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STOCK STRUCTURE OF SWORDFISH (*Xiphias gladius*) IN THE PACIFIC OCEAN USING MICROSATELLITE DNA MARKERS

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Abstract

The genetic structure of swordfish (*Xiphias gladius*) in the Pacific Ocean was assessed by analyzing 594 individuals from 6 different regions, genotyped with 13 microsatellite loci. The results showed very low genetic differentiation among the different geographical areas, which was not statistically significant. There was a slight evidence for isolation by distance across a U-shaped corridor, as it had been demonstrated by Reeb et al. (2000) using mtDNA, but more samples and a better geographical coverage are necessary to support this finding. These data confirmed the low genetic differentiation of swordfish within Pacific, which has been already found with other genetic markers.

Introduction

Fisheries science is based on the notion of an "idealized unit stock", a discrete entity with its own origin, demographics and fate (Waldman, 2005). An understanding of stock structure is necessary for designing appropriate management regulations in fisheries where multiple stocks are differentially exploited (Ricker, 1981). A wide range of methods are used to identify and delineate fish stocks like tagging, life history characteristics, parasites, otolith microchemistry, morphology and genetics (Cadrin et al., 2005). Genetic methods can be powerful tools in fisheries management, by enabling the identification of genetically differentiated populations, referred to as "*genetic stocks*" (Jamieson, 1973; Ovenden, 1990), i.e. units that are more or less reproductively isolated from each other, and thus they will react independently to exploitation. These populations may also differ in key parameters used in fishery models (e.g. growth, mortality, recruitment) (Hauser and Ward, 1998). However, there may be fish populations that are not genetically differentiated, but will still react independently to exploitation. These independent units represent different "*harvest stocks*", defined as "locally accessible fish resources, in which fishing pressure on one resource has no effect on the abundance of fish in another contiguous resource" (Gauldie, 1988). These later units are of interest to fisheries managers, but this concept does not imply any genetic or phenotypic differences between stocks. Both stock definitions (*genetic* and *harvest*) are interpretations of the more general stock definition of Ihssen *et al*. (1981), who defines stock as "an intraspecific group of randomly mating individuals with temporal and spatial integrity". This reflects the fact that there is lack of a universally applicable definition of the term "stock" and therefore the difficulty and practical problems faced when fish stocks have to be recognized.

The swordfish *Xiphias gladius* is a migratory large pelagic fish distributed in tropical, sub-tropical and temperate seas, worldwide, and it is important for the world fisheries. Its life history characteristics and its high mobility suggest a high gene flow and little genetic subdivision among individuals and pose difficulties in defining and delineating its stocks. Its spawning grounds has been also difficult to be identified because of the solitary nature of the fish, its long spawning season, the widespread area where larvae are found and the different methods used to survey ichthyoplankton (Grall et al. 1983).

The global genetic population structure of swordfish has been partly elucidated so far. Several genetic studies have demonstrated that swordfish populations are subdivided, mainly on an ocean-basin scale, with a highly distinct stock in the Mediterranean, two stocks in the Atlantic (North and South) with disputed boundary, and an Indo-Pacific stock (Magoulas et al. 1993; Kotoulas et al. 1995; Rosel and Block 1996; Alvarado Bremer et al. 1996; Chow and Takeyama 2000). However, there are indications for further subdivision within Indian (Lu et al. 2006) and Pacific Ocean (Grijalva-Chon et al. 1996; Reeb et al. 2000; Alvarado Bremer et al. 2006).

For the Pacific Ocean, a number of studies failed to detect genetic differentiation (e.g. Rosel and Block 1996, Chow and Takeyama 2000). On the other hand, Grijalva-Chon et al. (1996) found genetic differentiation at three allozymic loci between samples from central Pacific (Hawaii) and east Pacific Ocean (Mexico). Reeb et al. (2000), using D-loop mitochondrial DNA (mtDNA) sequences, observed a U-shaped pattern of genetic connectivity, which indicates that northern and southern populations in the eastern Pacific are genetically continuous, while those in the west (Japan vs. pooled NW and NE Australian samples) have diverged. Alvarado Bremer et al. (2006) used nuclear sequences of the intron 6 of the lactate dehydrogonase-A (*ldh*-A) gene and found evidence of genetic differentiation within Pacific: southeast Pacific and north-central Pacific were different from each other and from northeast and southwest Pacific, while the two latter samples were not different from each other.

