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Worldwide genetic structure of albacore *Thunnus alalunga* revealed by microsatellite DNA markers

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ABSTRACT: One of the most common problems in fisheries is the definition of management units. Albacore *Thunnus alalunga* is an important species for commercial fisheries. Its population structure is still partially unknown; however, on the basis of fisheries data, tagging experiments, and morpho-ecological studies, 6 management units are currently accepted for this species. The main objective of this study was to define genetic entities within *T. alalunga* and to discuss the appropriateness of current management units. For this purpose, 13 microsatellite loci were applied to 551 albacore samples collected worldwide, and the population genetic structure was assessed. The most relevant differences between management and genetic units were that (1) Atlantic and Indian Ocean samples are genetically indistinguishable, and (2) possible differentiation exists within the Pacific Ocean and also within the Mediterranean Sea. Thus, this study provides genetic information to clarify albacore population delimitation, which is a key factor to reach the demanded sustainable management of this resource.

KEY WORDS: Albacore · Management units · Microsatellite DNA · SSRs · Population genetics · Conservation · Fisheries · Stock

INTRODUCTION

Albacore *Thunnus alalunga* is an epi- and mesopelagic oceanic tuna which supports important commercial fisheries (Santiago 2004). It is cosmopolitan, highly migratory, and widely distributed mostly in temperate waters between latitudes 45° N and 45° S, including the Mediterranean Sea. Stocks of this species are currently overexploited, and there is an urgent need to improve its conservation and management efforts (Collette et al. 2011). For conservation purposes, marine organisms should be managed at the population level since the extent and dynamics of population structuring underlies resilience and sustainability (Carvalho et al. 2010). Therefore, an improvement of *T. alalunga* population structure assessment is necessary.

Based on fisheries distribution, identification of separate spawning areas, different estimated growth rate, morphological differences, and tagging experiments, the International Commission for the Conservation of Atlantic Tunas (ICCAT), the Indian Ocean Tuna Commission (IOTC), the Western and Central Pacific Fisheries Commission (WCPFC), and the Inter-American Tropical Tuna Commission (IATTC) established 6 management units for this species, 1 per

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ocean and hemisphere: the North Atlantic, South Atlantic, Indian, North Pacific, South Pacific, and Mediterranean stocks. The level of consistency among these 6 management units with regard to the population genetic structure of this species is only partially known.

Genetic approaches are becoming increasingly important to pelagic fish management procedures, since they have demonstrated enough resolution to detect intra-oceanic variability (Chow et al. 2000). They have already provided relevant information for direct management in several marine pelagic fish species such as sailfish *Istiophorus albicans* (Graves & McDowell 1995), swordfish *Xiphias gladius* (Chow & Takeyama 2000), bigeye tuna *Thunnus obesus* (González et al. 2008), bluefin tuna *T. thynnus* (Riccioni et al. 2010), and albacore (Arrizabalaga et al. 2004).

Genetic studies of natural populations often use highly polymorphic microsatellite loci because of their high statistical power for population genetics (Brooker et al. 1994, O'Connell & Wright 1997, Oleksiak 2010). Thus, nuclear-encoded DNA microsatellite markers could shed light on the genetic variability and population genetic structure of albacore, as it has been previously reported for other tuna species, such as bluefin (Riccioni et al. 2010) and bigeye tuna (González et al. 2008). Indeed, in albacore, Takagi et al. (2001) reported differentiation within and between the Pacific and Atlantic Oceans based on 4 microsatellite loci. Additionally, based on 12 microsatellite loci, Davies et al. (2011) described genetic differentiation within the Mediterranean Sea and between North Atlantic and South Pacific samples. Previous studies on the genetic structure of albacore populations used other types of markers, such as allozymes (Pujolar et al. 2003), mtDNA (Chow & Ushiama 1995, Yeh et al. 1997, Viñas et al. 1999, 2004, Nakadate et al. 2005, Wu et al. 2009), and blood groups (Arrizabalaga et al. 2004). Taken together, these studies showed genetic differences between the North Pacific and Indian Oceans, but barely detected, heterogeneity within oceans (Chow & Ushiama 1995, Nakadate et al. 2005), or even between North Atlantic and Mediterranean stocks (Viñas et al. 1999, Pujolar et al. 2003). In addition, no study to date has included samples from all of the management units.

