

**SPATIO-TEMPORAL GENETIC PATTERNS IN MEDITERRANEAN
BLUEFIN TUNA: POPULATION STRUCTURING AND
RETENTION OF GENETIC DIVERSITY**

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SUMMARY

We assessed spatio-temporal genetic patterns in Mediterranean samples of bluefin tuna (BFT) to look for signs of population structuring and genetic erosion, which might be related to stock overexploitation and depletion. We analyzed microsatellite variation at seven neutral loci on seven contemporary bluefin tuna samples collected from the Alboran Sea to the Levant Sea (N=316) and in two historical samples (N=99) collected from 1911 to 1926 in the southern Tyrrhenian and Adriatic Seas. We found signs of widespread and deep spatial genetic structuring in both contemporary (mean $F_{ST} = 0.014$, $P < 0.0001$) and historical bluefin tunas ($F_{ST} = 0.020$, $P < 0.0001$). These findings coherently confirm previous data obtained on a limited number of samples and reinforce the possibility that the Mediterranean is inhabited by reproductively isolated sub-populations. Temporal genetic analyses did not find evidence of genetic bottlenecks in Mediterranean bluefin tuna samples. However, since samples displayed heterogeneity of population demographic parameters, Mediterranean bluefin tuna subpopulations might have partially independent dynamics. Our results illustrate that in Mediterranean, structured bluefin tuna populations retain a high level of genetic diversity across space and time, despite possible demographic declines and population changes.

RÉSUMÉ

Nous avons évalué les schémas génétiques spatio-temporels dans des échantillons méditerranéens de thon rouge (BFT) en vue de rechercher des indices de structuration de la population et d'érosion génétique, lesquels pourraient être liés à la surexploitation et à la raréfaction du stock. Nous avons analysé la variation micro-satellitaire chez sept locus neutres de sept échantillons contemporains de thon rouge prélevés de la mer d'Alboran à la mer Levantine (N=316) et dans deux échantillons historiques (N=99) prélevés de 1911 à 1926 dans la mer Tyrrhénienne méridionale et la mer Adriatique. Nous avons trouvé des indices de structuration génétique spatiale très marquée et étendue dans les deux échantillons de thon rouge, contemporains (moyenne $F_{ST} = 0,014$, $P < 0,0001$) et historiques ($F_{ST} = 0,020$, $P < 0,0001$). Ces découvertes confirment de façon cohérente les données antérieures obtenues sur un nombre limité d'échantillons et renforcent la possibilité selon laquelle la Méditerranée est habitée par des sous-populations isolées sur le plan de la reproduction. Les analyses génétiques temporelles n'ont trouvé aucune preuve de goulets d'étranglement génétiques dans les échantillons de thon rouge de la Méditerranée. Toutefois, comme les échantillons ont fait apparaître une hétérogénéité dans les paramètres démographiques de la population, les sous-populations de thon rouge de la Méditerranée pourraient avoir des dynamiques partiellement indépendantes. Nos résultats illustrent le fait qu'en Méditerranée, les populations structurées de thon rouge font apparaître un niveau élevé de diversité génétique dans le temps et dans l'espace, malgré d'éventuelles chutes démographiques et de possibles changements de population.

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RESUMEN

Se han evaluado los patrones genéticos espaciotemporales en las muestras mediterráneas de atún rojo (BFT) para hallar indicios de estructuración de la población y de erosión genética, que podrían estar relacionados con la sobreexplotación y agotamiento del stock. Se analizó la variación microsatélite en siete loci neutrales de siete muestras de atún rojo contemporáneas recogidas desde el mar de Alborán hasta el mar de Levante ($N=316$) y en dos muestras históricas ($N=99$), recopiladas desde 1911 hasta 1926 en el mar Adriático y Tirreno meridional. Se hallaron signos de estructuración genética espacial muy marcada y extendida en ambas muestras de atún rojo, contemporáneas (media $F_{ST}=0,014$; $P < 0,0001$) e histórica ($F_{ST}=0,020$; $P < 0,0001$). Estos hallazgos confirman con coherencia los datos anteriores obtenidos para un número limitado de muestras y refuerzan la posibilidad de que el Mediterráneo esté habitado por subpoblaciones aisladas desde el punto de vista reproductivo. Los análisis genéticos temporales no evidenciaron embudos genéticos en las muestras de atún rojo del Mediterráneo. Sin embargo, dado que las muestras presentaban cierta heterogeneidad en los parámetros demográficos de población, las subpoblaciones de atún rojo del Mediterráneo podrían tener dinámicas parcialmente independientes. Nuestros resultados ilustran el hecho de que, en el Mediterráneo, las poblaciones estructuradas de atún rojo muestran un algo grado de diversidad genética en el tiempo y en el espacio, a pesar de los posibles descensos demográficos y cambios en la población.

