64, pp. 321 – 335.

Falush, D., Stephens, M. and Pritchard, J. K. (2003). Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*, 164, pp. 1567 – 1587.

Farnham, T. T. (2003). Genetic variation in Atlantic yellowfin tuna (*Thunnus albacares*) to assess stock structure and reproductive variance. Unpublished Master of Science Thesis. Texas A&M University.

Feldheim, K. A., Gruber, S. H. and Ashley, M. V. (2001). Population genetic structure of the lemon shark (*Negaprion brevirostris*) in the western Atlantic: DNA microsatellite variation. *Molecular Ecology*, 10, pp. 295 – 303.

Feron, R. C. V., De Ruijter, P. M. and Oskam, D. (1992). Ring shedding in the Agulhas Current System. *Journal of Geophysical Research*, 97(C6), pp. 9 467 – 9 477.

Garcia, A., Cecchetti, S., Santos, M. N., Mattiucci, S., Nascetti, G. and Cimmaruta, R. (2011). Population structure of Atlantic Swordfish (*Xiphias gladius* L. 1758) (Teleostea, Xiphiidae) using mitochondrial DNA analysis: implications for fisheries management. *Animal Biodiversity and Conservation,* 34, pp. 133 – 140.

García-Cortés, B., Mejuto, J. and Quintans, M. (2003). Summary of swordfish (*Xiphias gladius*) recaptures carried out by the Spanish surface longline fleet in the Atlantic Ocean: 1984-2002. *Collective Volume of Scientific Papers ICCAT*, 55(4), pp. 1476 – 1484.

Gillespie, J. H. (1998). Population Genetics: A Concise Guide. Baltimore: The Johns Hopkins University Press.

Gjøsæter. J., Jørstad, K. E., Nævdal, G. and Thorkildsen, S. (1992). Genotype distributions of cod from the Norwegian Skagerrak coast. *Sarsia*, 76, pp. 225 – 259.

Gold, J. R., King, T. L., Richardson, L. R., Bohlmeyer, D. H. and Matlock, G. C. (1994). Genetic studies in marine fishes. VII: allozyme differentiation within and between red drum (*Sciaenops ocellatus*) from the Gulf of Mexico and Atlantic Ocean. *Journal of Fish Biology*, 116, pp. 517 – 185.

González, *E.G.*, Beerli, P. and Zardoya, R. (2008). Genetic structuring and migration patterns of Atlantic bigeye tuna, *Thunnus obesus* (Lowe, 1839). *BMC Evolutionary Biology*, 8, pp. 252.

Goudet, J. (2001). FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3.2). Available at: http: //www2.unil.ch/popgen/softwares/fstat.htm [Accessed 1st February 2016].

Govender, A., van der Elst, R. and James, N. (2003). Swordfish. Global lessons. 1st ed. [pdf] South Africa: WWF-South Africa. Available at: http://hdl.handle.net/1834/921 [Accessed 1 February 2016].

Graves, J. E. and McDowell, J. R. (2001). A gentic perspective on the stock structures of blue marlin and white marlin in the Atlantic Ocean. *Collective Volume of Scientific Papers ICCAT*, 53, pp. 180 – 187.

Graves, J. E. and McDowell, J. R. (2003). Stock structure of the worlds Istiophorid billfishes; a genetic perspective. *Marine and Freshwater Research*, 54, pp. 287 – 298.

Graves, J. E. and McDowell, J. R. (2006). Genetic analysis of white marlin (*Tetrapturus albidus*) stock structure. *Bulletin of Marine Science*, 79, pp. 469 – 482.

Greig, T. W., Alvarado Bremer, J. R. and Ely, B. (1999). Preliminary results from genetic analyses of nuclear markers in swordfish, *Xiphias gladius*, reveal concordance with mitochondrial DNA analysis. *Collective Volume of Scientific Papers ICCAT*, 49, pp. 476 – 482.

Grewe, P. (1997). An assessment of bigeye (*Thunnus obesus*) population structure in the Pacific Ocean, based on Mitochondrial DNA and DNA microsatellites analysis. Working paper for the 7th Meeting of the Western Pacific Yellowfin Tuna Research Group, Nadi, Fiji, June 18 – 20.

Grijalva-Chon, J. M., Numachi, K., Sosa-Nishizaki, O. and de la Rosa-Velez, J. (1994). Mitochondrial DNA analysis of North Pacific swordfish *Xiphias gladius* population structure. *Marine Ecology Progress Series*, 115, pp. 15 – 19.

Hartl, D.L. and Clark, A.G. (1997). Principles of Population Genetics, 3rd ed. Sinauer Associates, Inc, Sunderland, MA.

Hellberg, M. E. (2009). Gene flow and isolation among populations of marine animals. *Annual Review of Ecology Evolution and Systematics*, 40, pp 291 – 310.

Herráeza, D. L., Schäfer, H., Mosner, J., Fries, H. R. and Wink, M. (2005). Comparison of microsatellite and single nucleotide polymorphism markers for the genetic analysis of a Galloway cattle population. *Zeitschrift für Naturforschung C*, 60, pp. 637 – 643.

Hinton, M. G., and Alvarado Bremer, J. (2007). Stock structure of swordfish in the Pacific Ocean. IATTC Working Group to Review Stock Assessments, 8th Meeting, La Jolla, CA, 7 – 11 May 2007. *Document SAR-8-11.*

Holdsworth, J. C., Sippel, T. J., and Paul, P. J. (2007). An investigation into swordfish stock structure using satellite tag and release methods. Biology Specialist Working Group Paper *WCPFC-SC3-2007/BI WP-3* [Accessed 15 February 2016].

Hubisz, M.J., Falush, D., Stephens, M. and Pritchard, J.K. (2009). Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources*, 9, pp. 1322 – 1332.

ICCAT (2013). Report of the Standing Committee on Research and Statistics (SCRS). [online] Madrid, Spain: ICCAT. Available at: http://www.iccat.es/en/meetings.asp [Accessed 1 February 2016].