In this paper, we are assessing genetic population structure of swordfish in the Pacific Ocean by analyzing samples with 13 polymorphic microsatellite markers.

Material and methods

Sample collection

Muscle tissues were obtained from 594 adult swordfish individuals, caught onboard by scientific observers during commercial longline activities and preserved in ethanol. Individuals were grouped into potential population units (samples), according to their geographic origin and collection date, as shown in Table 1. Samples were tried to be as uniform as possible with reference to their geographic origin and the collection date, and at the same time, to be large enough for robust statistical analyses. The geographic distribution of the specimens is shown in Figure 1.

Microsatellite analyses

Total DNA was extracted following the salt protocol of Miller et al. (1988). All individuals were genotyped for 13 microsatellite loci. Six of the loci (Xg55, Xg56, Xg66, Xg144, Xg166, Xg379) had been developed by Reeb et al. (2003), five were developed in HCMR (Xg31, Xg51, Xg41b, Xg42, SauR1) (Kasapidis et al. 2008; Kasapidis et al. unpublished data) and two had been developed in other fish and optimized for swordfish (DLASTR14, VBC201). The loci were PCR-amplified in four multiplex reactions and the PCR products were electrophoresed on an ABI 3700 automated sequencer. Alleles were scored using STRand program version 2.3.94 (Veterinary Genetics Laboratory, University of California, Davis). PCR amplifications were repeated for several individuals to fill in missing data due to PCR or electrophoresis failures, and to confirm genotypes.
The software MIKRO-CHECKER 2.2.1 (van Oosterhout et al. 2004) was used to check for technical artifacts such as null alleles, stuttering and large allele dropout.

Statistical analyses

Allele frequencies, observed (H_O) and expected (H_E) heterozygosities, mean number of alleles per locus and gene diversity were calculated using program GENETIX 4.01 (Belkhir et al. 2000). Fis and Fst, were calculated using the computer program GENEPOP version 3.4 (Raymond and Rousset 1995). This program was also used to test for linkage disequilibrium, deviation from Hardy-Weinberg equilibrium (HWE) and for allelic and genotypic differentiation among population pairs. Exact HWE probability tests (Guo and Thompson 1993), tests for heterozygote deficiency (Rousset and Raymond 1995) and exact tests for allelic and genotypic differentiation among populations were performed with Markov chain lengths obtained with 100 batches and 5000 iterations per batch and using a dememorization value of 10000. Significance levels for multiple tests were adjusted according to the sequential Bonferroni correction using a global significance level of 0.05 (Rice 1989).

Pairwise Fst's for all pairs of samples were calculated with program ARLEQUIN ver. 3.1 (Excoffier et al. 2005) and p-values were obtained by permutating data 1000 times. A hierarchical analysis of molecular variance (AMOVA; Excoffier et al. 1992) was carried out using the program SAMOVA.1.0 (SAMOVA, Spatial Analysis of MOlecular VAriance) (Dupanloup et al. 2002). This method implements an approach to define groups of populations that are geographically homogeneous and maximally differentiated from each other. The method is based on a simulated annealing procedure that aims at maximizing the proportion of total genetic variance due to differences between groups of populations. As geographical co ordinations of the samples were given those of their geographical centers. The method was run by assuming 2, 3 and 4 groups of populations.

Data were tested for Isolation by Distance, i.e. whether there is a correlation between genetic and geographical distances, by using a Mantel test implemented in software IBD v1.52 (Bohonak 2002). Geographical distances were measured in km between the geographical centers of the samples. Following the findings of Reeb et al (2000), two data sets were tested: one with geographic distances measured assuming that gene flow occurs along direct linear paths between any two demes and another assuming that gene flow occurs across a U-shaped pattern (see Fig. 1).

Results

In total, 594 individuals were genotyped at 13 microsatellite loci. The total amount of missing genotypes in this data set was less than 0.4%, indicating very high genotyping success, with none of the individuals containing more than two missing loci. Microsatellite loci exhibited different levels of polymorphism

with locus Xg144 being the less polymorphic with 7 alleles and mean gene diversity of 0.598, and locus Xg55 the most polymorphic with 57 alleles and mean gene diversity of 0.965 (Table 2).

