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Genetic heterogeneity of Atlantic bluefin tuna caught in the eastern North Atlantic Ocean south of Iceland

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Carlsson, J., McDowell, J. R., Carlsson, J. E. L., Ólafsdóttir, D., and Graves, J. E. 2006. Genetic heterogeneity of Atlantic bluefin tuna caught in the eastern North Atlantic Ocean south of Iceland. – ICES Journal of Marine Science, 63: 1111–1117.

Atlantic bluefin tuna (*Thunnus thynnus*) are currently managed by the member nations of the International Commission for the Conservation of Atlantic Tunas (ICCAT) as distinct western and eastern stocks, separated by the 45°W meridian. Previous studies of Atlantic bluefin tuna caught in the northeast Atlantic south of Norway suggested mixing of putative stocks in the region, based on abrupt shifts in the size and condition of fish during the fishing season. By contrast, more recent studies south of Iceland reported only small differences in size of tuna caught at different times of the season in that area. To better understand the stock structure and composition of Atlantic bluefin tuna in the region, we surveyed genetic variation at eight microsatellite loci for 800 Atlantic bluefin tuna collected in experimental commercial fishing operations south of Iceland during 1999 and 2002. We tested for heterogeneity between years, between seasons within a year, between two fishing areas within the region, and between sexes. Analysis of molecular variation demonstrated slight, but significant, genetic divergence between collections of fish caught early and late in the season over the two years. These results are consistent with prior observations of Atlantic bluefin tuna of different conditions entering the fishery through the season, and suggest that the northeast Atlantic fishery represents a mixed-stock fishery including animals migrating from different areas and recruited from different spawning grounds.

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Keywords: highly migratory, intra-season variation, microsatellites, mixed stock, teleost, *Thunnus thynnus*.

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Introduction

The Atlantic bluefin tuna (*Thunnus thynnus*) is a highly migratory species targeted by a variety of fisheries throughout the North Atlantic. Catches increased dramatically through the 1980s and 1990s, and the species is now considered to be severely overfished (ICCAT, 2003). The International Commission for the Conservation of Atlantic Tunas (ICCAT) has recognized separate western and eastern management units of Atlantic bluefin tuna since 1982 (Magnuson *et al.*, 1994). The adoption of the two-stock management approach was supported by discontinuities in catches across the North Atlantic and the presence of two spatially separated spawning areas: the Mediterranean Sea in the eastern Atlantic, and the Gulf of Mexico in the western Atlantic (Mather *et al.*, 1995). However, considerable information has accrued causing scientists and managers to question the validity of the two-stock model (Magnuson *et al.*, 1994). For example, it is now known that Atlantic bluefin tuna are distributed across the North Atlantic Ocean, and a pelagic longline fishery for the species has developed in the central North Atlantic (Matsuoda, 1998). Moreover, conventional and electronic tagging studies have demonstrated a relatively high frequency of transoceanic movements of the species (Mather *et al.*, 1995; Block *et al.*, 2001, 2005). Block *et al.* (2005) recently suggested that Atlantic bluefin tuna comprise distinct stocks that are geographically isolated at the time of spawning, but mix on feeding grounds throughout much of the North Atlantic.

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Fisheries landings data provide indication of a mixed-stock fishery for Atlantic bluefin on northern feeding grounds. Scientists monitoring catches in the North Sea and off the west coast of Norway during the 1950s and 1960s noticed shifts in the size and condition of individuals caught early and late in the fishing season (Hamre, 1960; Tiews, 1963). However, analyses of bluefin tuna caught in Iceland's exclusive economic zone (EEZ) between 1997 and 2001 only detected small and gradual declines in fish length during the autumn fishing season (Ólafsdóttir and Ingimundardóttir, 2003).