IOTC (2013). Report of the Sixteenth Session of the IOTC Scientific Committee. [online] Busan, Republic of Korea: IOTC. Available at: http://www.iotc.org/meetings/16th-session-scientific-committee [Accessed 1 February 2016].

Jakobsson, M. and Rosenberg, N. A. (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*, 23, pp. 1801 – 1806.

Jarne, P. and Lagoda, P. J. L. (1996). Microsatellites, from molecules to populations and back. *Trends in Ecology and Evolution*, 11, pp. 424 – 429.

Jean, C., Bourjea, J., Jouen, E. and Taquet, M. (2006). Stock structure of the swordfish (*Xiphias gladius*) in the Southwest Indian Ocean: a preliminary study. *Bulletin of Marine Science*, 79, pp. 521 – 526.

Kai, E. T. and Marsac, F. (2010). Influence of mesoscale eddies on spatial structuring of top predators' communities in the Mozambique Channel. *Progress in Oceanography*, 86, pp. 214 – 223.

Kalinowski, S. T., Taper, M. L. and Marshall, T. C. (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, 16, pp. 1099 – 1106.

Karlsson, S. and Mork, J. (2005). Deviations from Hardy –Weinberg equilibrium, and temporal instability in allele frequencies at microsatellite loci in a local population of Atlantic cod. *ICES Journal of Marine Science*, 62, pp. 1588 –1596.

Kasapidis, P., Mejuto, J., Tserpes, G., Antoniou, A., García-Cortés, B., Peristeraki, P., Oikonomaki, K., Kotoulas, G. and Magoulas, A. (2006). Genetic structure of the swordfish (*Xiphias gladius*) stocks in the Atlantic using microsatellite DNA analysis. *Collective Volume of Scientific Papers ICCAT*, 61, pp. 89 – 98.

Kasapidis, P., Magoulas, A., García-Cortés, B., and Mejuto, J. (2008). Stock structure of swordfish (*Xiphias gladius*) in the Pacific Ocean using microsatellite DNA markers. Biology Specialist Working Group Paper *WCPFC-SC4-2008/BI WP-4*. [Accessed 15 February 2016].

Kasapidis, P., Pakaki, V., Kotoulas, G. and Magoulas, A. (2009). Isolation and characterization of 18 new polymorphic microsatellite loci for the swordfish, *Xiphias gladius*. *Molecular Ecology Resources*. 9, pp. 1383 – 1386.

Keigwin, L. D. (1982). Isotopic paleoceanography of the Caribbean and east Pacific: Role of Panama uplift in Late Neogene time. *Science,* 217, pp. 350 – 352.

Knutsen, H., Jorde, P. E., Andre, C. and Stenseth, C. H. R. (2003). Fine-scaled geographical population structuring in a highly mobile marine species: the Atlantic cod. *Molecular Ecology*, 12, pp. 385 – 394.

Kotoulas, G., Mejuto, J., Tserpes, G., García-Cortés, B., Peristeraki, P., De la Serna, J. M. and Magoulas, A. (2003). DNA microsatellite markers in service of swordfish stock-structure analysis in the Atlantic and Mediterranean. *Collective Volume of Scientific Papers ICCAT*, 55, 1632 – 1639.

Kotoulas, G., Mejuto, J., Antoniou, A., Kasapidis, P., Tserpes, G., Piccinetti, C., Peristeraki, P., García-Cortés, B., Oikonomaki, K., De la Serna, J. M. and Magoulas, A. (2006). Global genetic structure of swordfish (*Xiphias gladius*) as revealed by microsatellite DNA markers. *Collective Volume of Scientific Papers ICCAT*, 61, 79 – 88.

Kunal, S. P., Kumar, G., Menezes, M. R. and Meena, R. M. (2013). Mitochondrial DNA analysis reveals three stocks of yellowfin tuna *Thunnus albacares* (Bonnaterre, 1788) in Indian waters. *Conservation Genetics*, 14, pp. 205 – 213.

Laconcha, U., Iriondo, M., Arrizabalaga, H., Manzano, C., Markaide, P., Montes I, Zarraonaindia, I., Velado, I., Bilbao, E., Goni, N., Santiago, J., Domingo, A., Karakulak, S., Oray, I. and Estonba, A. (2015). New nuclear SNP markers unravel the genetic structure and effective population size of albacore tuna (*Thunnus alalunga*). *PLoS ONE*, 10: e0128247.

Latch, E. K., Dharmarajan, G., Glaubitz, J. C. and Rhodes, O. E. (2006). Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. *Conservation Genetics*, 7, pp. 295 – 302.

Lessios, H. A. and Weinberg, J. R. (1993). Migration, gene flow and reproductive isolation between and within morphotypes of the isopod *Excirolana* in two oceans. *Heredity*, 71, pp. 561 – 573.

Li, Y., Bence, J. R. and Brenden, T. O. (2015). An evaluation of alternative assessment approaches for intermixing fish populations: a case study with Great Lakes lake whitefish. *ICES Journal of Marine Science*, 72, pp. 70 – 81

Li, W., Chen, X., Xu, Q., Zhu, J., Dai, X. and Xu, L. (2015) (1). Genetic population structure of *Thunnus albacares* in the central Pacific Ocean based on mtDNA COI gene sequences. *Biochemical Genetics*, 53, pp. 8 – 22.

Lu, C. P., Chen, C. A., Hui, C. F., Tzeng, T. D. and Yeh, S. Y. (2006). Population genetic structure of the swordfish, *Xiphias gladius* (Linnaeus, 1758), in the Indian Ocean and West Pacific inferred from the complete DNA sequence of the mitochondrial control region. *Zoological Studies*, 45, pp. 269 –279.

Lundy, C. J., Rico, C. and Hewitt, G. (2000). Temporal and spatial genetic variation in spawning grounds of European hake (*Merluccius merluccius*) in the Bay of Biscay. *Molecular Ecology*, 9, pp. 2067 – 2079.

Luschi, P., Lutjeharms, J. R. E., Lambardia, P., Mencacci, R., Hughes, G. R. and Hays, G. C. (2006). A review of migratory behaviour of sea turtles off Southeastern Africa. *South African Journal of Science*, 102, pp. 51 – 58.