The main objective of the present study was to assess the population genetic structure of *Thunnus alalunga* on a worldwide scale in order to identify the genetically defined stocks within the species and, based on these findings, discuss the appropriateness of current management units.

MATERIALS AND METHODS

Samples

Tissue samples of 551 albacore were collected between 2003 and 2009 (see Table S1 in the supplement at www.int-res.com/articles/suppl/m471p183_supp.pdf), from the 6 defined stocks (Fig. 1). Samples were stored in 96% ethanol at –20°C until DNA extraction. All samples were provided by AZTI Tecnalia with the exception of those from the Balearic and Adriatic Seas, which were provided by the Department of Life Sciences of the Galway Mayo Institute of Technology (GMIT).

Genetic loci

Thirteen microsatellite loci were included in this study. All of these were originally characterized in tuna species other than *Thunnus alalunga*. Four

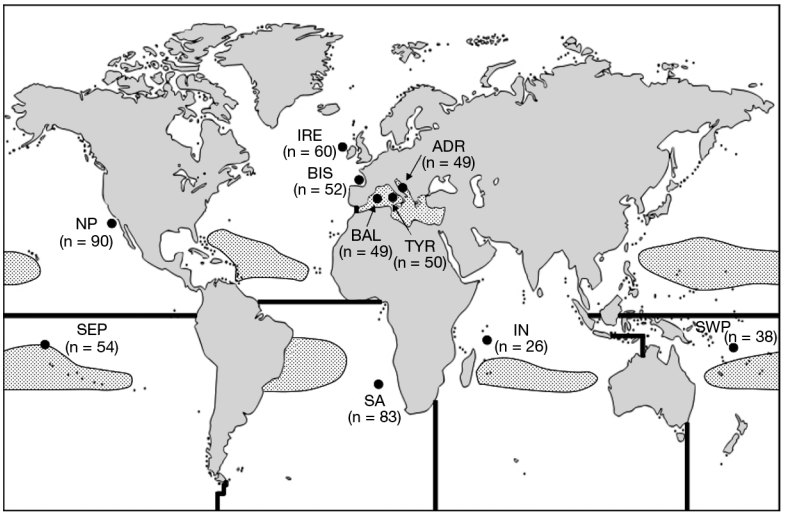

Fig. 1. *Thunnus alalunga*. Sampling locations (black dots), current stock boundaries (black lines), and albacore spawning areas (dotted areas). Information collected from Joseph et al. (1998). Abbreviations for sample locations: Adriatic Sea (ADR), Tyrrhenian Sea (TYR), Balearic Islands (BAL), Bay of Biscay (BIS), Celtic Sea (IRE), South Atlantic (SA), Indian Ocean (IN), North Pacific Ocean (NP), South-East Pacific Ocean (SEP), South-West Pacific Ocean (SWP)

microsatellite loci were originally described in Pacific bluefin tuna *T. orientalis*: *Ttho-1*, *Ttho-4*, *Ttho-6*, and *Ttho-7* (Takagi et al. 1999), and 9 microsatellite markers were originally characterized in Atlantic bluefin tuna *T. thynnus*: *Tth-5*, *Tth-21* (McDowell et al. 2002), *tth4*, *tth62*, *tth1-31*, *tth14*, *tth157*, *tth178*, and *tth226* (Clark et al. 2004).