KEYWORDS

Bluefin tuna, depleted stocks, DNA, population genetics, population structure, stock identification, Thunnus thynnus

1. Introduction

Fisheries data suggest that *Thunnus thynnus* (BFT) populations have been declining for many years (Fromentin and Powers 2005, Anon. 2007) and the biomass of adults (spawning stock biomass) was in 2006 at its lowest, with a worsening in the last 5-10 years (Anon. 2008). BFT is currently managed by ICCAT under the hypothesis of two management units, the western stock (spawning in the Gulf of Mexico and distributed along North America coasts) and the eastern stock (spawning in the Mediterranean and distributed also in the North East Atlantic), although mixing between the units occur (Block *et al.* 2005, Rooker *et al.* 2008).

In the eastern stock, fishing pressure has changed the spatial distribution of large adult BFTs since the 1950s-1970s. Currently, BFT appears to be rare in the Bay of Biscay, North Sea, Norwegian Sea and in parts of the Ionian Sea and it disappeared from the Black Sea in 1987 (see references in (MacKenzie *et al.* 2009)). The evidences of decline in biomass and of geographic range contraction indicate that stock is overexploited. One of the priority measures for conservation of the species is to increase the survival of juvenile tuna by reducing the catches in 2007-2010 and setting the minimum size of captured fish up to 30 kg (ICCAT recovery plan). Exploited fish populations often show changes in the size and age structure and this can affect individual relative fecundity and offspring viability.

MacKenzie *et al.* (2009) evaluated the effectiveness of this recovery plan for the eastern BFT by assessing population demography trajectories using simulations and different dynamic models. Such models were based on different assumptions of demographic parameters and fishing mortality estimates. This study shows that the current recovery plan of ICCAT could cause a fall of recorded catches and the collapse of BFT populations and fisheries. Simulations show that the adult population size will decrease to small size and these changes can affect downstream the recruitment because of i) the small number of spawners, ii) the potential effect on reproductive success and survival of individuals, and iii) the increased risk of recruitment failures due to the experiencing of adverse environmental conditions. In general, a depleted population shows less viability in changing environment conditions because of a lack of evolutionary potential to adaptation. Several indicators of population status suggest that BFT is overexploited, as age structure and reproductive demographics describe a population with reduced reproductive potential and increased vulnerability to ecosystem changes. All in all, the simulations performed are dependent on several assumptions of fishing mortality and population biology

(growth rate, maturity schedules, reproduction rate) and some variables included in the analysis (estimated catch) are uncertain and can influence the tuna spawner biomass projections (MacKenzie *et al.* 2009).

The concern for the BFT persistence in the Mediterranean pairs with the still unknown complex population dynamics, even if several progress have been obtained using electronic tagging surveys (Block *et al.* 2005, Sibert *et al.* 2006) and genetic analysis (Carlsson *et al.* 2004, Carlsson *et al.* 2007). A more complex demographic scenario could affect the outcomes of recovery plan which, for example, does not consider movements and connectivity between Mediterranean Sea and North Atlantic. Fromentin (2009) performed a comparison between Nordic and Mediterranean fisheries data available from 1900 to 2000. The comparative analysis showed that periodic collapses of BFT fishery might result from changes in migration pattern caused by herring stock collapse, water cooling and/or overfishing. Moreover, fishing had influenced the species dynamics since 1950s even if the interactions between different factors (environmental, trophic and fishing factors) may have impacted the migration pattern and recruitment of ABFT (Fromentin 2009).