Magoulas, A., Kotoulas, G., de la Serna, J. M., De Metrio, G., Tsimenides, N. and Zouros, E. (1993). Genetic structure of swordfish (*Xiphias gladius*) populations of the Mediterranean and the eastern side of the Atlantic: analysis by mitochondrial DNA markers. *Collective Volume of Scientific Papers ICCAT*, 40, 126 – 136.

Magoulas, A., Castillho, R., Caetano, S., Marcato, S. and Patarnello, T. (2006). Mitochondrial DNA reveals a mosaic pattern of phylogeographical structure in Atlantic and Mediterranean populations of anchovy (*Engraulis encrasicolus*). *Molecular Phylogenetics and Evolution*, 39, pp. 734 – 746.

Manel, S., Gaggiotti, O. E. and Waples, R. S. (2005). Assignment methods: Matching biological questions with appropriate techniques. *Trends in Ecology and Evolution*. 20, pp. 136 – 142.

May, B. and Krueger, C. C. (1990). Use of allozyme data for population analysis. In: Whitmore, D.H., ed., *Electrophoretic and Isoelectric Focusing Techniques in Fisheries Management*. Boston: CRC Press, pp. 157 – 171.

McDowell, J. R. and Graves, J. E. (2008). Population structure of striped marlin (*Kajikia audax*) in the Pacific Ocean based on analysis of microsatellite and mitochondrial DNA. *Canadian Journal of Fisheries and Aquatic Sciences*, 65, pp. 1307 – 1320.

McPherson, A. A., O'Reilly, P. T., McParland, T. L., Jones, M. W. and Bentzen, P. (2001). Isolation of nine novel tetranucleotide microsatellites in Atlantic herring (*Clupea harengus*). *Molecular Ecology Notes,* 1, pp. 31 – 32.

McQuinn, I. H. (1997). Metapopulations and the Atlantic herring. *Reviews in Fish Biology and Fisheries*, 7, pp. 297 – 329.

Menezes, M. R., Noguchi, D., Nakajima, M. and Taniguchi, M. (2008). Microsatellite development and survey of genetic variation in skipjack tuna *Katsuwonus pelamis*. *Journal of Fish Biology*, 73, pp. 463 – 473.

Montes, I., Iriondo, M., Manzano, C., Arrizabalaga, H., Jiménez, E., Pardo, M. A., Goñi, N., Davies, C. A. and Estonba, A. (2012). Worldwide genetic structure of albacore (*Thunnus alalunga*) revealed by microsatellite DNA markers. *Marine Ecology Progress Series*, 471, pp. 183 – 191.

Morin, P. A., Archer, F. I., Pease, V. L., Hancock-Hanser, B. L., Robertson, K. M., Huebinger, R. M., Martien, K. K., Bickham, J. W., George, J. C., Postma, L. D. and Taylor, B. L. (2012). Empirical comparison of single nucleotide polymorphisms and microsatellites for population and demographic analyses of bowhead whales. *Endangered Species Researc*h, 19, pp. 129 – 147.

Mork, J., Ryman, N., Ståhl, G., Utter, F., and Sundnes, G. (1985). Genetic variation in Atlantic cod (*Gadus morhua*) throughout its range. *Canadian Journal of Fisheries and Aquatic Sciences*, 42, pp. 1580 – 1587.

Muths, D., Grewe, P., Jean, C. and Bourjea, J. (2009). Genetic population structure of the Swordfish (*Xiphias gladius*) in the southwest Indian Ocean: sex-biased differentiation, congruency between markers and its incidence in a way of stock assessment. *Fisheries Research*, 97, pp. 263 – 269.

Muths, D., Le Couls, S., Evano, H., Grewe, P. and Bourjea, J. (2013). Multi-genetic marker approach and spatio-temporal analysis suggest there is a single panmictic population of Swordfish *Xiphias gladius* in the Indian Ocean. *PloS ONE*, 8: e63558.

Nakadate, M., Viñas, J., Corriero, A., Clarke, S., Suzuki, N. and Chow, S. (2005). Genetic isolation between Atlantic and Mediterranean albacore populations inferred from mitochondrial and nuclear DNA markers. *Journal of Fish Biology*, 66, pp. 1545 – 1557.

Nakamura, I. (1985). FAO species catalogue. Vol 5. Billfishes of the world. An annotated and illustrated catalogue of marlins, sailfishes, spearfishes and swordfishes known to date. FAO fisheries symposium. 5, pp. 65.

Nei, M. (1972). Genetic distance between populations. *American Naturalist*, 106, pp. 283 – 291.

Neilson, J. D., Paul, S. D. and Smith, S. C. (2007). Stock structure of swordfish (*Xiphias gladius*) in the Atlantic: a review of the non-genetic evidence. *Collective Volume of Scientific Papers ICCAT*, 61, pp. 25 – 60.

Neilson, J., Arocha, F., Calay, S., Mejuto, J., Ortiz, M., Scott, G., Smith, C., Travassos, P., Tserpes, G. and Andrushchenko, I. (2013). The recovery of Atlantic swordfish: the comparative roles of the regional fisheries management organization and species biology. *Reviews in Fisheries Science*, 21, pp. 59 – 57.

Nesbø, C. L., Rueness, E. K., Iversen, S. A., Skagen, D. W. and Jakobsen, K. S. (2000). Phylogeography and population history of Atlantic mackerel (*Scomber scombrus* L.): a genealogical approach reveals genetic structuring among the eastern Atlantic stocks. *Proceedings of the Royal Society of London B: Biological*. 267, pp. 281 – 292.

Nomura, S., Kobayash,i T., Agawa, Y., Margulies, D., Scholey, V., Sawada, Y. and Yagishita, N. (2014) Genetic population structure of the Pacific bluefin tuna *Thunnus orientalis* and the yellowfin tuna *Thunnus albacares* in the North Pacific Ocean. *Fisheries Science*, 80, pp. 1193 – 1204.

O'Connell, M. and Wright, J. M. (1997). Microsatellite DNA in fishes. *Reviews in Fish Biology and Fisheries*, 7, pp. 331 – 363.