Laboratory analysis

Genomic DNA was extracted from 50 to 75 mg of muscle tissue for each individual using a Qiagen DNeasy 96 Blood and Tissue Kit. The amount and quality of DNA from each sample was subsequently quantified in a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific). The 13 microsatellite loci were amplified in 4 independent multiplex polymerase chain reactions (PCR). TUNAPLEX1 reaction contained 4 microsatellite loci (*Ttho-1, Ttho-4, Ttho-6*, and *Ttho-7*; annealing temperature, T_a = 52°C), TUNAPLEX2 contained 2 loci (*Tth-5* and *Tth-21*; T_a = 54°C), TUNAPLEX3 contained 2 loci (*tth4* and *tth62*; T_a = 60°C), and TUNAPLEX4 contained 5 loci (*tth1-31*, *tth14*, *tth157*, *tth178*, and *tth226*; T_a = 57°C; Table S2 in the supplement). All PCR reactions were carried out in a final volume of 10 µl containing 7.5 to 20 ng DNA, 1 to 1.5 mM $MgCl_2$, 0.2 to 0.6 µM of each primer, 1× buffer, 0.2 to 0.8 µM dNTPs, and 0.5 to 2.5 units of AmpliTaq Gold DNA Polymerase (Applied Biosystems; Table S3). All multiplex PCRs were performed using the following conditions: 10 min initial denaturation at 95°C, followed by 33 cycles of denaturation for 1 min at 95°C, 30 s at annealing temperature, and 1 min at 72°C for extension. An extra final extension for 10 min at 72°C was included after the last cycle. Two independent runs were performed in an ABI Prism 3100 Genetic Analyzer (Applied Biosystems): the first run for TUNAPLEX1 to -3 and the second for TUNAPLEX4 (Table S2). A 50 to 500 bp size standard (LIZ500) was used to determine allele size. Raw data were processed with GENESCAN 3.7 and analyzed with GENOTYPER 3.7 software (Applied Biosystems).

Descriptive statistics

In order to evaluate deficit or excess of heterozygotes within each sample, the F_{IS} statistic (Weir & Cockerham 1984) was calculated using FSTAT v.2.9.3 (Goudet 2001). The Fisher exact test was used to test for departures from the expectations of Hardy-Weinberg equilibrium (HWE), as implemented using GENEPOP v4.0 software (Rousset 2008). HWE was evaluated for each locus and each sample. FSTAT v.2.9.3 software (Goudet 2001) was used to estimate the mean number of alleles (MNA), allelic richness (AR), observed heterozygosity (H_o), and expected heterozygosity (H_e). The false discovery rate (FDR) procedure (Benjamini & Hochberg 1995) was applied to correct the obtained HWE p-values. The presence and frequency of null alleles was estimated using the EM algorithm implemented in FreeNa (Chapuis & Estoup 2007) for each locus and sample.

Genetic population structure analyses

The existence of population structure in the whole sample was revealed in the Bayesian model-based clustering algorithm implemented using STRUCTURE v2.3.3 software (Pritchard et al. 2000). We used the admixture model and the LOCPRIOR option, and the number of ancestral clusters, k, was determined by comparing log-likelihood ratios in 10 runs for values of k between 1 and 10. Each run consisted of 10 000 iterations with a burn-in period of 10 000. Best k was estimated as proposed by Pritchard et al. (2000). CLUMPP v1.1.2 software (Jakobsson & Rosenberg 2007) was used to determine the optimal assignation of clusters for the analyzed individuals, maximizing similarity between the different structure replications. The resulting clusters were visualized using DISTRUCT v1.1 software (Rosenberg 2004).

All microsatellite loci were tested for natural selection using LOSITAN software (Antao et al. 2008). When using polymorphic DNA markers with high mutation rates, such as microsatellites, to analyze weakly genetically differentiated populations, as in the present case, the role of mutation in genetic data should be tested. To elucidate the role of mutation, the R_{ST} distribution (pR_{ST}) with 95% confidence intervals (CIs; Hardy et al. 2003) was constructed using SPAGeDi v1.2 (Hardy & Vekemans 2002).

To assess genetic diversity, pairwise F_{ST} (Weir & Cockerham 1984) among albacore samples was performed using FSTAT v.2.9.3 software (Goudet 2001). Global corrected p-values for pairwise F_{ST} were obtained from FSTAT software. This software combines p-values of individual loci, weighting them according to their polymorphism level (Petit et al. 2001). A hierarchical analysis of molecular variance (AMOVA; Excoffier et al. 1992) was used to partition the genetic variance between subsamples within groups (F_{SC}) and among groups (F_{CT}) using ARLEQUIN v3.0 (Excoffier et al. 2005).

