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GENETIC DATA AND ELECTRONIC TAGGING INDICATE THAT THE GULF OF MEXICO AND MEDITERRANEAN SEA ARE REPRODUCTIVELY ISOLATED STOCKS OF BLUEFIN TUNA (*THUNNUS THYNNUS*)

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SUMMARY

Population subdivision was examined in Atlantic bluefin tuna (*Thunnus thynnus thynnus*) through sequencing of the control region of the mitochondrial genome. A total of 160 samples obtained from the known spawning grounds in the Gulf of Mexico and Mediterranean Sea were analyzed. Bluefin tuna populations from the Gulf of Mexico and the Mediterranean Sea were found to be genetically distinct based on Φ_{st}, sequence nearest neighbor and AMOVA analyses, supporting the hypothesis that these two major spawning areas are independent stocks. These data are in agreement with electronic tagging studies that indicate distinct migratory paths of the Gulf of Mexico and Mediterranean Sea spawning populations. We hypothesize that the fidelity to individual spawning grounds observed in electronic tracking records of up to 4 years maintains the genetic differentiation observed between stocks.

KEYWORDS

Bluefin tuna, Population Genetics, Electronic Tagging, Spawning Migrations

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1. Introduction

Atlantic bluefin tuna is a wide ranging species found throughout the North Atlantic and Mediterranean Sea. These tuna are currently managed as two distinct populations with individual spawning grounds - the Gulf of Mexico/Bahamas in the West Atlantic and the Mediterranean Sea in the east (Mather *et al*., 1995; ICCAT, 2004). Spawning areas have been identified in several regions in the Mediterranean Sea with the main centers of spawning activity being the Ionian, Tyrrhenian Seas and Balearic Seas (Mather *et al*., 1995, Nishida *et al*., 1998; Garcia *et al*., 2005). While the Mediterranean Sea supports bluefin tuna throughout the year, bluefin are found in the GOM only during or prior to the spawning season (occupation from December – June) and all bluefin in the Gulf of Mexico at this time are believed to be mature spawners (Nemerson *et al*., 2000). Support for distinctness of the Gulf of Mexico (GOM) and Mediterranean Sea spawning grounds comes mainly from differences in key life history parameters, specifically age to first spawning and spawning season (ICCAT, 2004). However, continuity of catches across the Atlantic Ocean and rapid movement of tagged fish from one side of the ocean to the other keep open the possibility of one Atlantic –wide stock (NRC, 1994).

In addition to studies using catch statistics and demographic traits, genetic analysis has been used to examine population structure within Atlantic bluefin tuna (Edmonds and Sammons, 1973; Thompson and Contin, 1980; Broughton and Gold, 1997; Alvarado-Bremer *et al*., 1998; Pujolar and Pla, 2000; Ely *et al*., 2002, Carlsson *et al.,* 2004). Many of these studies were hampered by small sample sizes, especially from the GOM spawning grounds and, therefore, were not able to address the possibility of genetic distinctness between GOM and Mediterranean Sea. Carlsson et al. (2004) were able to show genetic subdivision of bluefin tuna between eastern and western basins of the Mediterranean Sea, supporting the hypothesis of multiple populations within the Mediterranean Sea. However, this study did not examine samples from the GOM so Atlantic-wide population structuring remains poorly defined.

Another methodology available to researches studying stock structure in fishes is through the use of tags. Recent electronic tracking studies initiated on Atlantic bluefin tuna have shown directed movements to spawning areas from feeding areas during the spawning seasons (Block *et al*., 2001, 2005; Teo *et al*., in press). These three studies also observed repeated movements by individual fish to both Mediterranean Sea and GOM spawning grounds for up to four years in a row, suggesting fidelity to a given spawning ground. No tagging study has observed movement to both known spawning grounds by any individual fish (Mather *et al*., 1995; Block *et al*., 2001, 2005). However, sample sizes of long term tracks have been small and tagging studies have not been able to determine the origin of tagged fish and, therefore, whether this movement to spawning grounds was natal homing. Genetic analyses have the potential to shed light on the question of population distinctness, as genetic differentiation between populations cannot exist without a significant level of fidelity to natal origin. In this study we sequenced the control region of the mitochondrial genome to test the null hypothesis of genetic homogeneity of bluefin tuna in the Atlantic Ocean.

