Microsatellite analysis of albacore tuna (Thunnus alalunga): population genetic structure in the Nord-East Atlantic Ocean and Mediterranean sea

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Microsatellite analysis of albacore tuna (*Thunnus alalunga*): population genetic structure in the North-East Atlantic Ocean and Mediterranean Sea

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Abstract Stock heterogeneity was investigated in albacore tuna (*Thunnus alalunga*, Bonnaterre 1788), a commercially important species in the North Atlantic Ocean and Mediterranean Sea. Twelve polymorphic microsatellite loci were examined in 581 albacore tuna from nine locations, four in the north-east Atlantic Ocean (NEA), three in the Mediterranean Sea (MED) and two in the south-western Pacific Ocean (SWP). Maximum numbers of alleles per locus ranged from 9 to 38 (sample mean, 5.2–22.6 per locus; overall mean, 14.2 ± 0.47 SE), and observed heterozygosities per locus ranged from 0.44 to 1.00 (overall mean: 0.79 ± 0.19 SE). Significant deficits of heterozygotes were observed in 20% of tests. Multilocus F_{ST} values were observed ranging from 0.00 to $\Theta = 0.036$ and $\Theta' = 0.253$, with a mean of $\Theta = 0.013$ and $\Theta' = 0.079$. Pairwise F_{ST} values showed that the SWP, NEA and MED stocks were significantly distinct from one another, thus corroborating findings in previous studies based on mitochondrial DNA, nuclear DNA (other than microsatellites) and allozyme analyses. Heterogeneity was observed for the first time between samples within the Mediterranean Sea. GENELAND indicated the potential presence of three populations across the NEA and two separate populations in the Mediterranean Sea. Observed genetic structure may be related to migration patterns and timing of movements of subpopulations to the feeding grounds in either summer or autumn. We suggest that a more intensive survey be conducted throughout the entire fishing season to ratify or refute the currently accepted genetic homogeneity within the NEA albacore stock.

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Introduction

Waldman (1999) defines a "stock" as an exploitable population with some degree of genetic integrity. Other definitions of stock have less or no emphasis on genetic structure (Cadrin et al. 2005). Stocks can be delineated from observations relating to various aspects of life history (Griffiths 1997). Discrimination of stock components into genetic stocks can be undertaken by molecular methods, such as allozyme analysis and mitochondrial DNA studies, or by directly targeting variations in nuclear DNA composition. The stock structure of albacore (*Thunnus*

alalunga) has been identified globally by a variety of methods, primarily from information gathered directly from the fishery. Catch rates from each location and catch at length data (incorporated with information from ages determined from the calcareous structures) have been used to determine differences in growth rates and stock abundance in each ocean basin (ICCAT 1996; Miyake et al. 2004). In addition, conventional tag-recapture studies using plastic floy tags attached to individual fish have provided information on the migratory movements of albacore. It is considered that separate north and south stocks are present in both the Atlantic and Pacific Oceans as there has been no evidence to date of cross-equatorial migration from conventional tag-recapture studies, with the latitudinal differences observed in catch rates and seasonality of spawning (ICCAT 1996; Ramon and Bailey 1996). Therefore, fish observed in the northern and southern hemispheres are managed as separate units. Beardsley (1969) proposed that small numbers of albacore may undertake inter-oceanic migrations between the South Atlantic Ocean and the Indian Ocean; however, such claims remain to be substantiated through tagging studies, and hence, the Indian Ocean population is managed as a separate stock (Chen et al. 2005). Results from tagging surveys by Arrizabalaga et al. (2002, 2003) have shown that only very limited migration occurs between the North Atlantic Ocean and Mediterranean Sea, and genetic differences have been observed between the two regions using nuclear DNA (Nakadate et al. 2005). Consequently, the Mediterranean stock is managed as a separate unit (ICCAT 1996). In summary, based on information gathered from the fishery, six populations of albacore are recognised as stock units: Northern Atlantic, Southern Atlantic, Mediterranean, Indian, Northern Pacific and Southern Pacific (Miyake et al. 2004; ICCAT 2007).

Stock identification by genetic methods may indicate previously unidentified population structuring (Hoarau et al. 2004; Carlsson et al. 2006; Was et al. 2008; Kovach et al. 2010). Results from molecular genetic studies presently support the recognised subdivision of albacore populations into the six recognised stocks. Despite the lack of differentiation in mitochondrial DNA (mtDNA) (using restriction endonuclease analysis) observed between albacore sampled in the South Atlantic and North Pacific Oceans (Graves and Dizon 1989), Chow and Kishino (1995) showed differentiation between North and South Atlantic and Indo-Pacific albacore populations using PCR–restriction fragment length polymorphism (RFLP) analysis of the mtDNA ATPase gene. Further analysis of the mtDNA D-loop region of albacore in the Indo-Atlantic region by Yeh et al. (1997) showed that populations in the South Atlantic and Eastern Indian Oceans were genetically distinct. Investigations into the genetic structure of North Atlantic and Mediterranean stocks, also using the mtDNA D-loop region (Viñas et al. 1999) as well as allozymes (Pujolar et al. 2003), showed genetic homogeneity between the two stocks. However, differences in morphometric characteristics, growth rates and reproductive areas had been previously reported for the two stocks (Megalofonou 2000). Viñas et al. (2004) conducted an additional study using the mtDNA control region in combination with nuclear DNA markers with their results indicating there was a small but significant difference between the two stocks. Nakadate et al. (2005) using nucleotide sequence variations of the glucose-6-phosphate dehydrogenase gene intron (G6PDH) and the mtDNA D-loop region corroborated their findings. Analysis of blood lectins (Arrizabalaga et al. 2004) indicated that the north-east Atlantic, South Atlantic and south-east Pacific populations were distinct but that South Atlantic and Indian Ocean populations were genetically similar.

Many of the previous studies address differences between stocks in different oceanic regions, with few investigating genetic heterogeneity within regions. Recently, Wu et al. (2009) studied albacore from three areas in the north-western Pacific Ocean (Taiwan, Japan and North of Hawaii) using analysis of mtDNA sequence data. Their findings showed that albacore tuna in the region constituted a single stock with no significant differences in geographic distributions. A preliminary study using microsatellites on albacore tuna revealed significant levels of differentiation between and within Atlantic and Pacific Oceans compared to mtDNA analyses of samples from the same areas (Takagi et al. 2001).