RESULTS

Genetic diversity within populations

Descriptive statistics across loci and samples, and for each microsatellite and sample are shown in Tables 1 & 2 and Table S4 in the supplement, respectively. H_e, AR, and MNA values were consistently high, similar to prevoius studies carried out with these markers in other species of the genus *Thunnus* (Takagi et al. 1999, McDowell et al. 2002, Clark et al. 2004). All loci proved to be highly polymorphic: between 5 and 33 alleles were detected per locus. *tth14* was the least variable locus (AR = 2.28) and *tth14* was the most variable (AR = 6.66; Table 1); for the analyzed samples, these values oscillated between 4.43 (South Atlantic, SA; see Fig. 1 for sample location codes) and 12.81 (North Pacific Ocean, NP; Table 2). All markers had high H_e (± SE) values ranging from 0.54 ± 0.05 to 0.94 ± 0.02 (Table 1). Similarly high H_e values were found in all analyzed samples, with values ranging from 0.69 ± 0.21 to 0.75 ± 0.19 (Table 2). Significant departures from HWE proportions were found for several microsatellite loci and samples. The single locus test across samples to assess departure from HWE showed no significant p-values for the *Ttho-1*, *Ttho-7*, *tth14*, *tth157*, and *tth226* markers (Table 1). The samples from Balearic Islands (BAL), Bay of Biscay (BIS), SA, Indian Ocean (IN), NP, and South-East Pacific Ocean (SEP) had non-significant p-values for the Fisher exact test for HWE (Table 2). The frequency of null alleles was tested, and 4 of the 13 microsatellite loci (*Ttho-6*, *Tth-5*, *tth62* and *tth178*) exhibited null allele frequencies higher than 0.05, which was considered the threshold value for the presence of null alleles. F_{IS} values for those 4 markers ranged from 0.16 to 0.60, whereas these values for the remaining 9 markers were low, between −0.06

Table 1. *Thunnus alalunga*. Descriptive statistics for the 13 microsatellite loci over all albacore samples analyzed. Number of genotyped individuals (n), allele number (k), mean number of alleles (MNA), allelic richness (AR), mean observed heterozygosity (H_o), mean expected heterozygosity (H_e), Hardy-Weinberg equilibrium p-values (HWE), F_{IS} statistic, mean null alleles frequencies. Mean values are ±SE. *Significant p-values after false discovery rate correction (Benjamini & Hochberg 1995)

Marker	n	k	MNA	AR	H_o	H_e	HWE	F_{IS}	Null
Tth-21	448	5	3.0 ± 0.8	2.45	0.55 ± 0.09	0.54 ± 0.05	0.0050*	−0.06	0.01 ± 0.02
Ttho-1	297	9	6.0 ± 1.7	3.33	0.60 ± 0.18	0.62 ± 0.14	0.0396	0.07	0.02 ± 0.04
Ttho-4	329	32	15.0 ± 3.5	5.69	0.78 ± 0.07	0.88 ± 0.05	0.0050*	0.06	0.03 ± 0.04
Ttho-7	292	19	11.0 ± 4.4	5.54	0.81 ± 0.10	0.86 ± 0.07	0.0349	0.04	0.02 ± 0.03
tth1-31	524	16	11.0 ± 1.9	4.42	0.73 ± 0.09	0.77 ± 0.02	<0.0001*	0.06	0.04 ± 0.04
tth14	521	9	5.0 ± 1.4	2.28	0.62 ± 0.07	0.61 ± 0.04	0.0503	−0.01	0.01 ± 0.02
tth157	535	8	6.0 ± 0.7	3.10	0.61 ± 0.06	0.58 ± 0.04	0.0135	−0.06	0.00 ± 0.01
tth226	529	27	17.5 ± 1.9	6.26	0.91 ± 0.05	0.92 ± 0.02	0.0529	0.02	0.01 ± 0.01
tth4	370	33	21.0 ± 5.2	6.66	0.90 ± 0.08	0.94 ± 0.02	<0.0001*	0.03	0.02 ± 0.02
Ttho-6	270	16	8.5 ± 2.0	4.15	0.59 ± 0.22	0.76 ± 0.09	<0.0001*	0.23	0.11 ± 0.10
Tth-5	473	24	17.0 ± 2.6	5.73	0.68 ± 0.19	0.88 ± 0.04	<0.0001*	0.25	0.11 ± 0.09
tth62	265	14	6.5 ± 2.5	4.09	0.24 ± 0.16	0.74 ± 0.08	<0.0001*	0.60	0.25 ± 0.10
tth178	506	17	11.0 ± 2.0	5.06	0.70 ± 0.06	0.83 ± 0.02	<0.0001*	0.16	0.06 ± 0.03