O'Leary, S. J., Hice, L. A., Feldheim, K. A., Frisk, M. G., McElroy, A. E., Fast, M. D. and Chapman, D. D. (2013) Severe inbreeding and small effective number of breeders in a formerly abundant marine fish. *PLoS One,* 8, e66126.

O'Reilly, P. and Wright, J. M. (1995). The evolving technology of DNA fingerprinting and its application to fisheries and aquaculture. *Journal of Fish. Biology*, 47, pp. 29 – 55.

O'Reilly, P. T., Canino, M. F., Bailey, K. M. and Bentzen, P. (2004) Inverse relationship between f-st and microsatellite polymorphism in the marine fish, walleye pollock (*Theragra chalcogramma*): implications for resolving weak population structure. *Molecular Ecology*, 13, pp. 1799 – 1814.

Okumuş, I. and Çiftci, Y. (2003). Fish population genetics and molecular markers: II- Molecular markers and their applications in fisheries and aquaculture. *Turkish Journal of Fisheries and Aquatic Sciences,* 3, pp. 51 – 79.

Paetkau, D., Slade, R., Burden, M. and Estoup, A. (2004). Direct, real-time estimation of migration rate using assignment methods: a simulation-based exploration of accuracy and power. *Molecular Ecology*, 13, pp. 55 – 65.

Patarnello, T., Volckaert, F. A. and Castilho, R. (2007). Pillars of Hercules: is the Atlantic–Mediterranean transition a phylogeographical break? *Molecular Ecology*, 16, pp. 4426 – 4444.

Pemberton, J. M., Slate, J., Bancroft, D. R. and Barrett, J. A. (1995). Nonamplifying alleles at microsatellite loci: a caution for parentage and population studies. *Molecular Ecology*, 4, pp. 249 – 252.

Piry, S., Alapetite, A., Cornuet, J. M., Paetkau, D., Baudouin, L. and Estoup, A. (2004). GeneClass2: a software for genetic assignment and first generation migrants detection. *Journal of Heredity*, 95, pp. 536 – 539.

Pla, C., Pujolar, J. M. and Viñas, J. (1998). Population genetics and stock structure of large pelagic species (*Thunnus thynnus, Thunnus alalunga, Xiphias gladius* and *Sarda sarda*) in the Mediterranean. *Collective Volume of Scientific Papers ICCAT*, 50, pp. 209 – 212.

Pogson, G. H., Mesa, K. H. and Boutilier, R. G. (1995). Genetic population structure and gene flow in the Atlantic cod *Gadus morhua*: a comparison of allozyme and nuclear RFLP loci. *Genetics*, 139, pp. 375 − 385.

Pogson, G. H. (2001). Nucleotide polymorphism and natural selection at the pantophysin (Pan I) locus in the Atlantic cod, *Gadus morhua* (L.). *Genetics*, 157, pp. 317 − 330.

Pritchard, J. K., Stephens, M. and Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, 155, pp. 945 – 959.

Pritchard, J. and Wen, W. (2004). Department of Human Genetics, University of Chicago, 920 E 58th st, CLCS 507, Chicago IL 60637, USA.

Provine, W. B. (2004). Ernst Mayr: Genetics and speciation. *Genetics*, 167, 1041 – 1046.

Pujolar, J. M., Roldán, M. I. and Pla, C. (2003). Genetic analysis of tuna populations, *Thunnus thynnus thynnus* and *T. alalunga*. *Marine Biology*, 143, pp. 613 – 621.

Puncher, G. N., Arrizabalaga, H., Alemany F., Cariani A., Oray I. K., Karakulak F. S., Basilone, G., Cuttutta, A., Mazzola, S., and Tinti, F. (2015). Molecular identification of Atlantic bluefin tuna (*Thunnus thynnus*, Scombridae) larvae and development of a DNA character-based identification key for Mediterranean scombrids. *PLoS ONE*, 10, e0130407.

Purcell, C. M., and Edmands, S. (2011). Resolving the genetic structure of striped marlin, *Kajikia audax*, in the Pacific Ocean through spatial and temporal sampling of adult and immature fish. *Canadian Journal of Fisheries and Aquatic Sciences*, 68, pp. 1861 – 1875.

Rannala, B. and Mountain, J. L. (1997). Detecting immigration by using multilocus genotypes. *Proceedings of the National Academy of Sciences USA*, 94, pp. 9197 – 9201.

Raymond, M. and Rousset, F. (1995). GENEPOP (version 4.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, 86, pp. 248 – 249.

Reeb, C. and Block, B. A. (1997). The usefulness of mitochondrial DNA studies to define management units of the swordfish, Xiphias gladius: a review of current literature. *Collective Volume of Scientific Papers ICCAT*, 46, pp. 390 – 392.

Reeb, C. A., Arcangeli, L. and Block, B. A. (2000). Structure and migration corridors in Pacific populations of the swordfish *Xiphius gladius*, as inferred through analyses of mitochondrial DNA. *Marine Biology*, 136, 1123 – 1131.

Reeb, C., Arcangeli, L., and Block, B. (2003). Development of 11 microsatellite loci for population studies in the swordfish, *Xiphias gladius* (Teleostei: Scombridae). *Molecular Ecology Notes*, 3, pp. 147 – 149.

Riccioni, G., Stagioni, M., Landi, M., Ferrara, G., Barbujani G, and Tinti, F. (2013). Genetic structure of bluefin tuna in the Mediterranean Sea correlates with environmental variables. *PLoS ONE,* 8, e80105.

Rice, W. (1989). Analyzing tables of statistical tests. *Evolution*, 43, pp. 223 – 225.

Richardson, P. L. (2007). Agulhas leakage into the Atlantic estimated with subsurface floats and surface drifters. *Deep Sea Research Part I: Oceanographic Research Papers*, 54(8), pp. 1361 – 1389.

Rosel, P. E., and Block, B. A. (1996). Mitochondrial control region variability and global population structure in the swordfish *Xiphias gladius*. *Marine Biology*, 125, pp. 11 – 22.