Collecting fisheries data with new evidence of reproductive behaviour and genetic differences between the Gulf of Mexico and Mediterranean Sea and, within this basin, between western and eastern Mediterranean, Fromentin (2009) advise the presence of at least three sub-populations: 1) a highly migratory stock in the North Atlantic, 2) a more resident stock in the Mediterranean and 3) a more resident stock in the West Atlantic. This hypothesis might be too simplistic because it does not take into account complex interplays between subpopulations. However, it is consistent with the evidence of several Mediterranean BFT subpopulations obtained with satellite tagging (De Metrio *et al.* 2005) and population genetic analysis (Carlsson *et al.* 2004; Vella 2009).

2. Aims

Within the framework of the Italian research project TUNING dealing with the characterization of the spatial and temporal patterns of genetic variation in the Mediterranean BFT, we analysed population structure and short-term temporal changes of genetic diversity in the Mediterranean BFT. The assessment of temporal genetic changes in the Mediterranean BFT assumes a practical relevance because it aims to detect a loss of genetic diversity in the overexploited and depleted populations. The estimate of demographic parameters is essential for the management of endangered species; in particular it is crucial to estimate the effective population size, the presence of signals of population contraction, of loss of evolutionary potential (low genetic diversity), and the extent of inbreeding and fragmentation (Frankham 1995). The effective population size (N_e) is a reliable indicator of the risk that a given species loses genetic diversity, because N_e is inversely proportional to the impact of genetic drift (i.e. the main process leading to fixation and loss of alleles and therefore to the loss of evolutionary potential). Moreover, the effect of genetic drift is known to be faster in fragmented and small populations and in stressful environments. In general smaller populations show relatively low mean fitness, due also to the accumulation of deleterious mutations as consequence of a less efficient natural selection.

3. Materials and Methods

For this analysis, we scored allele frequencies at seven microsatellite loci in nine Mediterranean BFT samples (**Figure 1**). Sampling design included two historical samples collected from 1911 to 1926 (Adriatic Sea, South Tyrrhenian Sea; $N = 99$) and seven contemporary samples collected from 1999 to 2007 (Levant Sea, Adriatic Sea, South Tyrrhenian Sea, Ligurian Sea, Sardinian Traps, Algerian coasts, Alboran Sea; $N = 316$). Microsatellite data were analysed with an array of dedicated population genetic tests and software. The description of methods and models we used is here omitted, but they are available from authors upon request.

4. Results and discussion

4.1 Spatial patterns of genetic variation

Here, we aimed to assess whether there is any genetic evidence of genetic variance, estimated by F_{ST} , show significant differences among contemporary population samples (**Table 1**) confirming and strengthening previous results obtained with the same markers on a lower number of samples (Carlsson *et al.* 2004, Carlsson *et al.* 2007). The overall genetic differentiation was of the same extent among contemporary samples ($F_{ST} = 0.014$, $P < 0.0001$) and between the two historical samples ($F_{ST} = 0.020$, $P < 0.0001$). This finding reveals that genetic differences apparently persisted across approximately the last century

and more than 10-12 generations and would suggest that genetic structuring in BFT appears to be a steady feature of the species rather than an anthropogenically-induced effect. Our F_{ST} estimates among adult and juvenile BFT population samples in the Mediterranean were of the same extent or slightly greater than those estimated in previous studies using yearlings samples (Carlsson *et al.* 2004, Carlsson *et al.* 2007) and in another tuna species, *Thunnus obesus* (Gonzalez *et al.* 2008). As a consequence, taking into account the ecological and reproductive BFT traits (reviewed in Fromentin and Powers 2005; Rooker *et al.* 2007), these levels of genetic structuring lead to reject the hypothesis that Mediterranean BFT samples belong to a single panmictic population. Overall, based on a high number of BFT samples and individuals, some collected from the most known spawning areas (e.g. Balearic, South Tyrrhenian, Levant Sea), we can suppose that BFT is subdivided in independent reproductive units in the Mediterranean, though it is difficult to unequivocally define these population units with highly mobile species such as BFT. Our findings coupled with those previously obtained by Carlsson *et al.* (2004, 2007) support the hypothesis that different sub-populations inhabit the Mediterranean and add further complexity to the population demography of BFT (Fromentin 2009).

4.2 Temporal patterns of genetic variation

Here, we aimed 1) to assess whether the Mediterranean BFT populations have suffered significant and consistent genetic bottlenecks; and 2) to get estimates of population demographic parameters (e.g. the effective size of BFT populations) which are independent from fishery based data.