Table 2. *Thunnus alalunga*. Descriptive statistics for 10 albacore samples over 9 microsatellite loci without null alleles. Number of genotyped individuals (n), mean number of alleles (MNA), allelic richness (AR), mean observed heterozygosity (H_o), mean expected heterozygosity (H_e), Hardy-Weinberg equilibrium p-values (HWE), F_{IS} statistic. Mean values are ±SE. Sample abbreviations as in Fig. 1. *Significant p-values after false discovery rate correction (Benjamini & Hochberg 1995)

Sample	n	MNA	AR	H_o	H_e	HWE	F_{IS}
ADR	49	9.44 ± 6.88	6.02	0.67 ± 0.24	0.69 ± 0.21	0.0056*	0.04
TYR	50	10.11 ± 5.58	9.42	0.72 ± 0.19	0.73 ± 0.18	0.0056*	0.01
BAL	49	10.89 ± 5.58	10.67	0.72 ± 0.16	0.75 ± 0.14	0.0348	0.04
BIS	52	12.22 ± 7.36	11.04	0.71 ± 0.13	0.75 ± 0.17	0.0556	0.04
IRE	60	10.00 ± 6.86	5.16	0.72 ± 0.20	0.75 ± 0.19	0.0001*	0.04
SA	83	9.22 ± 6.06	4.43	0.71 ± 0.15	0.73 ± 0.17	0.0529	0.04
IN	26	10.56 ± 7.11	10.30	0.72 ± 0.19	0.72 ± 0.18	0.0557	0.01
NP	90	13.33 ± 7.53	12.81	0.75 ± 0.17	0.74 ± 0.17	0.0135	0.00
SEP	54	11.56 ± 7.07	11.22	0.73 ± 0.13	0.75 ± 0.16	0.0396	0.02
SWP	38	10.44 ± 5.57	10.01	0.73 ± 0.09	0.75 ± 0.15	0.0001*	0.02

and 0.07. F_{IS} values for the samples oscillated between 0.00 and 0.04.

Genetic diversity between populations: population genetic structure

Due to the presence of null alleles, 4 microsatellite loci (*Ttho-6*, *Tth-5*, *tth62*, and *tth178*) were eliminated from subsequent analyses. STRUCTURE analysis evidenced population structure, with k = 3 (see Fig. S1 in the supplement at www.int-res.com/articles/suppl/m471p183_supp.pdf) being the most likely number of clusters (Fig. 2): the Mediterranean group, the Indo-Atlantic cluster, and the Pacific cluster.

Natural selection was examined for the 9 microsatellite markers. The Beaumont-Nichols test suggested directional natural selection at 1 microsatellite locus (*Ttho-7*). A BLAST search revealed that this locus was highly similar (e-value = 2×10^{-4}, 78% similarity) to cpu-UA/3 MHC class I antigen gene (Genbank accession number AY008848.2) from the teleost fish *Ictalurus punctatus* (Antao et al. 2001). This marker was removed from further analyses since F_{ST} was calculated with neutral markers, in order to detect aspects of migration and drift that might otherwise be obscured by effects of selection. Removing this marker lead to a change in the number of populations from k = 3 to k = 2; 1 group consists of Mediterranean samples and the remaining samples were included in the other group (results not shown). Consequently, the *Ttho-7* marker likely differentiates the South Pacific from the Indo-Atlantic group at the level of STRUCTURE analysis.

The overall multilocus R_{ST} value (R_{ST} = 0.023) did not exceed the 95% CI of pR_{ST} values (pR_{ST} = 0.009; CI: –0.001 to 0.024), and therefore was not significant (p = 0.060; Table S5 in the supplement). Pairwise F_{ST} values ranged from –0.007 (SA versus South-West Pacific Ocean, SWP) to 0.026 (Adriatic Sea, ADR versus NP and ADR versus SEP). Interestingly, in the Mediterranean Sea, the differentiation observed between ADR and BAL was significant (F_{ST} = 0.012 ± 0.012; p = 0.005). We did not detect significant differences between the samples from the Atlantic and Indian Oceans (BIS, Celtic Sea, IRE and SA versus IN), nor between Pacific Ocean samples (all pairwise comparisons using NP, SEP and SWP; Table 3).