MICRO-CEHECKER did not detect problems that could indicate stuttering or large allele dropout, but detected heterozygosity deficiency possibly due to null alleles in most samples for locus DLASTR14, in two samples for loci Xg144 and Xg51, and in one sample for loci Xg55 and Xg56. The probability tests did not reject the null hypothesis of HWE for any population at any locus, while the exact tests for heterozygote deficiency gave similar results with MICRO-CEHECKER. Observed and expected heterozygosities over all loci were similar in each sample as well as among samples (Table 3). All loci were in linkage equilibrium. The global multilocus Fis was 0.0238, with loci DLASTR14 and Xg144 having the highest values (0.1331 and 0.101, respectively), indicating a homozygote excess, probably due to the presence of null alleles (Table 2).

Exact tests for allelic and genotypic differentiation did not reveal any difference between any pair of samples when 8 samples were considered (SWPA1, SWPA2, SPA, SEPA00, SEPA01, MEPA, MPA and NWPA; SEPA99 was omitted because of its small size). The null hypothesis of no differentiation still could not be rejected when 6 samples were considered (SWPA, SPA, SEPA, MEPA, MPA, NWPA). The same results were obtained when locus DLASTR14, which had null alleles, was removed from the analyses.

Pairwise multilocus F_{ST}s were significant only between SWPA1 and SWPA2 (F_{ST}=0.00381, P=0.02051) and between SWPA1 and NWPA (F_{ST}=0.00299, P=0.00879), but not after a Bonferroni correction was applied. The global multilocus Fst was estimated to be 0.0002.

The analysis of molecular variance with SAMOVA, showed that almost all variation (>99.8%) was due to differences among individuals within populations. By assuming two, three or four groupings, the among group variation ranged between 0.11-0.13% but it was not statistically significant in any case (Table 5).

Mantel tests for correlation between genetic and geographical distances did not produce a significant result when using the shortest geographical distance between samples (p=0.919). They showed a marginally significant correlation (p=0.02) when geographic distances were measured along a U-shaped corridor and after pooling samples SWPA1 and SWPA2 into a single sample (Fig. 2).

Discussion

The analyses of 594 swordfish individuals from different parts of Pacific Ocean genotyped for 13 polymorphic microsatellite loci did not reveal any statistical significant differentiation apart from a slight differentiation between SWPA1 vs. SWPA2 and SWPA1 vs. NWPA, which was not statistically significant after a Bonferroni correction. There was a slight evidence for isolation by distance across a U-shaped corridor, as it had been demonstrated by Reeb et al. (2000) for mtDNA, but only after samples SWPA1 and SWPA2 were pooled together. More samples and a better geographical coverage are necessary to confirm this finding.

The current analysis should be considered quite reliable since it contains large samples in general, the geographic origin of samples is known with accuracy since they were collected onboard by scientific observers, several loci were used, each sample had been collected within a relatively small time frame, the data set had very few missing genotypes and only few loci showed evidence of null alleles. The geographical coverage of the samples was quite good apart from the Northwest Pacific and the east Pacific close to the Indonesian archipelago. Nevertheless, the genetic signal was very low with a global multilocus F_{ST} of 0.0002 and the maximum pairwise F_{ST} equal to 0.00381. For this level of differentiation and for the sample sizes used, it is difficult to discern genetic signal from noise, according to the formula proposed by Waples (1998) (for sample size S=150, F_{ST} should be > 0.0033 to surpass genetic noise, while for S=400 F_{ST} should be > 0.00125).

These results confirm the low genetic differentiation within Pacific observed in other studies, which had used other genetic markers. The genetic studies contacted on swordfish in the Pacific Ocean so far, have not provided unequivocal evidence for population structuring. Reeb et al. (2000), using mtDNA D-loop sequences, detected a U-shaped pattern of genetic connectivity within Pacific; nevertheless, this differentiation was weakly supported statistically. The only statistically significant F_{ST} value found was between a pooled Australian sample that included many individuals from Indian Ocean and Japan.

Alvarado Bremer et al. (2006), using nuclear *ldh*-A intron 6 sequences found statistically significant evidence of genetic heterogeneity in the Pacific only after samples were pooled into larger units, which were not homogenous temporally and spatially. The most genetically distinct region found was southeast Pacific, which contrasts the findings of Reeb et al. (2000) and that of the current study. Another intron locus, Calmodulin gene intron 4 (*CaM***)** did not show any differentiation within Indo-Pacific (Chow and Takeyama 2000). The use of several intron loci in a single study may provide a more reliable picture of swordfish genetic structure in the Pacific.

