NEW GENETIC TOOLS FOR ATLANTIC BLUEFIN TUNA MONITORING

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SUMMARY

The Grand Bluefin Tuna Year Programme (GBYP), established to enhance scientific advice for Atlantic bluefin tuna management and conservation, has supported the application of genetic approaches to address this aim during more than 7 yearly phases. The knowledge on Atlantic bluefin tuna population structure complexity gained during the last GBYP phases rejected the previous assumed paradigm of two non-mixing isolated populations. These findings revealed some limitations of the results obtained using the 96 SNP chip that was previously designed for genetic origin assignment and highlighted the need to develop a new genetic monitoring tool for a comprehensive individual genetic make-up analysis. We have developed a powerful SNP array, which makes it possible to obtain information from ~8,000 genetic markers valid for population connectivity and adaptive potential studies, among others. The newly developed SNP array has also been proven useful for kinship analysis, being appropriate for the implementation of close-kin mark-recapture models. Given these advances and the high-resolution insights provided at a competitive cost, we recommend adopting the new SNP array, developed under the GBYP, for future Atlantic bluefin tuna monitoring.

RÉSUMÉ

Le Programme de recherche sur le thon rouge englobant tout l'Atlantique (GBYP), mis en place pour renforcer l'avis scientifique sur la gestion et la conservation du thon rouge de l'Atlantique, a soutenu l'application d'approches génétiques pour atteindre cet objectif pendant plus de 7 phases annuelles. Les connaissances sur la complexité de la structure des populations de thon rouge de l'Atlantique, acquises lors des dernières phases du GBYP, ont rejeté le paradigme précédemment supposé de deux populations isolées qui ne se mélangent pas. Ces conclusions ont révélé les limites des résultats obtenus en utilisant la fiche de 96 SNP précédemment conçue pour l'assignation de l'origine génétique et ont mis en évidence la nécessité de développer un nouvel outil de suivi génétique aux fins d'une analyse exhaustive de la composition génétique individuelle. Nous avons développé un puissant jeu de SNP, qui permet d'obtenir des informations provenant de ~8.000 marqueurs génétiques valables pour les études sur la connectivité des populations et le potentiel d'adaptation, entre autres. Le nouveau jeu de SNP récemment développé s'est aussi avéré utile pour les analyses de parenté et approprié pour la mise en œuvre des modèles de marquage-recapture de spécimens étroitement apparentés. Compte tenu de ces avancées et des informations à haute résolution fournies à un coût compétitif, nous recommandons d'adopter le nouveau jeu de SNP, développé dans le cadre du GBYP, pour le futur suivi du thon rouge de l'Atlantique.

RESUMEN

El Programa de investigación sobre atún rojo para todo el Atlántico (GBYP), establecido para mejorar el asesoramiento científico para la ordenación y la conservación del atún rojo del Atlántico, ha apoyado la aplicación de enfoques genéticos para abordar este objetivo durante más de 7 fases anuales. Los conocimientos sobre la complejidad de la estructura del stock del atún rojo del Atlántico adquiridos durante las últimas fases del GBYP rechazaron el paradigma asumido anteriormente de dos stocks aislados que no se mezclan. Estos hallazgos revelaron algunas limitaciones de los resultados obtenidos utilizando el chip de 96 SNP que se había diseñado previamente para la asignación del origen genético y pusieron de relieve la necesidad de desarrollar una nueva herramienta de seguimiento genético para un análisis exhaustivo de la composición genética individual. Hemos desarrollado una potente gama de SNP, que permite

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obtener información de ~8.000 marcadores genéticos válidos para estudios de conectividad de las poblaciones y potencial de adaptación, entre otros. La gama de SNP recientemente desarrollada también ha demostrado su utilidad para el análisis de parentesco, siendo apropiada para la implementación de modelos de recuperación de marcas de parentesco cercano--. Dados estos avances y la información de alta resolución proporcionada a un coste competitivo, recomendamos adoptar la nueva gama de SNP, desarrollada en el marco del GBYP, para el seguimiento futuro del atún rojo del Atlántico.