Similar results were obtained using AMOVA analysis. All possible combinations of populations were tested, grouping the populations in levels from 3 to 5 groups. Results for a minimal F_{SC}/F_{CT} ratio (least variance within and highest between groups) for each grouping level are shown in Table 4. In the 3-cluster option, the best combination of populations was coincident with the STRUCTURE results. The 5-cluster option showed the lowest F_{SC}/F_{CT} ratio, composed of (1) East Mediterranean Sea

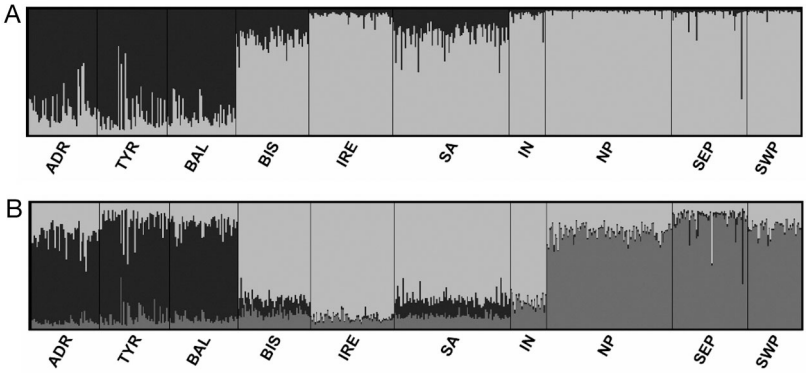

Fig. 2. *Thunnus alalunga*. Model-based clustering method for using multilocus genotype data to infer population structure. Population structuring test with STRUCTURE 2.3.3 software based on 9 microsatellite loci. (A) k (number of clusters) = 2; (B) k = 3. Sample abbreviations as in Fig. 1

Table 3. *Thunnus alalunga*. Pairwise F_{ST} values (below the diagonal) and p-values (above the diagonal) among samples. Sample abbreviations as in Fig. 1. *p < 0.05

Sample	ADR	TYR	BAL	BIS	IRE	SA	IN	NP	SEP	SWP
ADR	–	0.381	0.005*	0.006*	0.027*	0.103	0.011*	0.000*	0.000*	0.002*
TYR	0.000	–	0.269	0.024*	0.072	0.469	0.011*	0.000*	0.000*	0.002*
BAL	0.012*	0.001	–	0.006*	0.056	0.329	0.003*	0.000*	0.011*	0.003*
BIS	0.011*	0.006*	0.009*	–	0.503	0.689	0.431	0.006*	0.013*	0.165
IRE	0.011*	0.009	0.010	–0.001	–	0.282	0.617	0.131	0.074	0.102
SA	0.008	0.000	0.002	–0.004	0.004	–	0.463	0.266	0.327	0.833
IN	0.012*	0.012*	0.014*	0.000	–0.002	0.000	–	0.214	0.029*	0.094
NP	0.026*	0.018*	0.011*	0.007*	0.006	0.003	0.002	–	0.550	0.083
SEP	0.026*	0.017*	0.007*	0.008*	0.009	0.002	0.008*	0.000	–	0.175
SWP	0.015*	0.014*	0.011*	0.003	0.008	–0.007	0.007	0.004	0.003	–

Table 4. *Thunnus alalunga*. Hierarchical analysis of molecular variance. Number of groups, grouped populations (indicated by brackets), notes about each grouping, and fixation indices: F_{ST} (variance among populations relative to the total variance), F_{SC} (variance among populations within groups), and F_{CT} (variance among groups relative to the total variance). Sample abbreviations as in Fig. 1. *p < 0.05

No. groups	Sample	F_{ST}	F_{SC}	F_{CT}	F_{SC}/F_{CT}	Notes
6	(ADR, TYR, BAL) (BIS, IRE) (SA) (IN) (NP) (SEP, SWP)	0.00839*	0.00335	0.00504	0.66100	6 stocks
5	(ADR, TYR) (BAL) (BIS, IRE, SA, IN) (NP, SEP) (SWP)	0.00931*	0.00000	0.00954*	0.00000	Best 5 groups
4	(ADR, TYR) (BAL) (BIS, IRE, SA, IN) (NP, SEP, SEP)	0.00974*	0.00069	0.00905*	0.07300	Best 4 groups
3	(ADR, TYR, BAL) (BIS, IRE, SA, IN) (NP, SEP, SWP)	0.00983*	0.00205	0.00778*	0.25800	STRUCTURE output and best 3 groups