Genetic analyses have provided some insights into the population structure of Atlantic bluefin tuna. Early studies of the Atlantic-wide genetic population structure of bluefin tuna using allozymes did not detect population structure (Edmunds and Sammons, 1973; Thompson and Contin, 1979), a result that is also consistent with more recent analyses surveying variation at nuclear and mitochondrial gene regions (Takagi *et al.*, 1999; Ely *et al.*, 2002; Pujolar *et al.*, 2003). Although a few studies surveying variation of nuclear microsatellite loci and the mitochondrial control region have reported significant heterogeneity among samples, the authors attributed the differences to small sample sizes (Broughton and Gold, 1997; Alvarado Bremer *et al.*, 1999). Recently, Carlsson *et al.* (2004) demonstrated genetic population structure among young-of-the-year (YOY) bluefin tuna from geographically separated areas in the western and eastern Mediterranean Sea. This finding argues for further analysis of Atlantic bluefin tuna population structure on an Atlantic-wide scale, as well as a thorough investigation of the population structure of fish taken in putative mixed-stock fisheries.

In the present study we analysed variability in 800 Atlantic bluefin tuna at eight microsatellite loci to test for genetic differences between spatial samples, between temporal samples, and between males and females to evaluate if the Icelandic Atlantic bluefin tuna catches represent a mixed-stock fishery.

Methods

Samples and genetic markers

Atlantic bluefin tuna tissue samples were acquired from experimental pelagic longline fishing operations south of Iceland during the 1999 and 2002 fishing seasons (Figure 1)

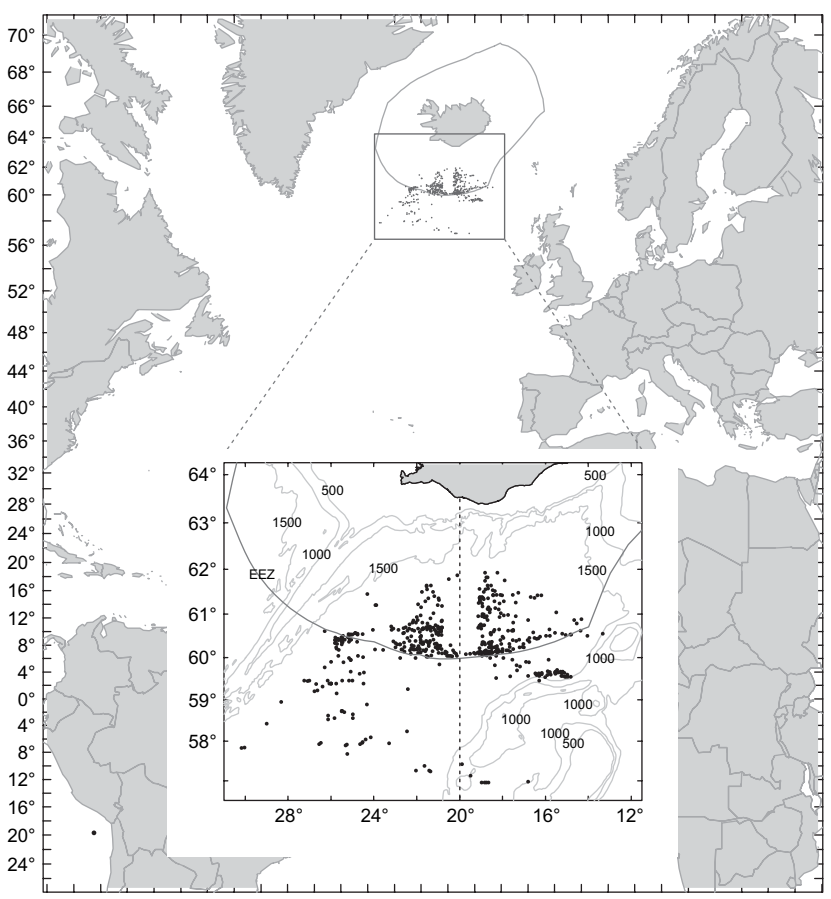

Figure 1. Map showing sampling areas in the Icelandic EEZ where Atlantic bluefin tuna were sampled in 1999 and 2002.

by subsampling catches. In 2002, a total of 200 tuna was caught in the most western and eastern fishing grounds in the Icelandic EEZ in both 1999 and 2002 (i.e. the total $n = 800$). Fish were sexed and measured to the nearest centimetre. A small sample of gill tissue was removed from each fish shortly after capture and stored in 95% ethanol.