KEYWORDS

Bluefin tuna; genetic monitoring; North Atlantic Ocean; stock-mixing; genetic traceability; population structure; migratory behavior; CKMR

1. Introduction

The Atlantic bluefin tuna (*Thunnus thynnus*, ABFT) has been managed during decades as two stocks: the Western stock (W-BFT) and the Eastern stock (E-BFT), separated by the 45ºW meridian line. However, electronic tagging (Block *et al.*, 2005) and otolith microchemistry (Rooker *et al.*, 2014) data showed that ABFT individuals can cross this boundary line. Moreover, more recent studies based on otolith chemistry and genetic makers suggest that the situation can be more complex that two isolated stocks that do not mix at feeding aggregates (Brophy *et al.*, 2020, Diaz-Arce *et al.*, 2023). As good understanding of the key biological and ecological processes is necessary for an effective fish stock assessment and management, the International Commission for the Conservation of Atlantic Tunas (ICCAT) adopted in 2008 the Grand Bluefin Tuna Year Programme (GBYP) to enhance the scientific advice for the assessment and management of the ABFT. One of the tasks carried out under this programme, which are funded in a yearly basis, has consisted in the application and development of genetic tools to address relevant questions relevant for the ABFT management and conservation.

Genetic markers-based tools have been proved useful to answer a diverse range of critical questions within the field of fisheries science. Genetic approaches useful for fisheries management and conservation are evolving hand in hand with the progress of sequencing and genotyping technologies, which during the last decades have allowed to obtain individual information at thousands of genetic markers in an increasingly cost-efficient manner. Genetic tools can be used to estimate population size, for stocks' traceability, to study fisheries target species population structure and connectivity and the delimitation of relevant management units, among others (Valenzuela-Quiñonez 2016, Bernatchez *et al.*, 2017). We have benefited from these numerous applications to further study the population structure and connectivity of the ABFT and to develop adequate monitoring tools.

2. Genetic origin assignment of Atlantic bluefin tuna captured at feeding aggregates using the 96 SNP chip

The evidence on ongoing stock mixing at feeding aggregates along the Atlantic Ocean, highlighted the need for the estimation of stock mixing proportions within different geographic regions for an efficient assessment of the ABFT. In this context a cost-effective tool was developed: the 96 SNP chip which allowed to trace genetic origin of individual samples based on a reduced subset of genetic markers (Rodríguez-Ezpeleta *et al.*, 2019). This tool was developed and validated during GBYP Phases 5 and 6 and showed a high assignment accuracy assigning individuals which were assumed to be originated from the Gulf of Mexico and Mediterranean Sea spawning grounds, corresponding to the Western and Eastern stocks respectively. Since then, results obtained using this tool have provided valuable information about the mixing proportions at the feeding aggregates along the Atlantic Ocean for the assessment and management of the ABFT. Moreover, the subset of markers included in the SNP chip as well as the baseline have been improved since their first design in GBYP Phases 5 & 6, and more than 3,000 ABFT have been assigned to its stock of origin under the GBYP Phases from 6 to 12 (**Figure 1**).

As the amount of analyzed individuals increased, one of our first observations was the relatively high proportion of unassigned individuals. Initially, it was interpreted as a methodological limitation, proposing that the baseline used would not capture the whole genetic diversity of both stocks and therefore the method was unable to assess origin at every case. However, following analysis using an improved baseline probed this hypothesis incorrect (GBYP Phases 9 to 11). In parallel to the development and application of the 96 SNP chip, further efforts have been made under the GBYP to understand the population structure complexity of the species. High resolution results based on thousands of genetic markers revealed the presence of genetically intermediate individuals at

feeding aggregates. Interestingly, these genetically intermediate individuals would result as unassigned individuals using the 96 SNP chip as they could not be assigned to either stock with sufficient confidence. Moreover, genetic analyses showed that individuals born in the newly found spawning area in the Slope Sea (Richardson *et al.*, 2016) are genetically intermediate as the result of individuals of Mediterranean Sea and Gulf of Mexico genetic origin spawning in that area, and that the migration rate of spawning Mediterranean individuals towards the spawning grounds located in the western Atlantic Ocean could be increasing (Diaz-Arce *et al.,* 2023).