Rosenberg, N. A. (2007). DISTRUCT: a program for the graphical display of population structure. Rosenberg lab at Stanford University. Available at: http://www.stanford.edu/group/rosenberglab/distruct.html [Accessed 1st February 2016].

Rousset, F. (2008). Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. *Molecular Ecology Resources*, 8, pp. 103 – 106.

Ruzzante, D. E., Taggart, C. T. and Cook, D. (1998). A nuclear DNA basis for shelf- and bank- scale population structure in northwest Atlantic cod (*Gadus morhua*): Labrador to Georges bank. *Molecular Ecology,* 7, pp. 1633 – 1680.

Ruzzante, D. E., Taggart, C. T. and Cook, D. (1999). A review of evidence for genetic structure of cod (*Gadus morhua*) populations in the NW Atlantic and population affinities of larval cod off Newfoundland and the Gulf of St. Lawrence. *Fisheries Research*, 43, pp. 79 – 97.

Saghai-Maroof, M. A., Soliman, K. M., Jorgensen, R. A. and Allard, R. W. (1984). Ribosomal DNAsepacer-length polymorphism in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proceedings of the National Academy of Sciences*, 81, pp. 8014 – 8019.

Saxton, B. L. (2009). Historical demography and genetic population structure of the blackfin tuna (*Thunnus atlanticus*) from the Northwest Atlantic Ocean and the Gulf of Mexico. Unpublished Master of Science Thesis. Texas A&M University.

Schneider, S., Roessli, D. and Excoffier, L. (2000). Arlequin: a software for population genetics data analysis. User manual ver 2.000. Ver. 2.000. Geneva: Genetics and Biometry Lab, Deptartment of Anthropology, University of Geneva.

Schrey, A. W. and Heist, E. J. (2003). Microsatellite analysis of population structure in the shortfin mako (*Isurus oxyrinchus)*. *Canadian Journal of Fisheries Aquatic Sciences*, 60, pp. 670 – 675.

Schrey, A. W. and Heist, E. J. (2007). Stock structure of pallid sturgeon analyzed with microsatellite loci. *Journal of Applied Ichthyology*, 23, pp. 297 – 303.

Shriver, M. D., Jin, L., Ferrell, R. E. and Deka, R. (1997). Microsatellite data support an early population expansion in Africa. *Genome Research*, 7, pp. 586 – 591.

Scoles, D. R. and Graves, J. E. (1993). Genetic analysis of the population structure of yellowfin tuna, *Thunnus albacares*, from the Pacific Ocean. *Fishery Bulletin*, 9(4), pp. 690 – 698.

Selkoe, K. A. and Toonen, R. J. (2006) Microsatellites for ecologists: A practical guide to using and evaluating microsatellite markers. *Ecological Letters*, 9, pp. 615 – 629.

Selkoe, K. A., Watson, J. R., White, C., Horin, T. B., Iacchei, M., Mitarai, S., Siegel, D. A., Gaines, S. D. and Toonen, R. J. (2010). Taking the chaos out of genetic patchiness: seascape genetics reveals ecological and oceanographic drivers of genetic patterns in three temperate reef species. *Molecular Ecology*, 19, pp. 3708 – 3726.

Shaklee, J. B. and Bentzen ,P. (1998) Genetic identification of stocks of marine fish and shellfish. *Bulletin of Marine Science*, 62, pp. 589 – 621.

Sharp, G. D. (1978). Behavioural and physiological properties of tunas and their effects on vulnerability to fishing gear. In: Sharp, G.D. and Dizon, A.E., ed., *The Physiological Ecology of Tunas*. New York: Academic Press, pp. 397 – 449.

Shaw, P. W., Turan, C., Wright, J. M., O'Connell, M. and Carvalho, G. R. (1999). Microsatellite DNA analysis of population structure in Atlantic herring (*Clupea harengus*), with direct comparison to allozyme and mtDNA RFLP analyses. *Heredity*, 83, pp. 490 – 499.

Simon, M. H., Arthus, K. L., Hall, I. R., Peeters, F. J. C., Loveday, B. R., Barker, S., Ziegler, M. and Zahn, R. (2013). Millennial-scale Agulhas current variability and its implications for salt-leakage through the Indian-Atlantic Ocean Gateway. *Earth and Planetary Science Letters*, 383, pp. 101-112.

Smith, B. L. and Alvarado Bremer, J. R. (2010). Inferring population admixture with multiple nuclear genetic markers and bayesian genetic clustering in Atlantic swordfish (*Xiphias gladius*). *Collective Volume of Scientific Papers ICCAT*, 65, pp. 185 – 190.

Smith, B. L., Lu, C-P., García-Cortés, B., Viñas, J., Yeh, S-Y. and Alvarado Bremer, J. R. (2015). Multilocus bayesian estimates of intra-oceanic genetic differentiation, connectivity, and admixture in Atlantic swordfish (*Xiphias gladius* L.). *PLoS ONE*, 10: e0127979.

Sorenson, L., McDowell, J. R., Knot, T. and Graves, J. E. (2013). Assignment test method using hypervariable markers for blue marlin (*Makaira nigricans*) stock identification. *Conservation Genetics Resources*, 5, pp. 293 – 297.

Takagi, M., Okamura, T., Chow, S. and Taniguchi, N. (2001). Preliminary study of albacore (*Thunnus alalunga*) stock differentiation inferred from microsatellite DNA analysis. *Fishery Bulletin*, 99, pp. 697 – 701.

Tserpes, G. and Tsimenides, N. (1995). Determination of age and growth of swordfish, *Xiphias gladius* L., 1758, in the eastern Mediterranean using anal-fin spines. *Fishery Bulletin*, 93(3), pp. 594 – 602.

Tzeng, T. D. (2007). Population structure and historical demography of the Spotted Mackerel (*Scomber australasicus*) off Taiwan inferred from mitochondrial control region sequencing. *Zoological Studies*, 46, pp. 656 – 663.

Van Oosterhout, C., Hutchinson, W. F., Wills, P. M. D. and Shipley, P. (2004). MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, 4, pp. 535 – 538.