In view of the paucity of information on the genetic structure of albacore tuna within the North Atlantic Ocean (NEA) and Mediterranean Sea, the main objective of the present study was to analyse spatial, seasonal and temporal genetic heterogeneity using 12 microsatellite markers in albacore tuna collected in consecutive years from 2005 to 2007, from four NEA areas (waters off the south-west of Ireland, towards the southern Bay of Biscay along the Porcupine Ridge and off the northern coast of Africa near the Canary Islands), and from central (Tyrrhenian and Southern Adriatic Seas) and western (Balearic Sea) Mediterranean Sea regions. All albacore sampled with the exception of those collected from near the Canary Islands were juveniles.

Materials and methods

Sampling

A total of 14 samples ($N = 581$) of albacore were collected from NEA (West of Ireland, South Bay of Biscay and Canary Islands) and Mediterranean Sea (Med) using a variety of fishing methods (Table 1). All samples were

Table 1 Details of albacore tuna (*Thunnus alalunga*) samples

Sample	Sample size	Location	Location name	Date	Survey vessel	Fishing method	Mean LF (cm)	Mean weight (kg)	Maturity	Age (years)/number of fish
Med_05	52	40 00′N 1 34′E	Western Mediterranean	Nov-05	–	L	63 ± 2. 6	4.68 ± 0.61	J	3/22, 4/28, 5/2
Med_06	50	39 49′N 13 00′E	Central Mediterranean	Nov-06	–	L	65 ± 4.0	5.06 ± 0.97	J	3/19, 4/17, 5/10, 6/1 (no information for 3 fish)
Med_07	50	39 49′N 13 00′E	Central Mediterranean	Dec-07	–	L	75 ± 3.4	7.90 ± 1.10	J	2/1, 3/8, 4/22, 5/16, 6/3
CS1_05	38	48 30′N 10 38′W	Celtic sea	Sep-05	MFV Maggie C	T	56 ± 5.7	–	J	2/31, 3/5, 4/0, 5/1, 6/2
CS2_05	50	47 34′N 12 28′W	Celtic sea	Jul-05	MFV Mellifont	T	62 ± 7.9	5.62 ± 2.07	J	2/23, 3/37, 4/9, 5/2
CS3_05	50	48 21′N 10 29′W	Celtic sea	Sep-05	MFV Supreme 2	P	66 ± 10.2	–	J	2/13, 3/17, 4/18, 5/2
CS1_06	57	47 32′N 9 30′W	Celtic sea	Aug-06	MFV Ocean Dawn	T	59.5 ± 8.5	5.00 ± 2.60	J	2/35, 3/11, 4/7, 5/2 (no information for 1 fish)
BB1_06	67	44 50′N 3 20′W	Bay of Biscay	Aug-06	MFV Skipper	P	74 ± 12.1	9.60 ± 4.50	J	2/13, 3/10, 4/17, 5/22, 6/3, 7/1, 8/1
BB2_06	16	43 36′N 2 23′W	Bay of Biscay	Sep-06	–	T	54 ± 9.9	1.40 ± 0.20	J	1/6, 2/8, 3/2
WI1_07	46	52 19′N 12 23′W	West of Ireland	Sep-07	MFV De Linn	T	72 ± 4.4	8.03 ± 1.69	J	3/7, 4/24, 5/11, 6/3 (no information for 1 fish)
WI2_07	50	51 33′N 13 51′ W	West of Ireland	Sep-07	MFV Teresa Mae	T	67 ± 5.0	–	J	2/1, 3/30, 4/17, 5/2
CAN_07	20	27 00′N 17 00′W	Canary Islands	Mar-07	–	–	106.8 ± 7.6	–	A	–
Pac_03	18	21 00′S 163 50′E	SW Pacific	Jul-03	–	–	89 ± 4.6	–	A	–
Pac_05	20	14 00′S 135 00′W	SW Pacific	Sep-05	–	–	98 ± 4.6	–	A	–

– indicates no information/data; fishing method, *L* pelagic longline, *P* pair trawling, *T* trolling; maturity based on size *J* juvenile, *A* adult

obtained from commercial fishing operations, which targeted aggregations of fish in summer feeding grounds. Fish were measured for fork length (L_F) to the nearest centimetre and weighed to the nearest 10 g. Maturity was assigned on the basis of age and length with fish <5 years and with a $L_F < 90$ cm considered immature or juveniles (see Santiago and Arrizabalaga 2005). Sample details are shown in Table 1. A 5-mm^3 piece of white muscle was removed from behind the head in each individual and stored in 96% ethanol. Two south-west Pacific Ocean (Pac) sample sets from archived freeze-dried tissue (2003 and 2005) were acquired to serve as out-group samples.

Microsatellite analysis

Genomic DNA was extracted from muscle tissue ($\sim$2 mm^3) using the phenol–chloroform method of Sambrook et al. (1989). DNA was diluted 1:5 in sterile deionised water to give a concentration of 30–100 ng μl^{-1}. The microsatellite loci developed for bluefin tuna were cross-amplified in albacore, and of those successfully amplifying, twelve were selected for analysis: *Ttho*4, *Ttho*6, *Ttho*7 (Takagi et al. 1999), *Tth*5 (McDowell et al. 2002), *Tth*4, *Tth*14, *Tth*17, *Tth*185, *Tth*254, *Tth*1-31, *Tth*12-29 and *Tth*16-2 (Clark et al. 2004). The reverse primer of each pair was end-labelled with fluorescent dye (700-IRD or 800-IRD, Li-COR, Lincoln, NE, USA). Polymerase chain reaction (PCR) was carried out using a reaction volume of 10 µl, containing 0.17U *Taq* polymerase, 1× reaction buffer (Bioline), 0.25 µM of each primer, 0.2 µM of mixed dNTPs, 0.2 mM $MgCl_2$ and 1 µl of the 1.5 dilution of template DNA. Thermocycling procedures for each locus were exactly those in Takagi et al. (1999); McDowell et al. (2002), and Clark et al. (2004).