2. Methods

Tissue samples were collected from 165 Atlantic bluefin tuna from the Gulf of Mexico (GOM), and Mediterranean Sea (Table 1). Samples from all areas were obtained from fisheries catch, in the course of tagging operations on the spawning grounds and also from fish electronically tagged outside the known spawning areas that subsequently traveled to one of these areas during or prior to the spawning season. This last group comprised fish tagged in both New England and North Carolina in conjunction with the Tag-A-Giant program (Block *et al*., 2001, 2005; Stokesbury *et al*., 2004). An 860 base pair section of the control region was amplified and sequenced using either a 373A automated sequencer (Applied Biosystems Inc., Foster City, CA, USA) equipped with 48 cm long read gel plates or an Applied Biosystems 3730 DNA sequencer. For 71 samples, it was possible to sequence the entire region in one reaction. For the remainder of the samples, sequencing was performed from both directions yielding an average of 408 base pairs of overlap between the two sequences.

To test for genetic differentiation among populations, pairwise Φ_{st} values were calculated with the program Arlequin 3.01c (Excoffier *et al*., 1992). In addition, the nearest neighbor statistic (S_{nn} – Hudson, 2000) was calculated using the program DNASP 4.0 (Rozas *et al*., 2003). The nearest neighbor statistic tests for population structure by calculating how often sequences that are most similar to each other are found in the same geographic sample area. In addition, a hierarchial analysis of molecular variance (AMOVA) was performed to compare Φ_{st} values among, between and within populations (Excoffier *et al*., 1992).

Five samples from the Mediterranean Sea contained control region sequences that were more similar to albacore tuna (*Thunnus alalunga*) and Pacific bluefin (*Thunnus thynnus orientalis*) tuna than to other Atlantic bluefin tuna. These samples were not included in any of the analyses or tables presented here. This clade was not found in samples collected in the GOM, while in the Mediterranean Sea it was present at a level of 4.5%.

Electronic tagging of bluefin tuna was conducted between the years 1996 and 2006 and more than 900 fish have been tagged in the North Atlantic as part f the Tag-A-Giant program. To date, 104 archival tags have been recovered throughout the Atlantic Ocean allowing for the reconstruction of fish tracks of up to 1,623 days (Block et al., 2005).

3. Results and Discussion

In the 160 mtDNA sequences examined, overall haplotype diversity was high (0.9861 ± 0.0053) with 134 haplotypes present. Overall nucleotide diversity ranged from 1.48 to 1.87% among sampling areas. Comparison of the two major spawning areas for Atlantic bluefin tuna, the Gulf of Mexico (GOM) and Mediterranean Sea, revealed significant divergence between the two regions ($\Phi_{st} = 0.01108$, $p = 0.0409$, Table 2). This result is corroborated by the sequence nearest neighbor statistic ($S_{nn} – 0.63592$), which revealed significant association between sequence similarity and sampling locality ($p = 0.0071$). The AMOVA examining levels of genetic variation within vs. between the GOM and Mediterranean Sea also found significant structuring between populations ($p = 0.03931$) even though the majority of variation (98.89%) was accounted for within populations. These results support the hypothesis of genetic isolation between the GOM and Mediterranean Sea stocks of Atlantic bluefin tuna.

Electronic tracking has been useful in elucidating the mechanisms through which genetic isolation has been maintained in Atlantic bluefin tuna. We have recovered the archival records from four fish that display tracks with repeated migrations to known spawning areas during the spawning season. Two fish have shown migrations to the GOM for periods of 2 to 3 consecutive years (Figure 1) and two fish have shown migrations to the western Mediterranean Sea for 3 to 4 consecutive years (Block et al., 2001, 2005). While it has not been possible to determine the natal origin of these fish, the high fidelity to the same spawning areas for multiple years in conjunction with the presence of genetic subdivision among spawning areas suggest that Atlantic bluefin tuna show natal homing. The high degree of spawning site fidelity observed and the significant genetic differentiation detected argue that the Gulf of Mexico and Mediterranean Sea support distinct stocks of bluefin tuna. These results should be taken into account when enacting future management decisions.

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5. References

ALVARADO-BREMER, J. R., Nasseri, I and Ely, B. (1998) A provisional study of northern bluefin tuna populations. ICCAT Coll Vol Sci Pap, SCRS/98/78, 127-129.

BLOCK, B.A., Dewar, H., Blackwell, S.B., Williams, T.D., Prince, E.D., Farwell, C.J., Boustany, A., Teo, S.L.H., Seitz, A., Walli, A., and Fudge, D. (2001) Electronic tags reveal migratory movements, depth preferences and thermal biology of Atlantic bluefin tuna. Science 293:1310-1314.

BLOCK, B.A., Teo, S.L.H., Walli, A., Boustany, A., Stokesbury, M.J.W., Farwell, C.J., Weng, K.C., Dewar, H., and Williams, T.D. (2005) Electronic tagging and population structure of Atlantic bluefin tuna. Nature 434(7037): 1121-1127.