Our analyses did not identify coherent and significant signals of genetic erosion, although the eastern Atlantic population has been under a long-lasting overexploitation leading to the steepest phase of decline in last 5–10 years (Fromentin and Powers 2005; Anon. 2007, 2008). The contemporary samples showed high values of allele diversity and heterozygosity and these values are of the same extent in the two historical samples (data not shown but available from authors upon request).

When looking for signatures of population bottlenecks, one reasonable assumption is that rare alleles have a higher chance to be lost than common alleles. This should result in a faster decline of allele number than the range in microsatellite allele size (M ratio, M_P_Val software; (Garza and Williamson 2001). Thus, we compared these quantities using a test for demographic bottlenecks.

The M-ratio bottleneck test proved to be rather sensitive to the choice of parameters used for the analysis. The M ratios in the Mediterranean BFT samples ranged from 0.76 to 0.89 (**Figure 2**). Thus, all BFT samples showed $M > 0.70$, that is an empirical diagnostic value of bottlenecked populations (Garza and Williamson 2001). However, all samples but three (the two samples from Adriatic and the sample from Alboran Sea) showed M ratios below 0.82, a cut-off value empirically-obtained for demographically stable natural populations. These values of M strengthen the presence of several subpopulations in the Mediterranean Sea each with its own demographic history as coherently detected by overall and pairwise significant F_{ST} values.

Although we did not find evidence of dramatic losses of genetic diversity, the signals displayed by our samples suggest that recent demographic declines potentially have impacted genetic variation in Mediterranean BFT populations, even if only slightly. These issues clearly illustrate that in the Mediterranean, structured BFT populations retain a high level of genetic diversity across space and time, despite a possible demographic decline and changes in the population age structure and reproductive demography (Anon. 2008) that can reduce its reproductive potential and increase its vulnerability to ecosystem changes (MacKenzie *et al.* 2009).

Nevertheless it should be kept in mind that genetic responses to demographic bottlenecks can be variable and depend on the species considered and population traits (Garza and Williamson 2001). The retention of diversity in the face of demographic instability is not unprecedented in bottlenecked populations of marine fish (Ruzzante *et al.* 2001, Shrimpton and Heath 2003, Consuegra *et al.* 2005) and several factors can contribute to buffer the loss of genetic diversity despite demographic decline. Indeed, species can show different genetic responses to demographic bottlenecks due to species' natural history, variable ecosystem conditions and population life traits (for example, generation time, ecological behaviour, fecundity). In a given population, to detect a loss of genetic variation, at least more than one generation has to be spent after the demographic decline (Garza and Williamson 2001). It could be possible that our analysis did not detect evident bottleneck signals in the contemporary because of a too recent steep demographic decline and a mean generation time of 8 years.

Values of short-term effective population size, N_e , were estimated with temporal methods for the Adriatic and South Tyrrhenian Seas using a generation time for BFT of 8 years. We obtained an approximate estimate of $N_e = 700$ for the Adriatic and $N_e = 400$ for the South Tyrrhenian. These estimates are within the range displayed by

marine fish and they straddle on the minimum threshold ($N_e = 500$) indicated to maintain genetic diversity and evolutionary potential across several generations in natural populations (Frankham *et al.* 2002). Under the present concern for the conservation of Mediterranean BFT, it could be of relevance to use N_e (obtained independently from the fishery data) to calculate empirical values of N , that is the census number of breeding individuals in a species. To do this, we used the average empirical value of the relationship N_e/N , that for marine fish is estimated around 10^{-4} (Hauser and Carvalho 2008). In the absence of more accurate assessment of this ratio for BFT, the N_e values inferred from our data and based on these assumptions, suggest that the census size of BFT subpopulations might be close to 10^6 .

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Table 1. Pairwise F_{ST} values among contemporary *Thunnus thynnus* samples. Significance was obtained on 10,100 permutations. *Values not significant at 5% level; ** Values not significant after Bonferroni correction.