Relative to less polymorphic nuclear and mtDNA, microsatellites are often considered more sensitive in the detection of fine-scale population structure. Nevertheless, there are cases where there is concordance among different markers in ability to detect population structure or cases where mtDNA demonstrates greater heterogeneity (Buonaccorsi et al. 2001 and references within). In swordfish, different studies published so far, showed concordance among different markers in detecting global population structure (i.e. Mediterranean, N. Atlantic, S. Atlantic, Indo-Pacific) (mtDNA: Alvarado-Bremer et al. 1996; mtDNA and *CaM*: Chow and Takeyama 2000; *ldh-A* and *aldC*: Greig et al.1999, Greig et al. 2000; microsatellites: Kasapidis et al. submitted). Therefore, there is no reason to assume that any of those markers is more powerful in detecting the population structure of swordfish.

When trying to interpret swordfish stock structure using genetic data, we have to take into account that the use of genetic methods to determine stock structure is not always straightforward. If there are statistically significant genetic differences between samples, and these differences are assumed to have arisen from restricted gene flow rather than local selection, then two stocks can be inferred. If no genetic differences are detected, then the results are inconclusive: either there is a single stock or there are actually two stocks, but the molecular markers used are unable to resolve them. Thus, a finding of sample heterogeneity allows more powerful conclusions concerning stock structure, than a finding of sample homogeneity (Ward, 2000). Moreover, a small amount of gene flow (few migrants per generation) is often sufficient to prevent detectable genetic differentiation between conspecific stocks (Hartl and Clark, 1997). Yet, for fisheries management, an exchange of as high as 10% between populations may justify their treatment as separate stocks. Even by using the most sensitive molecular markers, the discrepancy in gene flow between harvest stock and genetic stocks still exists, and molecular markers alone may not suffice to identify stocks with small degree of isolation (Hauser and Ward, 1998).

There are evidence from analyses of gonad indices and sex-ratio at size (Mejuto et al. 2008) and larvae distribution (Nishikawa and Ueyanagi 1974) that support the hypothesis of a very broad and probably *quasi continuous* maturity-spawning region, mostly linked with warm waters of the central-western intertropical band (using 50 m deep temperature as a proxy indicator), which probably allows extensive gene flow between different parts of the Pacific, thus diminishing genetic differentiation.

In order to obtain more reliable genetic results for swordfish in the Pacific Ocean, given its low genetic differentiation there, it is necessary to use large samples, with temporal replicates, in order to discern genetic signal from noise, as has been suggested by Waples (1998). The use of more genetic markers as well as a more focused sampling, though difficult, on larval stages may provide more robust evidence for genetic differentiation in the Pacific. The combination of different methods (larva distribution, catches, otoliths, tagging with an appropriate strategy, parasites) will definitely help to better define stocks in the Pacific Ocean.

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References

Alvarado Bremer J.R., Mejuto J., Greig T.W., Ely B. 1996. Global population structure of the swordfish (*Xiphias gladius* L.) as revealed by analysis of the mitochondrial DNA control region. J. Exp. Mar. Biol. Ecol. 197:295-310.

Alvarado Bremer J.R., Hinton M.G., Greig T.W. 2006. Evidence of spatial genetic heterogeneity in Pacific swordfish (*Xiphius gladius* L.) revealed by the analysis of ldh-A sequences. Bull. Mar. Sci. 79:493-504.

Belkhir K. 2000. GENETIX, Logiciel Sous WindowsTM Pour la Génétique Des Populations. Laboratoire Génome, Populations, Interactions CNRS UMR 5000, Université de Montpellier II, Montpellier, France.

Bohonak A.J. 2002. IBD (Isolation By Distance): a program for analyses of isolation by distance. J. Hered. 93:153-154.

Cadrin S.X., Friedland K.D., Waldman J.R. 2005. Stock identification methods. Applications in Fishery Science. Academic Press, London, 719pp.

Chow S., Takeyama H. 2000. Nuclear and mitochondrial DNA analyses reveal four genetically separated breeding units of the swordfish. Journal of Fish Biology 56:1087-1098.