Total genomic DNA was isolated from tissue samples by proteinase K/chelex extraction (Estoup *et al.*, 1996), and it served as a template for PCR amplification (PCR conditions will be made available by the authors upon request) of the following eight microsatellite loci: *Tth5*, *Tth8*, *Tth10*, *Tth21*, and *Tth34* (McDowell *et al.*, 2002); and *Ttho-1*, *Ttho-4*, and *Ttho-7* (Takagi *et al.*, 1999). Microsatellite polymorphisms were analysed on a Li-Cor 4200 Global IR^2 automated sequencer (Li-Cor, Lincoln, NE, USA) with a size standard (50−350 base pairs, Li-Cor, Lincoln, NE, USA) run at the centre and at both extremes of the gel to determine allele size. At least four lanes in each run consisted of individuals for which the allele sizes were known to ensure identical allele scoring across runs. To ensure repeatability of amplification and scoring, approximately 20% of the samples were run again. Fragment length polymorphisms were analysed with GENE IMAGIR software (Li-Cor, Lincoln, NE, USA).

Data analyses

The microsatellite data set was analysed with the Microchecker 2.2.1 software (van Oosterhout *et al.*, 2004) to ensure that it was not severely affected by null-alleles, large allele dropout and stuttering (1000 randomizations). The software package Arlequin (Schneider *et al.*, 1997) was used to determine whether genotype frequencies were consistent with the expectations of Hardy−Weinberg equilibrium (exact tests; Guo and Thompson, 1992), as well as to estimate observed (H_O) and expected (H_E) heterozygosities. The total observed variance was partitioned between years (1999 and 2002), between seasons (individuals caught early and late in the fishing season), between locations (Atlantic bluefin tuna caught southwest and southeast of Iceland), and between males and females. These tests were performed using Arlequin (AMOVA, 10 000 permutations). In all cases with multiple tests, significance levels were adjusted using the sequential Bonferroni technique (Rice, 1989).

Results

Atlantic bluefin tuna were collected during the 1999 fishing season from August through October. In 2002, the Atlantic bluefin tuna fishery south of Iceland occurred later in the year, and our sample originated from catches made from September through November. The mean size of fish used in the study was 202.51 cm (s.d. = 25.09, $n = 400$) and 203.34 (s.d. = 19.97, $n = 400$) in 1999 and 2002, respectively.

Genetic variability was analysed separately for the samples collected in 1999 and 2002 (Table 1). The number of alleles per microsatellite locus within temporal samples varied from three at locus *Tth21* (both years) to 25 at locus *Tth34* (2002 collection). Observed heterozygosity varied from 0.42 in the 2002 sample at locus *Tth21* to 0.84 in the 2002 sample at locus *Ttho-7*, and expected heterozygosity varied from 0.44 in the 2002 sample at locus *Tth21* to 0.85 in the 1999 sample at locus *Ttho-7* (Table 1). The

Table 1. Summary statistics for eight microsatellite loci among Atlantic bluefin tuna collections, number of individuals (n), number of alleles (a), allele size range in base pairs (as), expected heterozygosity (H_E), and observed heterozygosity (H_O).

	Loci								
Sample	*Tth5*	*Tth8*	*Tth10*	*Tth21*	*Tth34*	*Tth01*	*Tth04*	*Th07*	Average
1999									
n	400	400	400	400	400	400	400	400	
a	5	13	4	3	18	8	17	21	
as	125−181	296−346	110−118	125−133	101−173	181−195	128−174	180−228	
H_E	0.52	0.78	0.45	0.46	0.81	0.63	0.81	0.85	0.66
H_O	0.50	0.79	0.44	0.45	0.79	0.63	0.79	0.83	0.65
2002									
n	400	400	400	400	400	400	400	400	
a	4	14	4	3	25	8	17	17	
as	125−133	296−346	110−188	125−133	101−201	181−195	128−166	180−224	
H_E	0.51	0.79	0.47	0.44	0.81	0.60	0.81	0.84	0.66
H_O	0.52	0.79	0.47	0.42	0.79	0.58	0.80	0.84	0.65
Average									
H_E	0.52	0.78	0.46	0.45	0.81	0.61	0.81	0.85	0.66
H_O	0.51	0.79	0.45	0.43	0.79	0.60	0.80	0.83	0.65