Amplification products were separated on 6% polyacrylamide gels using a Li-COR 4300 automated sequencer (Li-COR, Lincoln, NE, USA). PCR products were diluted 1:5–1:15 with deionised water and 1 µl of the dilution mixed 1:3 with bromophenol blue in formamide loading buffer. A sizing standard (50–350 base pairs, Li-COR, Lincoln, NE, USA) was run in the centre and at both ends of the gels to calibrate allele size. An internal reference sample consisting of individuals where allele sizes had been predetermined was included to ensure consistency in genotype scoring across runs. Fragment length polymorphisms were scored with GENE IMAGIR software (Li-COR, Lincoln, NE, USA).

Data analysis

Allelic distribution, observed (H_O) and unbiased expected (H_E) heterozygosity estimates for the 14 samples were computed for each locus individually and as a multilocus estimate using GENETIX 4.05.2 (Belkhir et al. 2002). Tests for conformance to Hardy–Weinberg equilibrium (HWE), single and multilocus F_{IS} (Weir and Cockerham 1984), significance of heterozygote deficiency and linkage disequilibrium between pairs of loci were performed using GENEPOP v.4.0 (Rousset 2008) with specified Markov chain parameters (10,000 dememorisation steps, 100 batches, 5,000 iterations per batch). Sequential goodness of fit (SGoF) (Carvajal-Rodriguez et al. 2009) was employed in all multiple testing in order to reduce Type I errors. Deviations from HWE expectations were manually checked for the evidence of heterozygote or homozygote excess, and rare allele combinations. In locus–sample combinations where significant heterozygote deficiency was detected, the frequency of null alleles was estimated by the EM algorithm (Dempster et al. 1977) in the program FreeNA (Chapuis and Estoup 2007).

Global and pairwise F_{ST} estimated using traditional (Θ) (Weir and Cockerham 1984) and heterozygosity-corrected estimators (Θ') (Hedrick 2005; Meirmans and Hedrick 2011) were employed to infer population differentiation. Two heterozygosity-independent methods were also employed to assess population structuring: Pairwise exact *G* tests were performed in GENEPOP v.4.0; correspondence analysis (CA) was performed using the Adegenet package in R (Jombart 2008). For the latter analysis, all loci were included, plots were centred in the origin and missing data were replaced using mean χ^2 distance. The first and second principle components (PC) with the highest eigenvalues were plotted to reveal the relative typology of the samples based on their multilocus allele distributions.

Microsatellite genotypes and sample spatial location data were analysed for all loci and samples in GENELAND package in R (Guillot et al. 2005, 2008). The geographical information was used to detect spatial delineation of genetic discontinuities, where the number of population units is treated as an unknown parameter. The number of populations (*K*) was inferred by running the Markov chain Monte Carlo (MCMC) analysis using numbers of iterations varying from 100,000 to 1,000,000. The maximum number of *K* after the initial analysis was set at a minimum of 1 and a maximum of 10. The MCMC analysis was run at 100,000 iterations with 100 burn-in generations. The analysis was run with correlated allele frequency models and true spatial and null allele models. Consistency across the best suite of parameters was assessed across ten independent runs.

Results

Genetic diversity and HWE

A total of 581 fish from 14 locations in NEA, SWP and MED were genotyped at 12 microsatellite loci. All loci

Table 2 Summary of genetic variation in albacore tuna (*Thunnus alalunga*) for twelve microsatellite loci at 14 locations

Pop	Ttho4	Ttho6	Ttho7	Tth4	Tth5	Tth14	Tth17	Tth185	Tth254	Tth1-31	Tth12-29	Tth16-2
MED_05												
N	52	52	52	51	52	52	52	52	52	52	52	52
N_A	21	9	11	25	16	7	17	18	16	11	6	7
N_E	10.052	3.671	5.485	11.309	7.748	2.749	5.859	7.988	9.089	5.175	2.569	2.439
H_O	0.827	0.673	0.885	0.882	0.885	0.519	0.846	0.827	0.712	0.673	0.596	0.500
H_E	0.901	0.728	0.818	0.912	0.871	0.636	0.829	0.875	0.890	0.807	0.611	0.590
F_{IS}	0.091*	0.085	−0.072	**0.042**	−0.006	0.193**	−0.011	0.064	**0.210****	0.175*	0.034	0.178**
MED_06												
N	50	50	50	49	49	50	50	50	50	50	50	50
N_A	21	9	9	22	17	5	18	17	17	16	8	8
N_E	10.101	3.828	4.480	12.344	9.252	2.484	10.753	9.225	8.897	5.097	3.287	2.585
H_O	0.860	0.720	0.820	0.918	0.837	0.600	0.860	0.920	0.780	0.740	0.720	0.500
H_E	0.901	0.739	0.777	0.919	0.892	0.597	0.907	0.892	0.888	0.804	0.696	0.613
F_{IS}	**0.056**	0.036	−0.046	0.011	0.072	0.006	0.021	−0.022	0.134*	0.067***	−0.047	**0.263*****
MED_07												
N	50	50	48	49	50	50	50	49	49	50	50	50
N_A	21	7	11	21	16	6	17	16	23	13	9	6
N_E	7.764	3.365	4.017	12.440	8.000	2.629	11.038	6.860	10.889	4.440	3.294	2.530
H_O	0.880	0.720	0.750	0.878	0.920	0.620	0.860	0.898	0.959	0.860	0.660	0.580
H_E	0.871	0.703	0.751	0.920	0.875	0.620	0.909	0.854	0.908	0.775	0.696	0.605
F_{IS}	0.000	−0.014	0.012	0.056	−0.413	0.010	**0.064****	−0.041	−0.046	−0.100	0.038	0.051
CS1_05												
N	38	38	38	36	38	38	37	36	38	38	38	38
N_A	19	11	15	27	18	5	19	19	23	9	7	5
N_E	9.110	3.297	5.730	18.000	9.286	2.337	11.904	12.284	13.248	4.143	2.664	2.657
H_O	0.921	0.579	0.816	0.972	0.842	0.711	0.838	0.861	1.000	0.868	0.632	0.605
H_E	0.890	0.697	0.825	0.944	0.892	0.572	0.916	0.919	0.925	0.759	0.625	0.624
F_{IS}	−0.021	0.182***	0.025	−0.015	0.070	−0.230	0.099	0.077	−0.068	−0.132	0.002	0.043
CS2_05												
N	50	50	50	49	50	50	50	50	50	50	50	50
N_A	21	11	16	27	21	7	21	21	18	11	8	6
N_E	9.804	3.615	8.143	15.196	12.788	2.585	10.846	15.385	9.653	5.097	2.790	2.773
H_O	0.940	0.720	0.860	0.939	0.920	0.580	0.860	0.960	0.800	0.860	0.620	0.600
H_E	0.898	0.723	0.877	0.934	0.922	0.613	0.908	0.935	0.896	0.804	0.642	0.639
F_{IS}	−0.037	0.015	0.030	0.005	0.012	0.064	**0.063**	0.017	0.118*	−0.060	0.044	**0.042**