Dupanloup I., Schneider S., Excoffier L. 2002. A simulated annealing approach to define the genetic structure of populations. Mol. Ecol. 11:2571-81.

Excoffier L., Laval G., Schneider S., 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1:47-50.

Gauldie R.W. 1988. Tagging and genetically isolated stocks of fish: a test of one stock hypothesis and the development of another. J. Appl. Ichth., 4:168-173.

Grall C., De Sylva D.P., Houde E.D. 1983. Distribution, relative abundance and seasonality of swordfish larvae. Trans. Am. Fish. Soc. 112:235-246.

Greig T.W., Alvarado Bremer J.R., Ely B. 1999. Preliminary results from genetic analyses of nuclear markers in swordfish *Xiphias gladius*, reveal concordance with mitochondrial DNA analyses. Col. Vol. Sci. Pap. ICCAT 49:476-482.

Greig T.W., Alvarado Bremer J.R., Ely B. 2000. Nuclear markers provide additional evidence for population subdivision among Atlantic swordfish. Col. Vol. Sci. Pap. ICCAT 51:1637-1640.

Grijalva-Chon J.M., De la Rosa-Velez J., Sosa-Nishizaki O. 1996. Allozyme variability in two samples of swordfish, *Xiphias gladius* L., in the North Pacific Ocean. Fishery Bulletin U. S. 94:589 - 594.

Guo S.W., Thompson E.A. 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48:361-372.

Hartl D.L, Clark A.G. 1997. Principles of Population Genetics. Third edition. Sinauer Associates Inc. Sunderland, Massachusetts.

Hauser L., Ward R.D. 1998. Population identification in pelagic fish: the limits of molecular markers. In: Carvalho, G.R. (Ed.), Advances in Molecular Ecology, IOS Press, pp. 191-224.

Ihssen P.E., Booke H.E., Casselman J.M., Payne N.R., Utter F.M. 1981. Stock identification: materials and methods. Can. J. Fish. Aquat. Sci. 38:1838-1855.

Jamieson A. 1973. Genetic “tags” for marine fish stocks. In: Sea Fisheries Research (eds. Hardin J.F.R), Elek Science, London, pp. 91-99.

Kasapidis P., Mejuto J., Tserpes G., Antoniou A., Garcia-Cortes B., Peristeraki P., Oikonomaki K., Kotoulas G., Magoulas, A. 2008. Genetic structure of the swordfish (*Xiphias gladius*) stocks in the Atlantic using microsatellite DNA analysis. ICCAT Col. Vol. Sci. Pap. 61:89-98.

Kotoulas G., Magoulas A., Tsimenides N., Zouros E., 1995, Marked mitochondrial DNA differences between Mediterranean and Atlantic populations of the swordfish, *Xiphias gladius*. Mol. Ecol. 4:473-481.

Lu C.P., Chen C.A., Hui C.F., Tzeng T.D., 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 45:269-279. Institute of Oceanography, National Taiwan.

Magoulas A., Kotoulas G., Tsimenides N., Zouros E., de la Serna J.M., de Metrio G. 1993. Genetic structure of swordfish (Xiphias gladius) populations of the Mediterranean and the eastern side of the Atlantic: analysis by mitochondrial DNA markers. ICCAT Col. Vol. Sci. Pap. 40:126-136.

Mejuto J., García-Cortés B., Ramos-Cartelle, A. 2008. Reproductive activity of swordfish (*Xiphias gladius*) in the Pacific Ocean on the basis of different macroscopic indicators. WCPFC-BI-WP-2008/06.

Miller S.A., Dykes D.D., Polesky H.F. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 16:1215.

Ovenden J.R. 1990. Mitochondrial DNA and marine stock assessment: a review. Australian Journal of Marine and Freshwater Research 41:835-853.

Raymond M., Rousset F. 1995. GENEPOP (version1.2): population genetics software for exact tests and ecumenism. Journal of Heredity 86:248-249.

Rousset F., Raymond M. 1995. Testing heterozygote excess and deficiency. Genetics 140:1413-1419.

Reeb, C.A., Arcangeli L., 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.A., Arcangeli L., Block B.A. 2003. Development of 11 microsatellite loci for population studies in the swordfish, *Xiphias gladius* (Teleostei: Scombridae). Mol. Ecol. Notes 3:147-149.

Rice W.R. 1989. Analyzing tables of statistical tests. Evolution 43:223-225.