samples, (2) a Balearic sample, (3) Atlantic and Indian samples, (4) North and Southeast Pacific samples, and (5) Southwestern Pacific samples. It is associated with the highest (and significant) differences among groups (F_{CT} = 0.00954), and the lowest differences among geographic samples within groups (F_{SC} = 0.00000). Nevertheless, using only our best 5 microsatellites (those with >80% scoring success), the pairwise F_{ST} values were lower (Table S6 in the supplement), and the F_{SC} value in AMOVA analysis was already 0 in the 3-cluster option.

DISCUSSION

Information about population genetic structure is critical for the optimal conservation of marine biological communities. In this study, we assessed the population genetic structure of albacore *Thunnus alalunga*. A total of 551 individuals from all current management units of albacore were genotyped for 13 microsatellite loci. Currently established management units for this species exhibit clear mismatches and discrepancies with genetic population structure. We found that (1) Atlantic and Indian Ocean samples were homogeneous, and (2) a possible differentiation exists within the Pacific Ocean and also within the Mediterranean Sea.

DNA markers applied to a worldwide sample of albacore showed that some management units could be included in a single genetic unit (Atlantic and Indian Oceans, and North Pacific Ocean and Southeast Pacific). On the other hand, it appears that at least 2 genetic groups of albacore are currently managed as one in the Mediterranean Sea (East and West). Genetic diversity within species, due to local adaptation or stochastic differentiation, should be taken into account for the optimal management and conservation of the species. Therefore, a closer look at the genetic structure of albacore populations in the Mediterranean Sea and in the Pacific Ocean is advised; since this is the first study to investigate the worldwide population genetic structure of albacore, by analyzing albacore samples from all of the stocks defined for this species.

Genetic diversity within populations

Overall, marine species display high intra-population levels of genetic diversity and weak genetic differentiation among populations (DeWoody & Avise 2000, Nielsen et al. 2009, Knutsen et al. 2011). In the present study, high values for allelic diversity (AR = 4.43 to 12.81; H_e = 0.69 to 0.75) were observed in samples from all studied locations. These values are similar to those previously reported for bluefin tuna (*Thunnus thynnus* and *T. orientalis*: Carlsson et al. 2004, AR = 5.33 to 5.63, H_e = 0.66 to 0.70; Carlsson et al. 2007: H_e = 0.65 to 0.66; Riccioni et al. 2010: AR = 8.5–10.0, H_e = 0.70 to 0.76) and albacore (Takagi et al. 2001: H_e = 0.65 to 0.86), and they are consistent with the average value for marine fishes (DeWoody & Avise 2000: $H_e = 0.66 \pm 0.22$). Furthermore, F_{IS} values obtained for albacore (F_{IS} = 0.00 to 0.04) are within the range of values reported for *T. thynnus* (Riccioni et al. 2010: F_{IS} = −0.05 to 0.09).

Genetic diversity between populations: population genetic structure

Our data support the existence of population structure within *Thunnus alalunga*, probably due to gen-

etic drift and not mutation, as the R_{ST} test result was non-significant as opposed to the F_{ST} pairwise comparisons, which identified weak, but significant differences. The low levels of genetic differentiation reported for albacore have previously been ascribed to their biology, such as reproduction in the open ocean, their highly migratory nature, and/or large effective population sizes (Durand et al. 2005, Poulsen et al. 2006).

With regard to the genetic delimitation of albacore populations, different statistical methods yielded different results on the number of inferred populations. First, STRUCTURE software detected 3 albacore genetic groups (k = 3): (1) the Mediterranean group, (2) the Indo-Atlantic group, and (3) the Pacific group. Second, pairwise F_{ST} analysis suggested the existence of a subdivision within the Mediterranean Sea: Tyrrhenian and Adriatic Sea samples were grouped together and could be differentiated from the Balearic Sea. Third, AMOVA analysis indicated another subdivision within the Pacific Ocean. Different sensitivity of the statistics might explain this lack of coincidence in the number of populations recovered, but they are likely showing different hierarchical levels of grouping within the species.