genotypic distributions for loci *Tth34* and *Tth10* deviated significantly from Hardy–Weinberg expectations in the 1999 and 2002 samples ($p = 0.0209$ and $p = 0.0056$, respectively). However, only the genotypic distribution at locus *Tth10* remained significant after sequential Bonferroni corrections (initial $\alpha = 0.05/8 = 0.00625$), and this locus was included in all subsequent analyses. No deviations from expected Hardy–Weinberg proportions were found in any of the subsamples: males and females, early and late caught, or in the samples from the southeast and southwest fishing grounds for either 1999 or 2002.

Temporal genetic differentiation was analysed between years and between seasons within a year. Comparison of the distribution of genetic variation of 400 Atlantic bluefin tuna collected in 1999 with 400 bluefin tuna from 2002 revealed no significant differences between the two sample years ($F_{st} = -0.00036$, $p = 0.871$). To investigate potential differences in stock composition within a year (between fish caught early and late in the season), the 1999 and 2002 samples were each subdivided into "early season" (the first 100 individuals in the collection, 1 August through 15 August in 1999, and 2 September through 11 September in 2002) and "late season" (the last 100 individuals in the collection, 12 October through 29 October in 1999, and 3 October through 5 November in 2002). No significant differences were found between early season Atlantic bluefin tuna caught in 1999 and 2002 ($F_{st} = 0.00026$, $p = 0.403$; Table 2), or between late season Atlantic bluefin tuna caught in the two years ($F_{st} = -0.00136$, $p = 0.836$; Table 2). A comparison of early and late season fish in 1999 and in 2002 revealed no significant differences in the distribution of genetic variation between seasons within years ($F_{st} = 0.00152$, $p = 0.175$, and $F_{st} = 0.00186$, $p = 0.117$, respectively; Table 2). However, when the early season samples from 1999 and 2002 were combined and compared with the combined late season samples, the AMOVA revealed slight, but highly statistically significant, genetic differences between seasons ($F_{ct} = 0.00154$, $p = 0.000$), but not within a season between years ($F_{sc} = 0.00028$, $p = 0.125$). In addition, the mean lengths of Atlantic bluefin tuna caught within the Icelandic EEZ decreased slightly over the fishing season in both 1999 and 2002 ($p = 0.001$, $r^2 = 0.051$, $n = 740$; $p = 0.006$, $r^2 = 0.023$, $n = 318$ in 1999 and 2002, respectively; Ólafsdóttir and Ingimundardóttir, 2003).

The 1999 and 2002 collections of Atlantic bluefin tuna each comprised 200 individuals collected from fishing grounds to the southeast of Iceland inside the EEZ and 200 from fishing grounds to the southwest, also inside the EEZ. No significant differences were found between bluefin caught in the southeast region in 1999 and 2002 ($F_{st} = 0.00071$, $p = 0.183$; Table 3), or between fish caught in the southwest region in the two years ($F_{st} = -0.00013$, $p = 0.537$; Table 3). No significant differences were revealed between southeast and southwest samples in 1999 ($F_{st} = -0.00066$, $p = 0.830$; Table 3), but in 2002 there was a significant difference between samples from the different fishing grounds ($F_{st} = 0.00223$, $p = 0.013$; Table 3). However, when the two samples from the southwest and the two samples from the southeast were grouped together for an AMOVA, no significant heterogeneity was revealed between locations ($F_{ct} = -0.00002$, $p = 0.342$), nor were there significant differences within locations between years ($F_{sc} = 0.00029$, $p = 0.096$).

To investigate the effect of sex on the distribution of genetic variation, the 1999 and 2002 collections were subdivided into males and females. Multilocus F_{st} values revealed no significant heterogeneity between females caught in 1999 ($n = 140$) and those caught in 2002 ($n = 212$), nor between males caught in the two years ($n = 172$ and $n = 187$, respectively; Table 4). Similarly, the AMOVA revealed no significant heterogeneity between sexes across years ($F_{ct} = -0.00037$, $p = 0.667$), or between sample years within a sex ($F_{sc} = -0.00075$, $p = 0.920$).