Rosel P., Block B.A. 1996. Mitochondrial control region variability and global population structure in the swordfish, *Xiphias gladius*. Mar. Biol. 125:11-22.

Ricker W.E. 1981. Changes in the average size and average age of Pacific salmon. Can. J. Fish. Aquat. Sci. 38:1636-1656

Van Oosterhout C., Hutchinson W.F., Wills D.P.M., Shipley P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535-538.

Waldman J.R. 2005. Definition of stocks: an evolving concept. In: Stock identification methods. Applications in Fishery Science, pp. 7-16. Ed. by Cadrin. S.X., Friedland K.D., and Waldman J.R., Academic Press, London, 719 pp.

Waples R.S. 1998. Separating the wheat from the chaff: Patterns of genetic differentiation in high gene flow species. J. Heredity 89:438-450.

Ward R.D. 2000. Genetics in fisheries management. Hydrobiologia 420:191-201.

Table 1. Sample name, geographical coordinates of samples, collection period and sample size (N)

Sample name	Coordinates	Collection Date (MM/YY)	N
Southwest Pacific 1 (SWPA1)	25º - 33ºS / 163º - 177ºE	08/04 – 10/04	54
Southwest Pacific 2 (SWPA2)	25º - 31ºS / 152º - 174ºW	07/04 – 10/04	51
SWPA (pooled)			105
South Pacific (SPA)	20º - 35ºS / 100º - 134ºW	11/04 – 01/05	59
Southeast Pacific 1999 (SEPA99)	20º - 39ºS / 77º - 84ºW	03/99 – 05/99	19
Southeast Pacific 2000 (SEPA00)	20º - 40ºS / 70º - 85ºW	04/00 – 07/00	121
Southeast Pacific 2001 (SEPA01)	20º - 35ºS / 75º - 80ºW	05/01 – 08/01	56
SEPA (pooled)			196
Mid-east Pacific (MEPA)	00º - 05ºN / 97º - 135ºW	10/98 – 02/99	24
Mid Pacific (MPA)	07ºS - 09ºN / 147º - 178ºW	04/04 – 07/04	67
Northwest Pacific (NWPA)	20º - 40ºN / 127º - 172ºE	06/05-11/05	143
TOTAL			**594**

Table 2. Microsatellite loci: locus name, number of alleles, mean gene diversity, F_{IS} and F_{ST} (after Weir and Cockerham) per locus.

Locus	N of alleles	Mean gene diversity	F_{IS}	F_{ST}
Xg166	10	0.637	-0.0266	0.0008
Xg144	7	0.598	0.1010	-0.0025
Xg31	15	0.804	-0.0151	-0.0015
Xg379	20	0.879	0.0048	-0.0004
Xg55	57	0.965	0.0351	-0.0016
Xg41b	43	0.962	0.0176	0.0009
Sau98R1	21	0.896	0.0001	0.0004
Xg42	11	0.714	-0.0054	-0.0010
Xg56	28	0.899	0.0366	-0.0004
Xg66	11	0.851	-0.0028	0.0019
DLASTR14	23	0.849	0.1331	-0.0013
Xg51	45	0.947	0.0446	0.0005
VBC201	12	0.809	-0.0196	0.0065
All:			**0.0238**	**0.0002**

Table 3. Number of individuals per sample, observed and expected hetrozygosities and F_{IS} per sample over all loci.

Sample	N	Hobs.	Hexp.	F_{IS}
SWPA1	54	0.8048 (0.1381)	0.8101 (0.1339)	0.0158
SWPA2	51	0.8243 (0.1146)	0.8201 (0.1150)	0.0045
SPA	59	0.8181 (0.1137)	0.8345 (0.1117)	0.0280
SEPA00	121	0.7978 (0.1216)	0.8243 (0.1158)	0.0363
SEPA01	56	0.8092 (0.1376)	0.8117 (0.1385)	0.0122
MEPA	24	0.8165 (0.1443)	0.7998 (0.1279)	0.0001
MPA	67	0.8106 (0.1132)	0.8198 (0.1203)	0.0187
NWPA	143	0.8051 (0.1258)	0.8285 (0.1176)	0.0318

Table 4. Pairwise F_{ST} values (below diagonal) and p values (above diagonal) between 8 samples genotyped for 13 microsatellite loci. Statistically significant F_{ST} and respective p values (before Bonferroni correction) are in bold.