Thus, the STRUCTURE analysis would only recognize the more basal clustering (Mediterranean, Indo-Atlantic, and Pacific clusters), whereas F_{ST} and AMOVA would detect more contemporary aspects of population structure: those found within the Mediterranean Sea (East versus West), and those within the Pacific Ocean, where the Southwest Pacific might be differentiated from the rest.

The genetic relationship between North Atlantic and Mediterranean Sea albacore populations has been the subject of much debate. While initial studies did not find differences, probably due to the small sample size used by Viñas et al. (1999) or the lack of resolution of allozyme markers in the study by Pujolar et al. (2003), genetic differences were detected using other types of genetic markers such as blood groups (Arrizabalaga et al. 2004) and mtDNA (Viñas et al. 2004, Nakadate et al. 2005). In the present study, Mediterranean samples showed the highest and most significant F_{ST} values when compared to the oceanic ones (pairwise comparisons). This was also supported by STRUCTURE analysis (k = 2), which distinguished the Mediterranean Sea population from the rest. These results are consistent with the Mediterranean Sea populations of albacore being the ones with the smallest gene flow to or from other populations, suggesting an isolation event leading to their differentiation by genetic drift. In fact, compared to other albacore populations, the Mediterranean albacore show the most specific traits: smaller maximum length (Megalofonou 2000), lower trophic level (Goñi et al. 2011), and earlier maturity (ICCAT 2010), likely resulting from a local adaptation to living in a relatively small area, compared to oceanic albacore. Tagging data have revealed low migration out of the Mediterranean during the last decades (Arrizabalaga et al. 2004), so it is likely that compared to oceanic albacore, migrations within the Mediterranean Sea require less energy, which, in turn, could be invested in reproduction at earlier ages. Small size was also hypothesized to be an adaptation to hotter temperatures, as it favors heat loss (Bard 1981).

Moreover, our study suggests the existence of population structure within the Mediterranean Sea, which might reflect 2 genetically defined units: the western (the Balearic Sea sample) and the central-eastern (Tyrrhenian and Adriatic) population, which is consistent with Davies et al. (2011). Although there are no pronounced oceanographic barriers which might limit gene flow among populations in the Mediterranean Sea, differentiation between Eastern and Western Mediterranean areas has also been reported for the congeneric bluefin tuna *Thunnus thynnus* (Carlsson et al. 2004, Riccioni et al. 2010) using mtDNA and/or microsatellite data. All of these results suggest that at least 2 distinct albacore spawning areas may exist in the Mediterranean, although there is only a single one described (ICCAT 2010; Fig. 1).

In contrast to the genetic differentiation observed in the Mediterranean Sea, the Atlantic Ocean and Indian Ocean samples behave as a single genetic group. Previous studies using mtDNA (Yeh et al. 1997) as well as blood groups (Arrizabalaga et al. 2004) reported differences between them, although Arrizabalaga et al. (2004) suggested genetic proximity. Based on catch statistics, Morita (1977) suggested migration of albacore between these 2 oceans off South Africa, which could be promoted by the strong Agulhas Current. The same phenomenon was also suggested by González et al. (2008) in the congeneric bigeye tuna *Thunus obesus*. Thus, the observed genetic homogeneity between South Atlantic and Indian Oceans might be regarded as evidence of effective migration between South Atlantic and Indian albacore populations. Nevertheless, a more exhaustive study of these regions should be carried out, enlarging sample sizes and including new samples from the eastern region of the Indian Ocean and the western Atlantic.

Finally, the genetic structure of albacore in the Pacific Ocean appears to be more complex than presently recognized in terms of the division of stocks. Using microsatellite markers, Takagi et al. (2001) described significant differences between the 2 Pacific hemisphere samples, as well as between Southwest and Southeast Pacific albacore. Our results failed to detect differences between North and South Pacific albacore, as did Chow & Ushiama (1995) using mtDNA, but they suggested genetic heterogeneity within South Pacific albacore.

Prospective analyses

None of the individuals included in the present study were young of the year (YOY). Therefore, given the highly migratory nature of albacore, they could originate from a large number of potential spawning grounds. Future studies could benefit from including eggs, larvae, and/or young of the year. As such, genetic differences should reflect the spawning areas of the species, as some homing behavior is expected (Fonteneau & Soubrier 1996), providing a genetic reference that is more consistent with the 6 stocks assummed for management purposes (Fig. 1).

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