Table 2 continued

Pop	Ttho4	Ttho6	Ttho7	Tth4	Tth5	Tth14	Tth17	Tth185	Tth254	Tth1-31	Tth12-29	Tth16-2
CS3_05												
N	50	50	50	49	48	50	48	49	50	50	50	50
N_A	21	10	14	24	21	6	23	22	25	12	7	4
N_E	7.289	4.437	5.086	16.908	11.077	2.604	9.521	12.005	13.966	4.608	2.505	2.537
H_O	0.900	0.720	0.800	0.878	0.833	0.640	0.833	0.837	0.980	0.800	0.720	0.640
H_E	0.863	0.775	0.803	0.941	0.910	0.616	0.895	0.917	0.928	0.783	0.601	0.606
F_{IS}	−0.033	0.081*	0.014	0.078*	0.094	−0.029	0.079**	0.098*	−0.046	−0.012	−0.188	−0.046
CS1_06												
N	57	57	57	55	56	57	57	57	57	57	57	57
N_A	21	13	14	26	20	6	23	23	20	13	8	6
N_E	9.038	3.893	6.377	14.439	9.664	3.165	14.188	11.301	10.830	5.967	2.861	2.814
H_O	0.842	0.649	0.825	0.964	0.929	0.561	0.877	0.895	0.825	0.860	0.754	0.614
H_E	0.889	0.743	0.843	0.931	0.897	0.684	0.930	0.912	0.908	0.832	0.651	0.645
F_{IS}	0.062	0.135**	0.031*	−0.026	−0.027	0.188**	0.065*	0.027	0.100***	−0.024	−0.148	**0.031***
BB1_06												
N	67	67	67	67	67	67	67	67	67	67	67	67
N_A	20	11	19	28	21	4	20	22	27	17	7	7
N_E	9.855	3.286	7.615	17.884	11.251	2.572	11.125	13.400	13.941	6.417	2.327	3.488
H_O	0.910	0.627	0.881	0.955	0.940	0.657	0.896	0.866	0.806	0.866	0.478	0.537
H_E	0.899	0.696	0.869	0.944	0.911	0.611	0.910	0.925	0.928	0.844	0.570	0.713
F_{IS}	0.001*	0.096	0.001*	−0.002	−0.023	−0.056	0.028*	0.061	0.147***	−0.048*	0.201*	**0.211****
BB2_06												
N	16	16	16	16	16	16	16	16	16	16	16	16
N_A	17	8	9	15	15	5	14	14	18	10	5	5
N_E	8.828	3.346	6.400	11.907	8.828	2.738	8.828	10.667	11.130	4.267	2.116	2.393
H_O	0.875	0.625	0.813	1.000	0.938	0.813	0.938	0.875	0.938	0.688	0.438	0.438
H_E	0.887	0.701	0.844	0.916	0.887	0.635	0.887	0.906	0.910	0.766	0.527	0.582
F_{IS}	0.046	0.140*	0.069	−0.060	−0.025	−0.250	−0.025	0.067	0.002	0.060	0.202	0.278
WI1_07												
N	46	46	46	46	45	46	46	46	46	46	46	46
N_A	19	10	17	25	20	4	24	21	24	13	7	6
N_E	6.551	4.157	6.107	17.782	11.066	2.411	15.223	12.824	12.447	4.853	2.519	2.427
H_O	0.870	0.804	0.761	0.891	0.867	0.522	0.913	0.913	0.891	0.826	0.478	0.500
H_E	0.847	0.759	0.836	0.944	0.910	0.585	0.934	0.922	0.920	0.794	0.603	0.588
F_{IS}	−0.015	−0.048	0.101	0.067	0.058	0.119	0.034	0.021	0.042	−0.030	0.186	0.127

Table 2 continued

Pop	Ttho4	Ttho6	Ttho7	Tth4	Tth5	Tth14	Tth17	Tth185	Tth254	Tth1-31	Tth12-29	Tth16-2
WI2_07												
N	50	50	50	49	50	50	49	50	50	50	50	50
N_A	26	11	17	29	19	5	23	20	23	13	9	5
N_E	8.929	3.362	8.532	15.540	8.503	2.525	13.527	11.655	13.812	5.482	2.607	2.438
H_O	0.860	0.580	0.940	0.878	0.880	0.660	0.878	0.880	0.920	0.940	0.540	0.460
H_E	0.888	0.703	0.883	0.936	0.882	0.604	0.926	0.914	0.928	0.818	0.616	0.590
F_{IS}	0.042	**0.178****	−0.056	0.054*	0.017	−0.081	0.065	0.052	0.021	−0.137	0.121	0.215
CAN_07												
N	20	19	17	17	17	20	18	17	16	17	20	20
N_A	14	7	14	17	13	4	17	12	15	8	5	3
N_E	5.970	2.051	5.959	12.844	8.758	2.204	11.172	9.966	9.309	5.207	2.564	2.228
H_O	0.850	0.579	0.706	1.000	1.000	0.650	0.944	0.941	1.000	0.824	0.750	0.500
H_E	0.833	0.512	0.832	0.922	0.886	0.546	0.910	0.900	0.893	0.808	0.610	0.551
F_{IS}	0.005	−0.103	0.181	−0.054	−0.099	−0.165	−0.009	−0.016	−0.088	0.011	−0.205	0.118
PAC_03												
N	18	18	18	18	18	18	18	18	18	18	18	18
N_A	15	9	17	15	15	5	16	18	16	10	5	4
N_E	7.281	3.340	9.127	11.172	10.983	2.582	9.529	12.706	11.172	6.113	3.057	2.464
H_O	1.000	0.556	0.833	0.833	0.944	0.722	0.944	1.000	0.889	0.944	0.556	0.444
H_E	0.863	0.701	0.890	0.910	0.909	0.613	0.895	0.921	0.910	0.836	0.673	0.594
F_{IS}	−0.131	0.234**	0.093	0.113*	−0.011	−0.151	−0.027	−0.057	0.052	−0.157	0.134	0.279
PAC_05												
N	20	20	20	20	20	20	20	19	20	20	20	20
N_A	18	7	15	16	16	4	14	17	15	9	6	4
N_E	7.921	2.909	8.163	11.594	8.602	2.192	6.154	12.033	11.111	4.188	3.077	1.891
H_O	0.850	0.700	0.900	0.900	0.850	0.600	0.900	1.000	0.950	0.850	0.750	0.500
H_E	0.874	0.656	0.878	0.914	0.884	0.544	0.838	0.917	0.910	0.761	0.675	0.471
F_{IS}	0.053	−0.041	0.000	0.041	0.064	−0.078	−0.049	−0.064	−0.018	−0.091	−0.086	−0.035