Discussion

Analyses of Atlantic bluefin tuna caught in the North Sea and along the Southwest Norwegian coast in the 1950s and 1960s showed that the length and condition of fish caught early and late in the fishing season differed

Table 2. Multilocus F_{st} estimates of Atlantic bluefin tuna caught in Icelandic waters, in the lower left diagonal, and probability estimate of F_{st} being different from zero in the upper right diagonal between samples caught during different time periods.

Sample	Early caught 1999	Early caught 2002	Late caught 1999	Late caught 2002
Early caught 1999		0.403	0.175	0.189
Early caught 2002	0.00026		0.687	0.117
Late caught 1999	0.00152	−0.00085		0.836
Late caught 2002	0.00147	0.00186	−0.00136	

Table 3. Multilocus F_{st} estimates of Atlantic bluefin tuna caught in Icelandic waters, in the lower left diagonal, and probability estimate of F_{st} being different from zero in the upper right diagonal between samples caught southwest and southeast off Iceland. Values in bold represent significant F_{st} estimates.

Sample	Southwest 1999	Southwest 2002	Southeast 1999	Southeast 2002
Southwest 1999		0.537	0.830	0.726
Southwest 2002	−0.00013		0.481	**0.013**
Southeast 1999	−0.00066	−0.00006		0.183
Southeast 2002	−0.00040	**0.00223**	0.00071	

Table 4. Multilocus F_{st} estimates of Atlantic bluefin tuna caught in Icelandic waters, in the lower left diagonal, and probability estimate of F_{st} being different from zero in the upper right diagonal between unlikely females and males.

Sample	Females 1999	Females 2002	Males 1999	Males 2002
Females 1999		0.821	0.993	0.993
Females 2002	−0.00029		0.870	0.821
Males 1999	−0.00175	−0.00078		0.962
Males 2002	−0.00168	−0.00066	−0.00117	

significantly, leading researchers to suggest the presence of a mixed-stock fishery in this area (Hamre, 1960; Tiews, 1963). However, a more recent study on Atlantic bluefin tuna catches from south of Iceland between 1997 and 2001 found only a slight decline in length and no differences in condition throughout the fishing season in autumn (Ólafsdóttir and Ingimundardóttir, 2003). Our genetic analysis of 800 adult Atlantic bluefin tuna from Icelandic waters revealed significant genetic heterogeneity. We found genetic differences between Atlantic bluefin tuna caught early and late in the season. In addition, there was a significant decrease in length over the fishing season in both 1999 and 2002. Significant genetic heterogeneity between Atlantic bluefin tuna caught southwest and southeast off Iceland was only present in 2002, and disappeared when 1999 and 2002 data were pooled. However, no genetic differences were found between males and females, suggesting that they did not originate from different spawning populations. These findings suggest that Atlantic bluefin tuna caught south of Iceland may represent a mixed-stock fishery, supporting earlier indications of separate influx of Atlantic bluefin tuna into northern areas in the North Atlantic based on shifts in size and condition throughout the fishing season (Hamre, 1960; Tiews, 1963; Ólafsdóttir and Ingimundardóttir, 2003).

Many marine fish have very large effective population sizes relative to their counterparts in freshwater systems (Smedbol *et al.*, 2002). In addition, highly migratory species have the capacity to make extensive migrations, and the population range of a given population of these species might encompass entire oceans (Mather *et al.*, 1995; Block *et al.*, 2001, 2005). Atlantic bluefin tuna is a prime example of a species characterized by all these properties, making the expected level of genetic differentiation within the species low. It is therefore important to evaluate whether observed genetic structure, though statistically significant, is also biologically meaningful (Waples, 1998).