BROUGHTON, R. E. and Gold, J. R. (1997) Microsatellite development and survey of variation in northern bluefin tuna (*Thunnus thynnus*). Mol Mar Bio Biotech. 6(4), 308-314.

CARLSSON, J., McDowell, J. R., Diaz-Jaimes, P., Carlsson, J. E. L., Boles, S. B., Gold, J. R., Graves, J. E. Microsatellite and mitochondrial DNA analyses of Atlantic bluefin tuna (*Thunnus thynnus thynnus*) population structure in the Mediterranean Sea. Mol Ecol. 13(11), 3345-3356.

CORT, J. L. (1991) Age and growth of the bluefin tuna Thunnus thynnus (L.) of the Northeast Atlantic. ICCAT Col. Vol. Sci. Pap. 35, 213–230.

ELY, B., Stoner, D. S., Dean, J. M., Alvarado Bremer, J. R., Chow, S., Tsuji, S., Ito, T., Uosaki, K., Addis, P., Cau, A., Thelen, E. J., Jones, W. J., Black, D. E., Smith, L. Scott, K., Naseri, I., Quattro, J. M., (2002) Genetic analysis of Atlantic northern bluefin tuna captured in the northwest Atlantic Ocean and the Mediterranean Sea. ICCAT Col. Vol. Sci. Pap. 54(2): 372-376.

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

GARCIA, A., Alemany, F., Velez-Belchí, P., López Jurado, J. L., Cortés, D., de la Serna, J.M., González Pola, C., Rodríguez, J.M., Jansá, J. and Ramírez, T. (2005) Characterization of the bluefin tuna spawning habitat off the Balearic archipelago in relation to key hydrographic features and associated environmental conditions. ICCAT Coll Vol Sci Pap. 58(2), 535-549.

HUDSON R. R. (2000) A new statistic of detecting genetic differentiation. Genetics. 155, 2001-2014.

ICCAT 2003 Report of the 2002 Atlantic bluefin tuna stock assessment session. ICCAT Col. Vol. Sci. Pap. ICCAT 55(3): 710-937.

MATHER, F. J., Mason, J. M. and Jones, A. C. (1995) Life history and fisheries of Atlantic bluefin tuna. NOAA Technical Memorandum, NMFS–SFSC. 370, 165 pp.

National Research Council (1994) An assessment of Atlantic bluefin tuna. Natl Acad Press, Washington, DC. 148 pp.

NEMERSON, D., Berkeley, S. and Safina, C. (2000) Spawning site fidelity in Atlantic bluefin tuna, *Thunnus thynnus*: The use of size-frequency analysis to test for the presence of migrant east Atlantic bluefin tuna on Gulf of Mexico spawning grounds. U S Natl Mar Fish Serv Fish Bull. 98(1), 118-126.

NISHIDA, T., Tsuji, S., Segawa, K. (1998) Spatial data analyses of Atlantic bluefin tuna larval surveys in the 1994 ICCAT BYP. ICCAT Coll Vol Sci Pap. 48(1), 107-110.

PUJOLAR, J. M. and Pla, C. (2000) Genetic differentiation between north-west Atlantic and Mediterranean samples of bluefin tuna (Thunnus thynnus) using isozyme analysis. ICCAT Coll Vol Sci Pap. 51, 882-891.

RICE, W. R. (1989) Analyzing tables of statistical tests. Evolution. 43, 223 –225.

ROZAS, J., Sánchez-DelBarrio, J. C., Messeguer, Rozas R. (2003) DnaSP,DNA polymorphism analyses by the coalescent and other methods. Bioinformatics. 19, 2496 –2497.

SCHNEIDER, S., Kueffer, J. M., Roessli, D., Excoffier, L. (1997) ARLEQUIN Version 1 .1:a Software for Population Genetic Data Analysis Genetics and Biometry Laboratory University of Geneva, Geneva.

STOKESBURY, M. J. W., Teo, S. L. H., Seitz, A., O'Dor, R. K., Block, B. A. (2004) Movement of Atlantic bluefin tuna (*Thunnus thynnus*) as determined by satellite tagging experiments initiated off New England. Can J Fish Aquat Sci. 61(10), 1976-1987.

THOMPSON, H. C., and Contin, R. F. (1980) Electrophoretic study of Atlantic bluefin tuna (*Thunnus thynnus*) from the eastern and western Atlantic Ocean. Bull Mar Sci. 30, 727-731.