All Pops	Ttho4	Ttho6	Ttho7	Tth4	Tth5	Tth14	Tth17	Tth185	Tth254	Tth1-31	Tth12-29	Tth16-2	Total
Mean N	41.714	41.643	41.357	40.786	41.143	41.714	41.286	41.143	41.357	41.500	41.714	41.714	41.423
SE N	4.398	4.426	4.466	4.393	4.431	4.398	4.431	4.501	4.505	4.484	4.398	4.398	1.238
Mean N_A	19.571	9.500	14.143	22.643	17.714	5.214	19.000	18.571	20.000	11.786	6.929	5.429	14.208
SE N_A	0.796	0.489	0.831	1.341	0.707	0.281	0.908	0.856	1.089	0.697	0.370	0.374	0.511
Mean N_E	8.464	3.469	6.516	14.240	9.700	2.556	10.690	11.307	11.392	5.075	2.731	2.547	7.391

Table 2 continued

All Pops	Tho4	Tho6	Tho7	Tth4	Tth5	Tth14	Tth17	Tth185	Tth254	Tth1-31	Tth12-29	Tth16-2	Total
SE N_E	0.359	0.152	0.420	0.699	0.397	0.066	0.715	0.596	0.487	0.194	0.093	0.094	0.326
Mean H_O	0.885	0.661	0.828	0.921	0.899	0.632	0.885	0.905	0.889	0.828	0.621	0.530	0.790
SE H_O	0.012	0.020	0.017	0.014	0.013	0.021	0.010	0.015	0.024	0.022	0.029	0.018	0.012
Mean H_E	0.879	0.703	0.838	0.928	0.895	0.605	0.900	0.908	0.910	0.799	0.628	0.601	0.799
SE H_E	0.006	0.017	0.011	0.003	0.004	0.010	0.008	0.006	0.004	0.007	0.013	0.014	0.010
F_{IS}	−0.007	0.059	0.012	0.008	−0.005	−0.045	0.016	0.003	0.023	−0.036	0.012	0.118	–
Mean F_{IS}	–	–	–	–	–	–	–	–	–	–	–	–	0.013
SE F_{IS}	–	–	–	–	–	–	–	–	–	–	–	–	0.012

N number of individuals, N_A number of alleles per locus, N_E effective number of alleles per locus, H_O observed heterozygosity, H_E unbiased expected heterozygosity (gene diversity), F_{IS} inbreeding coefficient (Weir and Cockerham 1984). The significance of F_{IS} depicted by * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. The HWE significant after multiple testing is shown in bold

were moderately to highly polymorphic with a maximum of 9–38 alleles per locus (mean 5.2–22.6; Table 2) with similar level of polymorphism across samples and effective numbers of alleles (N_E) ranged from 1.89 to 18. Of the 301 alleles detected at the 12 loci, 43 were rare alleles occurring within a single sample with frequencies no higher than 0.006. Observed heterozygosity values ranged from 0.44 for *Tth*16-2 to 1.00 for *Tth*4 and *Ttho*4. Mean multilocus observed heterozygosities were similar across all samples (0.74–0.82).

Ten locus–sample combinations significantly deviated from HWE after multiple testing (Table 2); however, only six of these combinations could be explained by heterozygosity deficiency, of which three were in locus Tth16-2. The estimate of null allele frequency in Tth16-2 ranged from 0.05 to 0.11. There was no consistent pattern across samples or the other loci; hence, only locus Tth16-2 was removed for analyses assuming HWE. No significant linkage disequilibrium was found among any combination of loci across all samples, and no loci combination within samples was significant after correction for multiple testing, indicating no evidence of physical linkage between all pairs of loci tested among all sampled areas.

Population differentiation between regions

Significant population structure was detected across samples, with three major clusters being identified; NEA, MED and SWP. Samples were consistently separated from each other for all analytical methods used. Global F_{ST} values for all loci tested were significant (Table 3). Pairwise multilocus F_{ST} estimates and exact *G* tests of genic proportions indicated that the two samples from the SWP (Pac_03 and Pac_05) were similar to each other but differed significantly from all samples from the MED and NEA with F_{ST} estimates ranging for Θ between 0.007 and 0.036 and for Θ' between 0.065 and 0.253 (Table 3). The correspondence analysis (CA) supports this as both SWP samples grouped separately from the northern hemisphere samples (Fig. 1). All three MED samples were found to be significantly distinct from all of the NEA samples based on pairwise F_{ST} estimates (Θ ranging from 0.011 to 0.026 and Θ' from 0.011 to 0.166) and exact *G* tests (Table 3). The difference is also illustrated in the CA plot (Fig. 1), in which samples from the MED and NEA are clearly separated from each other.