It is unlikely that the weak but significant genetic heterogeneity we observed was caused by the significant deviation from Hardy−Weinberg expatiations at the *Tth10* locus in the 2002 collection. The deviation was not caused by homozygosity or heterozygosity excess (cf. Table 1 and Microchecker analysis), and it is improbable that the marker biased our results and caused significant genetic differences (the single locus F_{st} at *Tth10* was not significant; data not shown). On the other hand, the heterogeneity could be caused by non-representative sampling. Our samples consisted of adults and are unlikely to represent only a few families (i.e. a family effect; Allendorf and Phelps, 1981). Moreover, we could not find any deviations from Hardy−Weinberg expectations in any of the temporal, fishing ground, or sex samples which would be expected if these were not representative. A third possibility is that the observed structure is an indication of a mixed-stock fishery. We tend to favour the latter explanation, because our samples were not strongly affected by technical artefacts (null-alleles, large allele dropout, or stuttering) or by non-representative samples (e.g. family effects). In addition, our suggestion of a mixed-stock fishery based on genetic and length data (Ólafsdóttir and Ingimundardóttir, 2003) is supported by results of earlier studies of length and condition factor observed in the Northeast Atlantic fisheries in the 1950s and 1960s (Hamre, 1960; Tiews, 1963). Our observation of decreased length of Atlantic bluefin tuna over the season was not as pronounced as the abrupt shifts observed in the Nordic fisheries in the past (i.e. Hamre, 1960; Tiews, 1963). If the observed shifts in size over the fishing season are related to mixed origin of the fish, then the altered patterns observed in the recent fisheries may be due to changed rates of mixing following the collapse in these fisheries in the early 1960s. An alternative explanation of these size differences (independent of mixed stocks) might be varied onset of migration of fish of different size within the same stock. Large fish may leave the spawning grounds earlier and/or swim faster, so arrive earlier than smaller fish at the feeding grounds. The larger fish may also leave the feeding grounds earlier, causing the observed decline in mean lengths later, in autumn. However, this suggestion does not explain the weak but significant genetic differences between early- and late-caught Atlantic bluefin tuna.

The slight but significant genetic heterogeneity we observed restrains us from drawing firm conclusions regarding the mixed-stock fishery. However, one possible scenario is that Atlantic bluefin tuna in Icelandic waters originate from different spawning populations and that they are mixing on the feeding grounds off Iceland. There are two areas in which Atlantic bluefin tuna are known to spawn: in the western Mediterranean and in the Gulf of Mexico (Mather *et al.*, 1995). The western Mediterranean spawning grounds consist of two disjunct spawning areas, one off the Balearic Islands and one in the south Tyrrhenian Sea. However, recent genetic studies have suggested that there is extensive gene flow between these two spawning areas that counteract genetic differentiation (Carlsson *et al.*, 2004). However, the potential for a third Atlantic bluefin tuna spawning area located somewhere in the eastern Mediterranean Sea is supported by the presence of

population genetic structure between the western and eastern basins (Carlsson *et al.*, 2004) and the occurrence of mature fish in both areas (Karakulak *et al.*, 2004).

No studies have shown population genetic structure between Atlantic bluefin tuna spawned in the Mediterranean spawning areas and Gulf of Mexico (Edmunds and Sammons, 1973; Thompson and Contin, 1979; Takagi *et al.*, 1999; Ely *et al.*, 2002; Pujolar *et al.*, 2003, but see Broughton and Gold, 1997; Alvarado Bremer *et al.*, 1999). However, the temporal and spatial heterogeneity in Atlantic bluefin tuna caught off Iceland could be an indication of Atlantic-wide population genetic structure (cf. the shallow but significant population genetic structure observed by Broughton and Gold, 1997; Alvarado Bremer *et al.*, 1999). On the other hand, the heterogeneity might correspond to mixing of the two postulated populations from the Mediterranean Sea (Carlsson *et al.*, 2004). At present, however, we cannot delineate which populations of Atlantic bluefin tuna might be mixing in Icelandic waters. Further population genetic studies, including robust samples from the spawning ground in the Gulf of Mexico and elsewhere, should be performed before the natal origin of the Atlantic bluefin tuna caught in Icelandic waters can be assessed and the postulated mixed-stock fishery evaluated.

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