	SWPA1	SWPA2	SPA	SEPA00	SEPA01	MEPA	MPA	NWPA
SWPA1		**0.02051 ±0.0043**	0.47461 ±0.0168	0.41895 ±0.0150	0.27734 ±0.0143	0.43164 ±0.0138	0.45508 ±0.0169	**0.00879 ±0.0029**
SWPA2	**0.00381**		0.15918 ±0.0083	0.50000 ±0.0143	0.38867 ±0.0161	0.43555 ±0.0175	0.66602 ±0.0145	0.26074 ±0.0132
SPA	0.00015	0.00156		0.49121 ±0.0109	0.64453 ±0.0168	0.45312 ±0.0168	0.45117 ±0.0164	0.15918 ±0.0145
SEPA00	0.00026	0.00004	0.00016		0.70605 ±0.0154	0.86719 ±0.0119	0.70020 ±0.0166	0.50195 ±0.0161
SEPA01	0.00084	0.00040	-0.00041	-0.00045		0.96484 ±0.0050	0.34961 ±0.0140	0.24121 ±0.0128
MEPA	0.00027	0.00019	0.00022	-0.00190	-0.00378		0.95215 ±0.0059	0.75586 ±0.0141
MPA	0.00011	-0.00062	0.00020	-0.00039	0.00044	-0.00311		0.21289 ±0.0123
NWPA	**0.00299**	0.00077	0.00107	0.00001	0.00079	-0.00112	0.00083	

Table 5. AMOVA analyses with SAMOVA software assuming two, three and four groups of populations.

	Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	P	F_{ST}
2 groups: (SWPA1), (rest)	Among groups	1	6.752	0.00678	0.13	0.332±0.013	0.00136
	Among populations within groups	6	32.63	0.00053	0.01		
	Within populations	1142	6124.157	5.36266	99.86		
	Total	1149	6163.539	5.36997			
3 groups: (SWPA1, SPA), (NWPA), (rest)	Among groups	2	13.921	0.00596	0.11	0.312±0.016	0.00068
	Among populations within groups	5	25.461	-0.00229	-0.04		
	Within populations	1142	6124.157	5.36266	99.93		
	Total	1149	6163.539	5.36633			
4 groups: (SWPA1), (SPA), (NWPA), (rest)	Among groups	3	19.394	0.00684	0.13	0.300±0.013	0.00070
	Among populations within groups	4	19.988	-0.00306	-0.06		
	Within populations	1142	6124.157	5.36266	99.93		
	Total	1149	6163.539	5.36644			