Population differentiation within regions

Within the NEA out of 36 pairwise comparisons, only 5 F_{ST} estimates and 9 exact *G* test were significant after multiple testing correction (Table 3). These samples were obtained from feeding grounds covering approximately

Table 3 Multilocus pairwise estimates of differentiation (Θ and Θ′) and significance of exact *G* test for albacore tuna (*Thunnus alalunga*)

	MED_05	MED_06	MED_07	CS1_05	CS2_05	CS3_05	CS1_06	BB1_06	BB2_06	WI1_07	WI2_07	CAN_07	PAC_03	PAC_05
MED_05		0.014 *	0.008 *	0.014 *	0.019 *	0.018 *	0.024 *	0.018 *	0.026 *	0.017 *	0.020 *	0.023 *	0.025 *	0.033 *
MED_06	0.124		0.002	0.012 *	0.020 *	0.015 *	0.021 *	0.017 *	0.018 *	0.013 *	0.019 *	0.024 *	0.026 *	0.034 *
MED_07	0.072	0.036		0.011 *	0.021 *	0.015 *	0.020 *	0.017 *	0.020 *	0.013 *	0.018 *	0.021 *	0.026 *	0.036 *
CS1_05	0.122	0.100	0.113		0.004	0.000	0.006 *	0.000	0.000	-0.002	-0.002	0.001	0.014 *	0.020 *
CS2_05	0.152	0.174	0.184	0.046		0.005 *	0.006 *	0.002	0.007	0.003	0.001	0.002	0.003	0.007 *
CS3_05	0.135	0.112	0.119	0.002	0.038		0.003	0.001	0.003	0.002	0.003	0.006	0.017 *	0.019 *
CS1_06	0.149	0.162	0.148	0.029	0.041	0.024		0.007 *	0.003	0.009 *	0.004	0.007	0.011 *	0.015 *
BB1_06	0.150	0.134	0.141	0.012	0.023	0.011	0.048		0.001	0.002	0.001	0.003	0.009 *	0.015 *
BB2_06	0.157	0.130	0.155	-0.011	0.047	0.003	0.034	-0.004		0.008	-0.001	0.002	0.009	0.014 *
WI1_07	0.166	0.125	0.132	0.004	0.019	0.008	0.036	0.021	0.039		0.000	0.002	0.014 *	0.019 *
WI2_07	0.154	0.167	0.145	0.009	0.018	0.023	0.019	0.024	0.001	-0.001		0.000	0.008 *	0.014 *
CAN_07	0.150	0.158	0.162	0.026	0.015	0.051	0.019	0.036	0.001	-0.002	0.016		0.009	0.014 *
PAC_03	0.189	0.211	0.194	0.132	0.046	0.128	0.094	0.105	0.073	0.115	0.088	0.082		-0.005
PAC_05	0.223	0.253	0.253	0.149	0.065	0.103	0.107	0.121	0.084	0.122	0.103	0.100	-0.015	

Tables correspond to pairs of *Θ* (above diagonal) and heterozygosity-corrected Θ′ (below diagonal). Significant values after multiple test correction are denoted with stars (*). The values have been shaded in grey for ease of interpretation: the darker the grey the higher the relative value among comparisons (within estimator). The lines represent the borders between samples in different basins: Mediterranean Sea (top and left), north-east Atlantic and Pacific (bottom and right). Differentiation values in grey font indicate exact *G* test comparisons that were not significant after correction for multiple testing

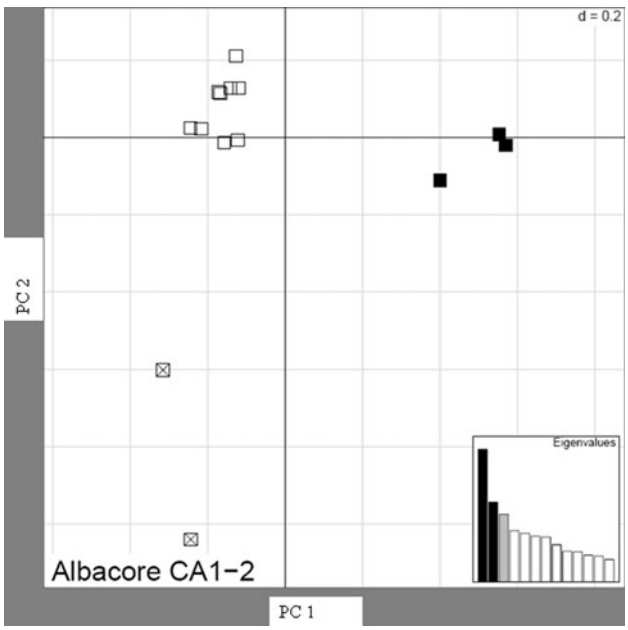

Fig. 1 Sample correspondence analysis (CA) of albacore tuna (*Thunnus alalunga*) representing the first (PC1) and second (PC2) principal components of multilocus allele distributions. Eigenvalues corresponding to the selected components are shown in *black* in the histogram at the *bottom right corner*. Sample indicated by: NEA = (*open square*), Med = (*filled circle*), SWP = (⊠)

2,500 km from south-west Ireland to the southern Bay of Biscay. There was evidence of genetic heterogeneity in pairwise comparisons involving the three samples: CS2_05, CS1_06 and BB1_06 (Table 3). Four significant pairwise F_{ST} and four exact *G* test comparisons indicated slight genetic heterogeneity between CS1_06 and other NEA samples (average $\Theta = 0.006$; $\Theta' = 0.031$). Significant genetic differentiation was also detected with both methods between CS2_05 and CS3_05 ($\Theta = 0.005$; $\Theta' = 0.038$). These three samples (CS2_05, CS1_06 and BB1_06) were caught early in the fishing season (July and August) (Table 1), while the other five juvenile NEA samples were caught between the end of September and October (Table 1).

All juvenile samples (CS1_05, CS2_05, BB2_06 and WI2_07) from the NEA were genetically undifferentiated from the adults collected from the Canary Islands (CAN_07), i.e. there were no significant differences in pairwise comparisons of F_{ST} values and exact tests for these five samples (Table 3).

The results of the GENELAND analysis are shown using a maximum of $K = 10$, at 100,000 iterations, thinning at a rate of 100, correlated allele frequency model, with 100 burn-in generations. GENELAND analysis consistently identified $K = 5$ within the NEA and MED samples. The map of probable sample membership to a particular cluster is shown in Fig. 2 (Individual membership can be viewed in supplementary Figures). The southern Bay of Biscay samples (BB1_06 and BB2_06) group together. The only sample in this study which was obtained from an adult population of albacore in the North Atlantic (CAN_07) forms its own group. The majority of the remaining NEA (CS and WI) samples are classified as belonging together.