	<i>CADR</i>	<i>CSTY</i>	<i>CLIG</i>	<i>CSAR</i>	<i>CALG</i>	<i>CALB</i>
<i>CSTY</i>	0.017					
<i>CLIG</i>	0.006*	0.021				
<i>CSAR</i>	0.013	0.017	0.011**			
<i>CALG</i>	0.018	0.004*	0.019	0.025		
<i>CALB</i>	0.018	0.012	0.022	0.025	0.015	
<i>CLEV</i>	0.017	-0.001*	0.020	0.023	0.001*	0.011

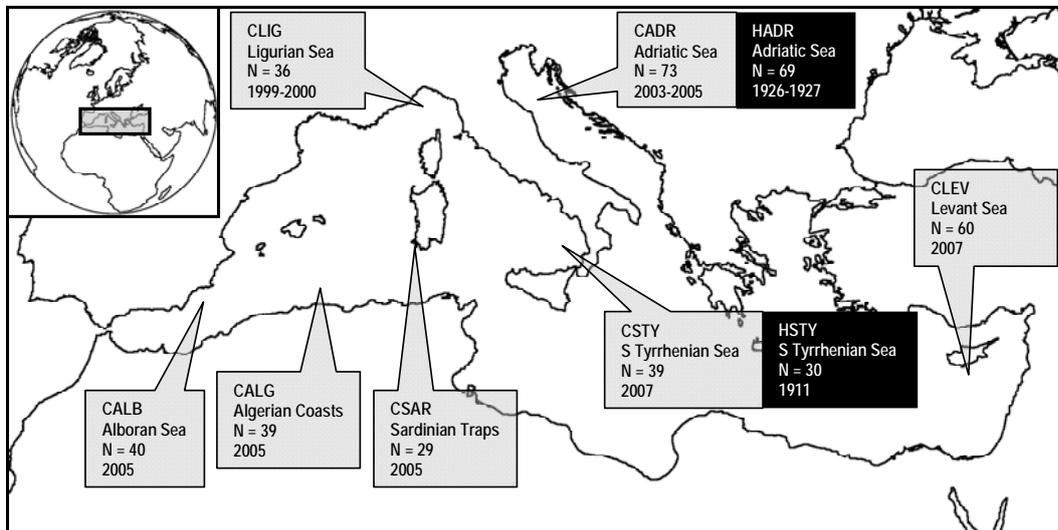


Figure 1. Map of the contemporary (C) and historical (H) samples of *Thunnus thynnus* collected in the Mediterranean. Beside location, acronym, sample size and year/s of collection are also given.

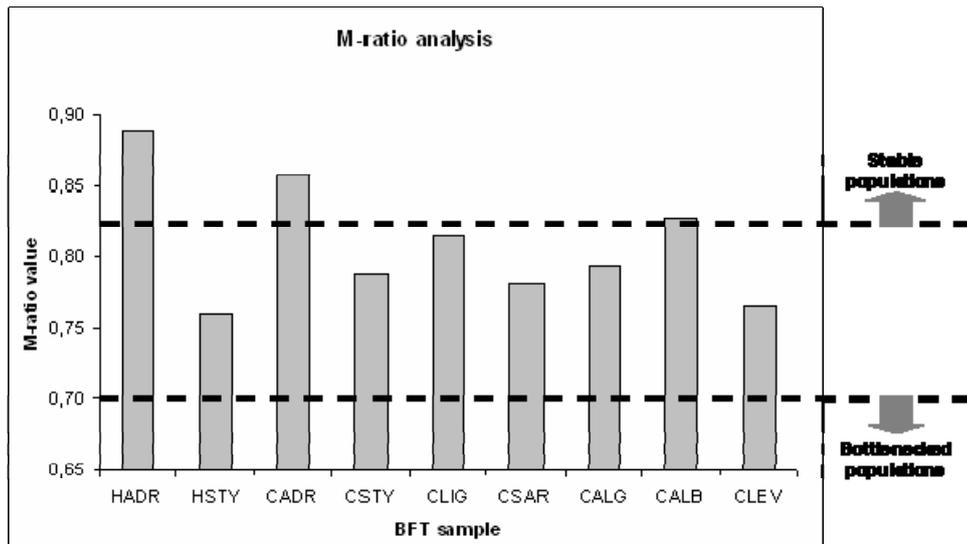


Figure 2. M-ratio analysis. Empirical threshold values are highlighted by dashed lines. 0.82 is the value for demographically stable populations, 0.70 is the cut-off value under which populations have suffered genetic bottleneck (Garza and Williamson 2001).