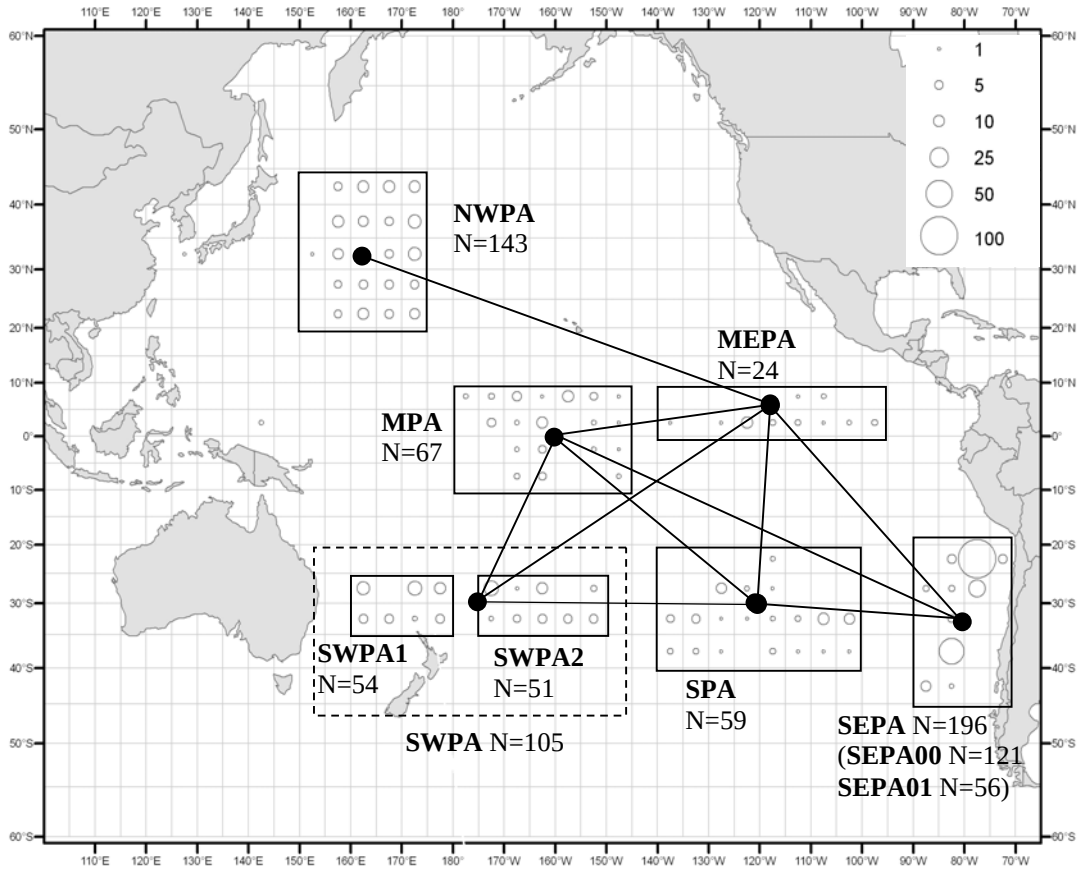

Figure 1. Location and sampling size (proportional to circle diameter) in 5ºx5º square of the swordfish samples genotyped for 13 microsatellite loci. Individuals were grouped into samples according to their geographical origin. Sample name and size is also mentioned. Lines indicate geographic distances among samples along the U-shaped pattern of gene flow proposed by Reeb et al. (2000).

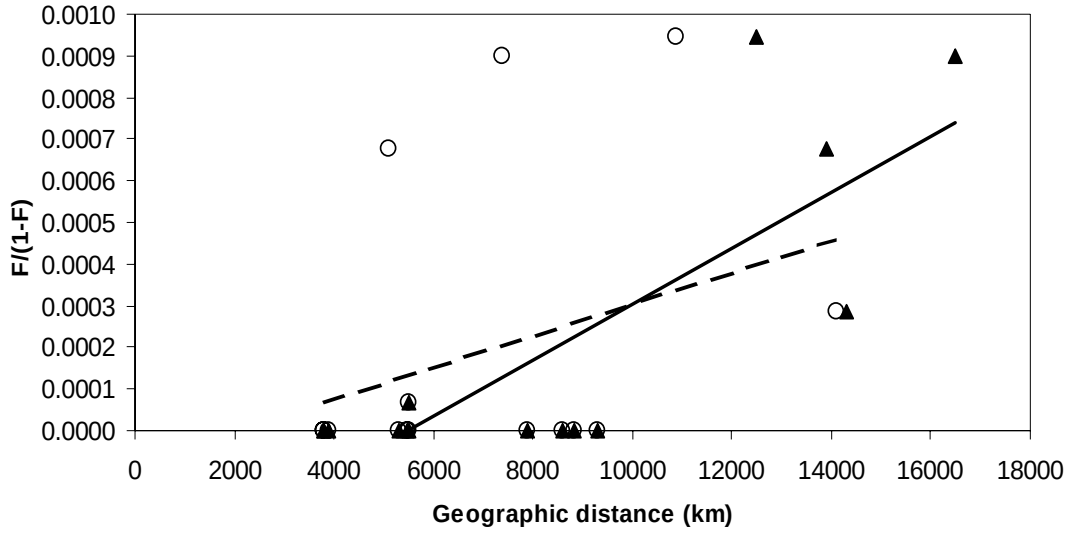

Figure 2. Linear regression analysis of transformed genetic distances vs. geographical distances between sample pairs assuming either a) migration follows direct straight paths (line A, open circles) or b) migration follows a U-shaped pattern as in Fig. 1 (line B, triangles). In the current analysis samples SWPA1 and SWPA2 were considered as one. Regression equations and coefficients as well as p values of Mantel tests are as follows: A: $y = 4 * 10^{-8}x - 0.00007$, $r^2 = 0.1024$, p=0.989; B: $y = 7 * 10^{-8}x - 0.0004$, $r^2 = 0.6549$, p=0.02.