Pairwise F_{ST} estimates and exact *G* tests showed that the sample collected from the western side of the Mediterranean Sea in 2005 (Med_05) differed significantly from the two samples from the central region: Med_06 ($\Theta = 0.014$; $\Theta' = 0.124$) and Med_07 ($\Theta = 0.008$; $\Theta' = 0.072$). Although the results from exact *G* tests also indicated significant allele frequency heterogeneity among all three MED samples (Table 3), differentiation between the two samples from the central region of the Mediterranean Sea was not significant. The GENELAND analysis provides a

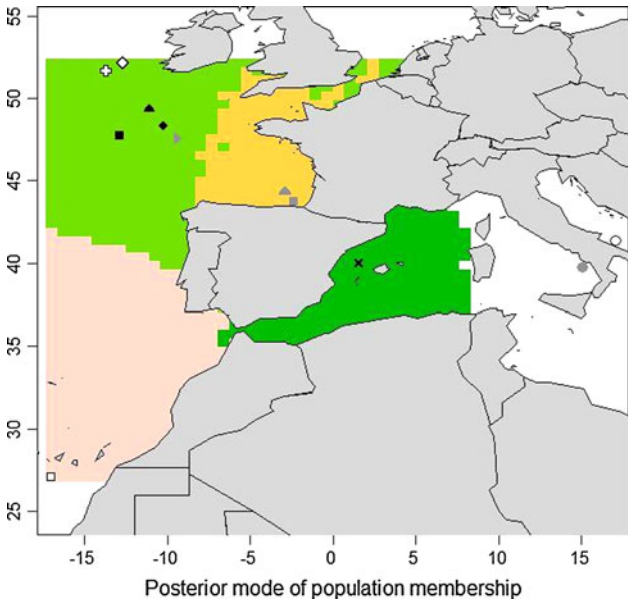

Fig. 2 Geographical locations of albacore tuna (*Thunnus alalunga*) sampling in Med_05 (X), Med_06 (*filled circle*), Med_07 (*open circle*), CS1_05 (*filled triangle*), CS2_05 (*filled square*), CS3_05 (*filled diamond*), CS1_06 (*shaded right pointed triangle*), BB1_06 (*shaded triangle*), BB2_06 (*shaded square*), WI1_07 (*open diamond*), WI2_07 (+) and CAN_07 (*open square*); the different *colours* indicate posterior probability of belonging to subpopulations 1–5 detected in the GENELAND analysis (colours are arbitrary to differentiate between population groupings)

graphical representation of such significant heterogeneity between the western and central Mediterranean samples.

The two samples from the SWP (Pac_03 and Pac_05) did not differ for either F_{ST} or exact G test pairwise comparisons in either data sets.

Discussion

Genetic diversity and HWE

The present study used microsatellite markers to investigate the genetic structure of albacore tuna both within and among different oceanic regions. Twelve microsatellites were screened, all of which were developed for bluefin tuna (Takagi et al. 1999; McDowell et al. 2002; Clark et al. 2004). Three of the microsatellites used in the present study had previously been utilised by Takagi et al. (2001) to evaluate genetic variation within and among albacore samples from the North and South Atlantic and Pacific Oceans. Similar numbers of alleles were observed in the two studies at loci *Ttho*6 ($N_A = 18$ (this study) and $N_A = 19$ (Takagi et al. 2001)). At *Ttho*-4 and *Ttho*7 in NE Atlantic samples, there were approximately twice as many alleles observed in this study (Mean $N_A = 32$ and 26, respectively) compared to Takagi et al. (2001) ($N_A = 11$ and 12, respectively). Mean heterozygosities per locus were similar in both studies for all loci and areas sampled; heterozygosities were similar in range to studies on other species of tuna (Appleyard et al. 2001; Carlsson et al. 2004).

Population differentiation between regions

Multilocus pairwise comparisons of F_{ST} values were low, ranging from 0.000 (negative value shown in Table 2) to $\Theta' = 0.253$ ($\Theta = 0.036$), with an average value of $\Theta' = 0.071$ ($\Theta = 0.013$). Despite relatively low F_{ST} values, 55 out of 91 (60%) were significant. Overall, results from pairwise F_{ST} estimates and exact tests, and the CA plot (Table 3; Fig. 1) indicate that NEA, MED and SWP are strongly differentiated from one another. The largest F_{ST} values were found between SWP and MED samples (SWP_05 and Med_07, $\Theta' = 0.253$; $\Theta = 0.036$, $P < 0.001$) and F_{ST} between MED and NEA ranged from $\Theta' = 0.100$ to 0.166 and $\Theta = 0.011$ to 0.026 (all significant after multiple testing correction). The finding of significant genetic differentiation between NEA and SWP albacore corroborates those of Chow and Ushiama (1995), where haplotype analysis of the mitochondrial ATPase gene indicated genetic heterogeneity between both the Atlantic and Pacific stocks but, showed homogeneity within both stocks. In addition to F_{ST} and CA results, the GENELAND analysis also supports genetic differentiation between NEA and MED albacore; this has also been reported by Arrizabalaga et al. (2004), Viñas et al. (2004) and Nakadate et al. (2005) for a variety of markers, such as blood lectins, mtDNA and nuclear markers. All these studies validate non-molecular differences reported between NEA and MED albacore (Megalofonou 2000). There is now ample genetic evidence to support the justification of managing albacore in the Mediterranean Sea as a separate entity to albacore in the North Atlantic Ocean.

The science of landscape ecology is increasingly being combined with population genetics to explain differentiation between populations of a species (Manel et al. 2003). Isolation by distance and physical barriers to gene flow are two factors often proposed to explain differences within species across different geographic areas, for example, as the basis for the separation of bluefin tuna into two subspecies, one which inhabits the Atlantic Ocean (*Thunnus thynnus thynnus*) and the other the Pacific Ocean (*T. t. orientalis*) (Ward 1995). Landscape genetics can be applied to marine studies where both visible and invisible oceanographic features, such as benthic topography and currents, can lead to the segregation of marine populations with pelagic stages in their life history (Jørgensen et al. 2005; Karlsson and Mork 2005; Was et al. 2008; Kovach et al. 2010). The observed genetic distinctness of North Atlantic

Ocean and Mediterranean Sea albacore is particularly interesting in the light of the geological separation of the two regions in the late Miocene period (~5.9 million years ago) and reconnection during the Pliocenic period of the late Cenozoic period, some 5.33 million years ago (Patarnello et al. 2007). The Mediterranean Sea is a fully enclosed sea except for the narrow and deep connection with the North Atlantic Ocean, with the majority of biota having colonised the Mediterranean Sea from the Atlantic Ocean through this entrance (Almada et al. 2001; Domingues et al. 2005). Albacore are similar in morphology (Pujolar et al. 2003) and environmental preference (Beardsley 1969; Chow and Kishino 1995) to bluefin tuna (*T. t. thynnus*), and both are considered part of the "bluefin" tuna group that occupy cooler waters, yet bluefin tuna migrate out of the Mediterranean Sea through the straits of Gibraltar (Carlsson et al. 2006), whereas for albacore, Atlantic-Mediterranean migrations have been shown to be limited (Arrizabalaga et al. 2002, 2003). The comparative differences in behaviour of these two species raises questions as to why limited Atlantic-Mediterranean movement is observed in albacore when these fish (as a fast moving pelagic species tolerant of cooler waters) have the physiology to cross oceanographic features such as the Almeria–Oran front and the Straits of Gibraltar.