While the SNP chip stands as a powerful and useful tool for estimating and monitoring stock mixing proportions at the feeding aggregates through space and time (analyzed samples captured across years allow to explore temporal data), the obtained results need to be interpreted taking into account that the SNP chip was developed under the assumption of two genetically isolated populations (corresponding with the Western and Easter stocks) and the assumption that hybrids between both populations would not exist, which have been proved to be violated by the presence of interbreeding in the Slope Sea and the detection of some first generation migrants in the Gulf of Mexico (Diaz-Arce *et al.,* 2023).

3. The SNP array: new genomic analysis tool for an integrated monitoring of ABFT

Given the new knowledge on population structure complexity and connectivity gained through the last GBYP phases, it became necessary to develop an improved and more powerful traceability tool independent from population structure assumptions which would recover more exhaustive information from analyzed individuals. This tool should be valid not only to monitor the stock mixing and distribution, but also to answer other questions relevant for management advice and conservation strategies of ABFT. To meet these needs, a new SNP array which included nearly 8,000 genetic markers was designed and manufactured under the GBYP Phase 10. The SNP array consisted in an Axiom Custom Genotyping Array which is a widely used tool for agrigenomic and aquaculture monitoring projects (available here and here) and can be easily applied by any institution around the world as it is based on standardized direct genotyping, meaning that the obtention of the results is not dependent of data processing and ensures replicability across laboratories at a competitive cost for sample processing and genotyping. In total, 1,152 samples have been already analyzed using the SNP array and additional 768 samples are planned to be processed during the upcoming GBYP Phase 13. The results obtained using this new tool were validated by the inclusion of 100 replicate samples that had been previously analyzed using high-throughput sequencing methods (Rodríguez-Ezpeleta *et al.*, 2019, Diaz-Arce *et al.*, 2023), providing with equivalent results (analysis made under the GBYP phases 10 to 12).

The SNP array contains different types of markers which provides complementary information about each analyzed sample. A summary of the types and number of markers included in the array, and their potential use is summarized in **Table 1**. The inclusion of thousands of neutral genetic markers allows the exploration of the actual individual's genetic profile not relying on a priori information as well as identification of kin pairs (analysis performed under GBYP phase 12 probed the SNP array as a reliable tool for kin pair detection) which could be complemented with motherhood information obtained through the included mitochondrial markers. Likewise, potentially adaptive markers identified in Diaz-Arce *et al.,* (2023) could provide information about the individual adaptive potential. The markers already included in the 96 SNP panel were also included to allow increasing the already large assignment available dataset analyzed using this tool. Finally, sex assignment markers adapted from (Suda *et al.*, 2019) included in this array probed to correctly assign sex to 21 out of 23 analyzed individuals for which sex was confirmed based on gonad inspection.

This newly developed tool provides high resolution genomic data, which will allow to better understand the migratory and reproductive behavior of the ABFT. Moreover, its suitability for kin pair detection makes it a valid genomic tool for the implementation of Close-Kin-Mark-Recapture promising model for ABFT. Therefore, we recommend future ABFT genetic monitoring to be based on the application of the newly developed SNP array, which will be accessible at a competitive price.

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Table 1. Type and number of markers included in the SNP array for integrated Atlantic bluefin tuna monitoring. The applicability of the information derived from each marker type is described.