Table 1. Information on bluefin tuna samples used for genetic analysis

Sample Group	Total N	Sub Grouping	Subgroup N	Dates Collected	Size Range (cm)
Gulf of Mexico	55	GOM Samples	40	Mar-June, 1999-2001	217 - 267
		NMFS Samples	8	Feb-June, 1995-2001	215 - 268
		Eletronically Tagged Fish	7	Jan-Mar, 1997-2005	171 - 268 *
Mediterranean Sea	105	Corsica Samples	20	Sept, 2000-2001	112 - 204
		Tyrrenhian Sea Samples	15	Aug-Sept, 1998	18 - 30
		Eletronically Tagged Fish	12	Dec-Mar, 1997-2004	185 - 224 *
		Ionian Sea Samples	47	Oct-Dec, 1998-2000	32 - 175
		Northern Tunisia Samples	3	Sept, 2003	40 - 44
		Eletronically Tagged Fish	8	Jan-Mar, 1997-2003	172 - 215 *

* Denotes length at tagging 0.25 to 4 years before visitation to a spawning ground

Table 2. Summary statistics comparing 55 Gulf of Mexico and 105 Mediterranean Sea bluefin tuna

Test	Statistic	p-value
Φ_{st}	0.01108	0.0409 *
Sequence Nearest Neighbor	0.63592	0.0071 **
AMOVA F_{st}	0.01108	0.03931 *

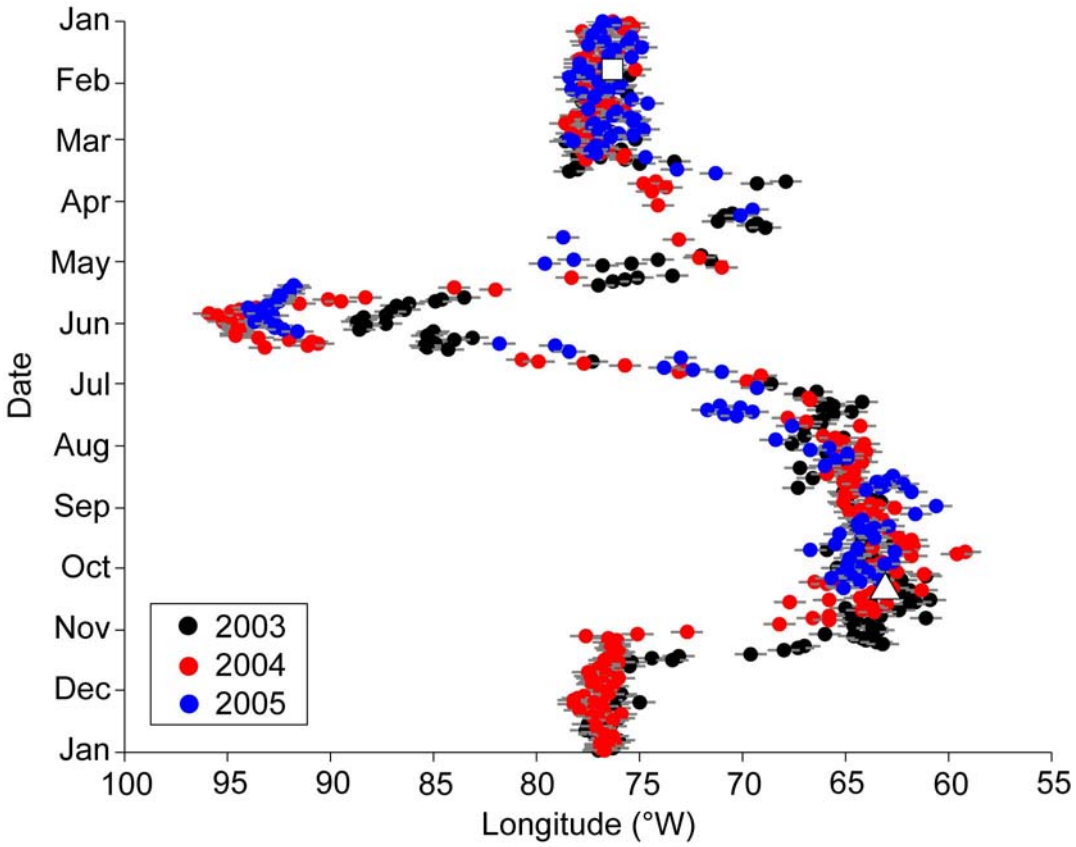

Figure 1. Three years of longitude for a fish electronically tagged off the coast of North Carolina, USA exhibiting three years of movement into the Gulf of Mexico during the spring spawning season. Tagged 25 January 2003 at 208 cm curved fork length