Population differentiation within regions

Albacore in the North Atlantic Ocean is currently managed as a single stock, and no genetic structuring is recognised within the population (ICCAT 2007) in spite of studies that indicate otherwise (Hue 1979, 1980; Arrizabalaga et al. 2004). Although there have been no previous studies on albacore, a study on bluefin tuna by Carlsson et al. (2006) was able to determine whether individual bluefin tuna in a feeding aggregation in the North Atlantic belonged to either the eastern or western stock. The two stocks have been shown to migrate to feeding grounds at different times but are present for a few months as a mixed feeding aggregation (Carlsson et al. 2006). The results presented here indicate that there may be both spatial and seasonal structuring within the North Atlantic albacore, with the Bay of Biscay (BB1_06 and BB2_06, caught in August and September) samples separating from the Celtic Sea (CS1_06, caught in September) samples in 2006 and West of Ireland samples (WI1_07 and WI2_07, caught in September) separating from the Canary Islands sample (CAN_07, caught in March) in 2007. The observed structuring of albacore in a transient population in the NEA may be based on different timing of migration to feeding areas and the observation of genetic structuring may be dependent on the month the samples are collected during the fishing season. The identification of three subpopulation clusters within the north-east Atlantic feeding aggregations (based on twelve microsatellite loci) indicates that management based on the whole population may mask issues with the health of subpopulations; therefore, caution must be used to prevent a genetic subpopulation (and hence the expression of available phenotypic plasticity) being exploited into possible extinction. This is fundamental to ensure the longevity of the populations/stocks within the whole catchment (Carvalho and Hauser 1994). Hue (1979, 1980) proposed that North Atlantic albacore are differentiated into at least two subpopulations with separate seasonally distinct migration routes (i.e. the "Classic" and the "Azores"); the fish that follow these separate migration routes can be characterised by observed differences in morphometric traits (head length vs. body length) and by the analysis of proteins from the eye lenses. Further information needs to be gathered to track the movement of the different components migrating into the feeding aggregations as well as collecting adult albacores from spawning grounds. Data on intra-oceanic migration pathways may be ascertained from archival tags, such as those used on larger fish (Sims et al. 2003). A further study including more intensive sampling throughout the fishing season would be needed to confirm or disprove the suggested structuring where different populations may be migrating to the feeding areas at different times. The combination of investigating migration pathway and timings with microsatellite data may provide further information in order to either refute or ratify genetic homogeneity within the North Atlantic stock.

Megalofonou (1990) and Cefali et al. (1986) cited in Megalofonou (2000) have shown that the distribution of albacore in the Mediterranean is discontinuous, with the highest concentrations found in the Tyrrhenian Sea in the Western Mediterranean Basin and the Ionian, Adriatic and Aegean Seas in the Eastern Mediterranean Basin. Previous studies have shown that oceanographic barriers appear to exist within the Mediterranean Sea, most notably the Almeria–Oran and the Siculo-Tunisian fronts, which separate the Mediterranean into the East and West basins. Carlsson et al. (2004) proposed possible heterogeneity of bluefin tuna within the Mediterranean, with the distinction being most evident between the Tyrrhenian and Ionian Seas (i.e. between the East and West basin separated by the Siculo-Tunisian front). Genetic heterogeneity between the East and West basins has been observed in other species, from those with sedentary and slow dispersal (sea grass, *Posidonia oceanica* (Arnaud-Haond et al. 2007) and cuttlefish, *Sepia officinalis* (Pérez-Losada et al. 2007)) to mobile species such as sea bass, *Dicentrarchus labrax* (Bahri-Sfar et al. 2000) and anchovy, *Engraulis encrasicolus* (Magoulas et al. 2006). In the current study, little difference in F_{ST} values between the Tyrrhenian (Med_06) and Southern Adriatic Seas (Med_07) was found, indicating

homogeneity in albacore population genetic structure around the Italian peninsula. However, large differences were observed in both the F_{ST} and GENELAND analysis between samples from the Balearic Sea (Med_05) and those around the Italian peninsula, indicating possible heterogeneity within albacore in the Western Mediterranean basin. Such findings have not been reported in other studies on tuna. It is therefore possible that further heterogeneity in addition to that observed in the present study may exist in albacore within the Mediterranean Sea.

In conclusion, significant population structuring was observed in both North Atlantic and Mediterranean albacore, despite potentially high gene flow by larval dispersal, high fecundity, large population size (ICCAT 2007), high fishery mortality, and the extensive trans-oceanic feeding and spawning migrations undertaken by albacore tunas. This study highlights that albacore in North Atlantic Ocean and Mediterranean Sea need to be managed at a smaller scale where substructuring is indicated. However, in order to define boundaries, more exactly further work should be undertaken; this includes collecting reference material from spawning aggregations in the Eastern Atlantic Ocean (Beardsley 1969), and throughout the entire Southern Mediterranean Sea (Piccinetti and Piccinetti Manfrin 1993) should be included. At present, the North Atlantic albacore stock is managed as a single unit, with the Mediterranean stock as a separate entity (ICCAT 2007). Heterogeneity may exist within both stocks on the basis of different migration patterns, discontinuous distribution, morphometric traits and molecular data, which may have implications for stock management if one subpopulation contributes more to the effective population size than another. Bias in stock assessment could lead to the possible elimination of some subpopulations (Carvalho and Hauser 1994) by overfishing of recruits or spawning stock.

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