Marker type	*Number*	*Applicability*
Neutral	7,719	Population connectivity studies Genetic profile estimation Kinship analysis
Mitochondrial markers	13	Harness kinship analysis (motherhood) Mitochondrial introgression
Potentially adaptive	109	Monitoring introgression and adaptive potential
Markers from 96 SNP chip	86	Genetic origin assignment
Sex Markers	4	Assign genetic sex

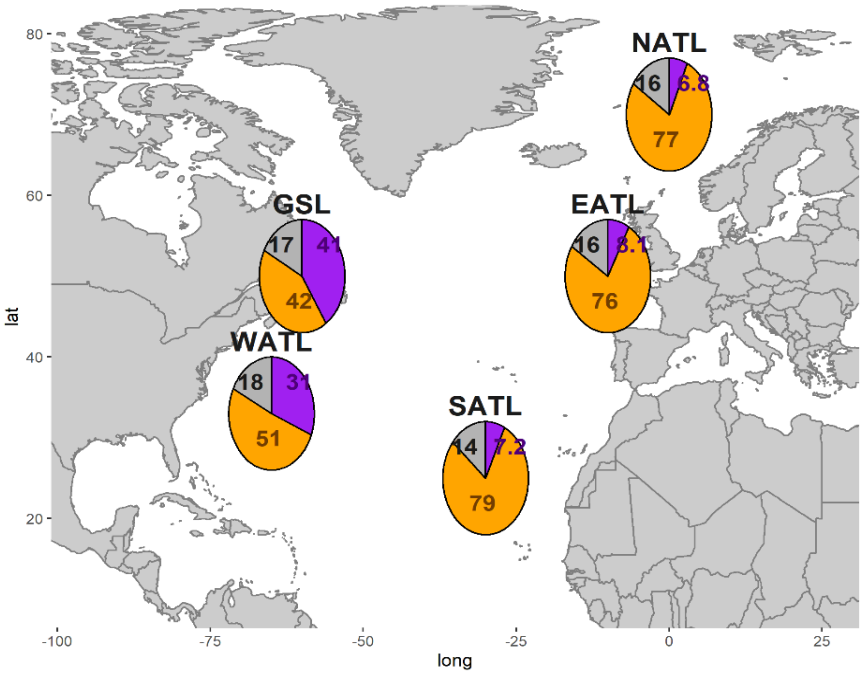

Figure 1. Proportion of Atlantic bluefin tuna (*Thunnus thynnus*) samples assigned to Mediterranean (orange) or Gulf of Mexico (purple) origin and unassigned (grey) calculated from the genetic assignment 3,242 individual samples captured from the different ICCAT areas (GSL= Gulf Saint Lawrence, WATL=West Atlantic, SATL=South Atlantic, EATL=East Atlantic and NATL=North Atlantic) using the reduced 96 SNP chip developed in Rodríguez-Ezpeleta *et al.* (2019) and analyzed under the GBYP from phases 5 to 12.

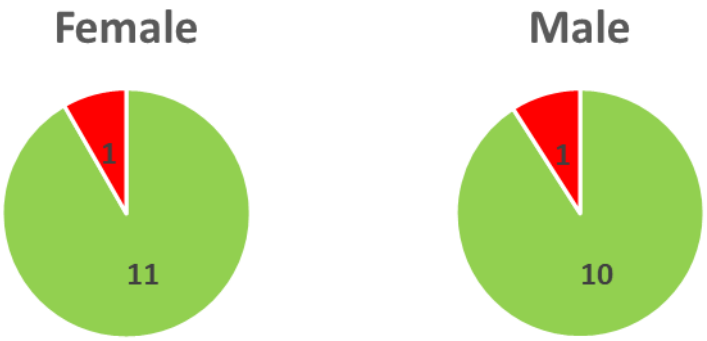

Figure 2. Pie charts show number of samples for which genetic sex was correctly (green) and uncorrecly (red) asigned based on the sex assignmen genetic markers included in the SNP array. Left and right plots include samples assigned to females and males respectively based on gonad histology inspection respectively.