Van Oosterhout, C., Joyce, D. A. and Cummings, S.M. (2006). Evolution of MHC class IIB in the genome of wild and ornamental guppies*, Poecilia reticulata*. *Heredity*, 97, pp. 111 – 118.

Viñas, J., Pujolar, J. M., Alvarado Bremer, J. Ely, B. and Pla, C. (1998). Population genetics of swordfish (*Xiphias gladius*) in the Mediterranean Sea, based on allozyme electrophoresis and mitochondrial DNA sequencing. *Collective Volume of Scientific Papers ICCAT*, 50, pp. 225 – 233.

Viñas, J., Alvarado Bremer, J. R. and Pla, C. (2004). Inter-oceanic genetic differentiation among albacore (*Thunnus alalunga*) populations. *Marine Biology*, 145, pp. 225 – 232.

Viñas, J., Alvarado Bremer, J., Mejuto, J., De la Serna, J. M., García-Cortés, B. and Pla, C. (2007). Swordfish genetic population structure in the North Atlantic and Mediterranean. *Collective Volume of Scientific Papers ICCAT*, 61, pp. 99 – 106.

Viñas, J., Pérez-Serra, A., Vidal, O., Alvarado Bremer, J. R. and Pla, C. (2010). Genetic differentiation between eastern and western Mediterranean swordfish revealed by phylogeographic analysis of the mitochondrial DNA control region. *ICES Journal of Marine,* 67(6), pp. 1222 – 1229.

Viñas J., Gordoa, A., Fernández-Cebrián, R., Pla, C., Vahdet, U. and Araguas, R. M. (2011). Facts and uncertainties about the genetic population structure of Atlantic bluefin tuna (*Thunnus thynnus*) in the Mediterranean. Implications for fishery management. *Reviews in Fish Biology and Fisheries,* 21, pp. 527 – 41.

Waples, R. S. (1998) Separating the wheat from the chaff: Patterns of genetic differentiation in high gene flow species. *Journal of Heredity*, 89, pp. 438 – 450.

Waples, R. S. and Gaggiotti, O. (2006). What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Molecular Ecology*, 15, pp. 1419 – 1439.

Ward, R. D. (2000). Genetics of fish populations. In: Hart, P.J.B., and Reynolds, J.D., ed., *Handbook of Fish Biology and Fisheries Management*. 1st ed. United Kingdom: Blackwell Publishing, pp. 200 – 224.

Ward, R. D., Woodwark ,M. and Skibinski, D. O. F. (1994). A comparison of genetic diversity levels in marine, freshwater, and anadromous fishes. *Journal of Fish Biology*, 44, pp. 213 – 232.

Ward, R. D., Elliot, N. G., Smolenski, A. J. and Grewe, P. M. (1997). Global population structure of yellowfin tuna, *Thunnus albacares*, inferred from allozyme and mitochondrial DNA variation. *Fishery Bulletin,* 95, pp. 566 – 575.

Ward, R. D., Reeb, C. A. and Block, B. A. (2001). Population structure of Australian swordfish, *Xiphias gladius*. [online]. Final Report to the Australian Fisheries Management Authority. Available at: http://www.soest.hawaii.edu/PFRP/pdf/afma_rpt.pdf. [Accessed 1 February 2016].

Witkke-Thompson, J. K., Pluzhnikov, A. and Cox, N. J. (2005). Rational Inferences about Departures from Hardy-Weinberg Equilibrium. *American Journal of Human Genetics*, 76(6), pp. 967 – 986.

Wasser, S., Shedlock, A., Comstock, K., Ostrander, E., Mutayoba, B., Stephens, M. (2004). Assigning African elephants DNA to geographic region of origin: applications to the ivory trade. *Proceedings of the National Academy of Sciences USA,* 101, pp. 14847 – 14852.

Wattier, R., Engel, C. R., Saumitou-Laprade, P., Valero, M. (1998). Short allele dominance as a source of heterozygote deficiency at microsatellite loci: experimental evidence at the dinucleotide locus Gv1CT in *Gracilaria gracilis* (Rhodophyta). *Molecular Ecology*, 7, pp. 1569 – 1573.

Weir, B. S. and Cockerham, C. C. (1984). Estimating F statistics for the analysis of population structure. *Evolution*, 38, pp. 1358 – 1370.

West, W. M., Kerwath, S. E., Da Silva, C., Wilke, C. G. and Marsac, F. (2012). Horizontal and vertical movements of swordfish tagged with pop up-satellite transmitters in the South-West Indian Ocean, off South Africa. 10th IOTC Working Party on Billfish. *IOTC-2012-WPB10-16*. [Accessed 15 February 2016].

West, W. M., Kerwath, S. K. (2016). The history and current status of the commercial tuna fisheries in South Africa. Unpublished.

White, C., Selkoe, K.A., Watson, J., Siegel, D.A., Zacherl, D.C., and Toonen, R.J. (2010). Ocean currents help explain population genetic structure. *Proceedings of the Royal Society B:Biological Sciences*, 277, pp. 1685 – 1694.

Wirth, T. and Bernatchez, L. (2001). Genetic evidence against panmixia in the European eel. *Nature*, 409, pp. 1037 – 1040.

Withler, R. E., Beacham, T. D., Schulze, A. D., Richards, L. J., and Miller, K. M. (2001). Co-existing populations of Pacific ocean perch, *Sebastes alutus*, in Queen Charlotte Sound, British Columbia. *Marine Biology*, 139: pp. 1 – 12.

Wright, S. (1978). Evolution and the Genetics of Population, Variability Within and Among Natural Populations. The University of Chicago Press, Chicago.

Wu, G. C. C., Chian, H. C., Chen, K. S., Hsu, C. C. and Yang, H. Y. (2009). Population structure of albacore (*Thunnus alalunga*) in the Northwestern Pacific Ocean inferred from mitochondrial DNA. *Fisheries Research*, 95, pp. 125 – 131.

Wu, GC-C., Chiang, H-C., Chou, Y-W., Wong, Y-R., Hsu, C-C., Chen C-Y. and Yang, H-Y. (2010) Phylogeography of yellowfin tuna (*Thunnus albacares*) in the Western Pacific and the Western Indian Oceans inferred from mitochondrial DNA. *Fisheries Research*, 105, pp. 248 – 253.

Wu, Z., Xu, Q., Zhu, J., Dai, X. and Xu, L. (2014). Genetic population structure of the bigeye tuna *Thunnus obesus* in the central Pacific Ocean based on mtDNA Cytb sequences. *Fisheries Science*, 80, pp. 415 – 426.

Zhan, A., MacIsaac, H. J. and Cristescu, M. E. (2010). Invasion genetics of the *Ciona intestinalis* species complex: from regional endemism to global homogeneity. *Molecular Ecology*, 19, pp. 4678 – 4694.

APPENDIX A

Table 14. A summary of population genetics studies conducted with various markers on large pelagic species and other fish species globally.

Species	Paper	Marker	Area	Species	Paper	Marker	Area
Swordfish	Alvarado Bremer *et al.,* 1995	mtDNA	Atlantic, Pacific, Mediterranean	Yellowfin tuna	Aguila *et al.,* 2015	Microsatellite	Pacific
	Alvarado Bremer *et al.,* 1996	mtDNA	Atlantic, Pacific, Mediterranean		Dammannagoda *et al.,* 2008	mtDNA, microsatellite	Indian
	Alvarado Bremer *et al.,* 1998	mtDNA	Atlantic, Pacific, Indian		Farnham, 2003	mtDNA, microsatellite	Atlantic
	Alvarado Bremer *et al.,* 2005	mtDNA	Atlantic, Pacific, Indian, Mediterranean		Kunal *et al.,* 2013	mtDNA	Indian
	Alvarado Bremer *et al.,* 2006	Nuclear	Pacific		Li *et al.,* 2015 (1)	mtDNA	Pacific
	Alvarado Bremer *et al.,* 2007	Nuclear	Atlantic, Indian		Nomura *et al.,* 2014	mtDNA, microsatellite	Pacific
	Chow and Takeyama, 2000	mtDNA, nuclear	Atlantic, Pacific, Indian, Mediterranean		Ward et al., 1997	Protein, mtDNA	Atlantic, Pacific, Indian
	Chow *et al.,* 1997	mtDNA	Atlantic, Pacific, Indian, Mediterranean		Wu *et al.* 2010	mtDNA	Pacific, Indian
	Chow *et al.,* 2002	Nuclear	Atlantic	Bigeye tuna	Appleyard *et al.,* 2002	mtDNA, microsatellite	Indian
	Chow *et al.,* 2007	SNP	North and South Atlantic		Chiang *et al.,* 2006	mtDNA	Pacific
	Garcia *et al.,* 2011	mtDNA	Atlantic		Chiang *et al.,* 2008	mtDNA	Indian
	Greig *et al.,* 1999	mtDNA, nuclear	Atlantic, North Pacific, Mediterranean		Chow *et al.,* 2000	mtDNA	Atlantic, Pacific, Indian
	Grivalja-Chon *et al.,* 1994	mtDNA	North Pacific		Durand *et al.,* 2005	mtDNA, nuclear	Atlantic, Pacific, Indian
	Kasapidis *et al.,* 2006	Microsatellite	Atlantic		Gonzalez *et al.,* 2008	Microsatellite	Atlantic, Pacific, Indian
	Kasapidis *et al.,* 2008	Microsatellite	Pacific		Wu *et al.,* 2014	mtDNA	Pacific
	Kotoulas *et al.,* 2003	Microsatellite	Atlantic, Mediterranean	Albacore tuna	Davies *et al.,* 2011	Microsatellite	Atlantic, Mediterranean
	Kotoulas *et al.,* 2006	Microsatellite	North and South Atlantic, southwest Pacific, southeast Indian and Mediterranean		Laconcha *et al.,* 2015	SNP	Atlantic, Pacific, Indian, Mediterranean
	Lu *et al.,* 2006	mtDNA	West Pacific, Indian		Montes *et al.,* 2012	Microsatellite	Atlantic, Pacific, Indian, Mediterranean
	Magoulas *et al.,* 1993	mtDNA	Northeast Atlantic, Mediterranean		Nakadate *et al.,* 2005	mtDNA, nuclear	Atlantic, Mediterranean
	Muths *et al.,* 2009	mtDNA, microsatellite	Indian		Pujolar *et al.,* 2003	Protein	Atlantic, Mediterranean
	Muths *et al.,* 2013	mtDNA, microsatellite	Indian		Takagi *et al.,* 2001	Microsatellite	Atlantic, Pacific
	Patarnello *et al.,* 2007	Review of previous studies	North Atlantic, Mediterranean	Southern bluefin tun	Wu *et al.,* 2009	mtDNA	Pacific
	Pla *et al.,* 1998	mtDNA	Mediterranean	Atlantic Bluefin tuna	Grewe, 1997	mtDNA, microsatellite	Pacific
	Reeb *et al.,* 2000	mtDNA	Pacific		Albaina *et al.,* 2013	SNP	Atlantic, Pacific, Indian, Mediterranean
	Rosel and Block, 1996	mtDNA	Atlantic, Pacific, Mediterranean		Boustany *et al.,* 2008	mtDNA	Atlantic, Mediterranean
	Smith and Alvarado Bremer, 2010	SNP, protein	Atlantic, Mediterranean		Carlsson *et al.,* 2004	mtDNA, microsatellite	Mediterranean
	Smith *et al.,* 2015	SNP	Atlantic		Clark *et al.,* 2004	Microsatellite	Atlantic
	Vinas *et al.,* 1998	Protein, mtDNA	Mediterranean		Riccioni *et al.,* 2013	Microsatellite	Mediterranean
	Vinas *et al.,* 2006	mtDNA	North Atlantic, Mediterranean	Skipjack tuna	Viñas *et al.,* 2011	mtDNA, microsatellite	Mediterranean
	Vinas *et al.,* 2010	mtDNA	Mediterranean		Dammannagoda *et al.,* 2011	mtDNA, microsatellite	Indian
	Ward *et al.,* 2001	mtDNA, microsatellite	Pacific, Indian	Other fish species	Schrey and Heist, 2003	Microsatellite	Atlantic, Pacific, Indian
Marlins	Graves and McDowell, 2001	mtDNA, nuclear, microsatell	Atlantic		Beacham *et al.,* 1999	Microsatellite	Southern British Colombia
	Graves and McDowell, 2006	mtDNA, microsatellite	Atlantic		Beacham *et al.,* 2002	Microsatellite	Newfounland, Labrador
	McDowell and Graves, 2008	mtDNA, microsatellite	Pacific		Feldheim *et al.,* 2001	Microsatellite	Western Atlantic
	Purcell *et al.,* 2011	mtDNA, microsatellite	Pacific		Schrey and Heist, 2007	Microsatellite	Missouri, Mississippi and Atchafalaya Rivers
	Sorenson *et al.,* 2013	mtDNA, microsatellite	Atlantic, Pacific		Shaw *et al.,* 1999	Microsatellite	Atlantic, Pacific

APPENDIX B

Table 15. Allele frequencies per locus for East (>20°E) and West (<20E°).

A8	Alleles	*212*	*216*	*220*	*224*	*228*	*232*	*236*
	East (>20°E)	0.003	0.003	0.04	0.857	0.079	0.016	0.003
	West (<20°E)	0	0	0.045	0.881	0.03	0.037	0.007
A7	Alleles	*263*	*267*	*271*	*275*	*279*	*283*	*293*
	East (>20°E)	0.02	0.705	0.094	0. 122	0.031	0.026	0.003
	West (<20°E)	0.024	0.734	0.081	0. 153	0.008	0	0
A113	Alleles	*196*	*210*	*214*	*218*	*222*	*226*	*230*
	East (>20°E)	0.003	0. 116	0.077	0.082	0.54	0. 173	0.009
	West (<20°E)	0	0. 169	0. 115	0.054	0.538	0. 123	0
B6	Alleles	*236*	*240*	*244*	*248*	*252*	*256*	*260*
	East (>20°E)	0.023	0.013	0.019	0.629	0.061	0. 226	0.029
	West (<20°E)	0.016	0	0.032	0.573	0. 113	0. 258	0.008

B108	Alleles	*161*	*165*	*169*	*173*	*177*	*181*	*185*	*189*	*193*	*197*	*201*	*205*	*209*	*213*	*217*	*225*	*229*
	East (>20°E)	0.006	0. 111	0.017	0.008	0.036	0.003	0.019	0. 133	0.381	0.05	0. 164	0.006	0.014	0.019	0.028	0	0.006
	West (<20°E)	0	0. 104	0	0.052	0	0.007	0.015	0. 164	0.328	0.082	0. 187	0.007	0.015	0	0.03	0.007	0

B112	Alleles	*188*	*192*	*196*	*200*	*204*	*208*	*212*	*216*	*220*	*224*	*228*	*232*	*236*	*240*	*244*	*248*
	East (>20°E)	0	0.006	0.006	0	0.09	0.085	0. 144	0. 138	0. 189	0. 121	0. 144	0.048	0.011	0.008	0	0.008
	West (<20°E)	0.008	0	0.025	0.008	0. 107	0.082	0. 172	0. 164	0. 189	0.074	0. 115	0.049	0	0	0.008	0

C8	Alleles	*143*	*147*	*151*	*155*	*159*	*163*	*167*	*171*	*175*	*179*	*183*	*187*	*191*	*195*	*199*	*203*	*207*	*211*	*215*	*219*	*223*	*227*	*231*	*235*	*239*	*251*	*255*
	East (>20°E)	0.012	0	0.006	0.054	0.03	0.048	0.057	0. 131	0.077	0. 11	0.068	0.068	0.08	0.054	0.039	0.045	0.036	0.009	0.003	0.018	0.018	0.012	0.009	0.012	0.003	0.003	0
	West (<20°E)	0	0.008	0.023	0.039	0.031	0.062	0.039	0. 141	0.047	0. 102	0.07	0. 102	0.062	0.047	0.039	0.039	0.016	0.031	0.031	0.031	0	0.008	0	0.016	0.008	0	0.008

C10	Alleles	*183*	*187*	*191*	*195*	*199*	*203*	*207*	*211*	*215*	*219*	*223*	*227*	*231*	*235*	*239*	*243*	*247*	*251*	*255*	*267*	*271*	*279*					

	East (>20°E)	0.003	0.009	0.021	0.034	0.034	0.043	0.046	0. 181	0. 11	0. 132	0. 107	0.058	0.074	0.071	0.04	0.021	0.006	0	0.003	0	0.003	0.003
	West (<20°E)	0.008	0.008	0.033	0.041	0.025	0.057	0. 115	0. 156	0. 115	0.09	0. 115	0.057	0.041	0.041	0.041	0.016	0.016	0.008	0.008	0.008	0	0
D2B	Alleles	*147*	*155*	*159*	*163*	*167*	*171*	*175*	*179*	*183*	*187*	*191*											
	East (>20°E)	0.003	0.017	0.006	0.097	0. 287	0.34	0. 171	0.052	0.014	0.011	0.003											
	West (<20°E)	0	0	0	0. 101	0. 283	0.348	0. 167	0.087	0.007	0.007	0											
Xg166	Alleles	*111*	*114*	*120*	*123*	*126*	*129*	*132*	*135*	*138*	*141*	*144*											
	East (>20°E)	0.006	0.006	0.022	0.003	0. 157	0.091	0.569	0.088	0.033	0.022	0.003											
	West (<20°E)	0	0	0.031	0	0. 115	0. 108	0.523	0. 192	0.015	0.015	0											
Xg144	Alleles	*110*	*119*	*125*	*128*	*131*	*134*	*137*	*140*	*152*	*155*	*158*	*161*	*164*	*167*	*170*							
	East (>20°E)	0.003	0.009	0.032	0.019	0.098	0.013	0.006	0.003	0.003	0.019	0.351	0.351	0.082	0.006	0.003							
	West (<20°E)	0.018	0.088	0.009	0.018	0.061	0	0	0	0	0.009	0.342	0. 281	0. 158	0.018	0							