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Population structure and provenance of tropical tunas: recent results from high throughput genotyping and potential implications for monitoring and assessment

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P. M. Grewe[1], H. E. Irianto[2], C. H. Proctor[1], M. S. Adam[3], A. R. Jauhary[3], K. Schafer[4], D. Itano[5], K. Evans[1], A. Killian[6] and C. R. Davies[1]

[1] CSIRO Oceans and Atmosphere, GPO Box 1538, Hobart Tasmania 7001, Australia
[2] Center for Fisheries Research and Development, Jakarta, Indonesia
[3] Marine Research Centre, Ministry of Fisheries and Agriculture, Male, Maldives
[4] Inter-American Tropical Tuna Commission, La Jolla, California, USA.
[5] 689 Kaumakani Street, Honolulu Hawaii. 96825, USA
[6] Diversity Arrays Technology, University of Canberra, Bruce, ACT, Australia

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Authors: P. M. Grewe[1], H. E. Irianto[2], C. H. Proctor[1], M. S. Adam[6], A. R. Jauhary[6], K. Schafer[3], D. Itano[4], K. Evans[1], A. Killian[5], C. R. Davies[1].

[1]CSIRO Oceans and Atmosphere, GPO Box 1538, Hobart Tasmania 7001, Australia
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[3]Inter-American Tropical Tuna Commission, La Jolla, California, USA.
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[5]Diversity Arrays Technology, University of Canberra, Bruce, ACT, Australia
[6] Marine Research Centre, Ministry of Fisheries and Agriculture, Male, Maldives.

Abstract

Tropical tuna fisheries are central to food security and economic development of many regions of the world. Contemporary population assessment and management generally assume these fisheries exploit single, well mixed spawning populations within ocean basins. To date, population genetic techniques have lacked the discriminating power required for conclusively testing this assumption. Recent developments in high throughput sequencing platforms have provided technical advances of DNA profiling for improved power of population discrimination at much reduced cost. We provide an overview of recent applications of these approaches to tropical tuna in the Indo/Pacific region. Results to date have demonstrated heterogeneous population structure for yellowfin tuna across the Pacific basin and suggest requirements for further implementation of these methods and associated sampling of tropical tuna species for comprehensive regional and/or basin scale levels. These studies demonstrate that high throughput sequencing methods can identify population structure in large pelagic species and assign provenance with a high level of confidence.

Introduction

International tuna fisheries, managed under the auspices of Regional Fisheries Management Organizations (RFMO), are central to global food security and underpin multi-billion dollar economic activity in the developed and developing world. International law and governance arrangements for these fisheries[†], which straddle international boundaries and large proportions of ocean basins, assume that the areas of competence of each RFMO equate to single panmictic stocks of the major target species[1]. These current arrangements largely reflect geo-politics, the post-WWII development pattern of industrial tuna fisheries and a reliance on fisheries dependent sampling to examine population structure and connectivity within and between RFMOs. Hence, the validity of this "unit stock" assumption and the appropriateness of current assessment and management arrangements are open to question.

Until recently, the resolving power of population genetic approaches (e.g. allozymes, mtDNA, and DNA microsatellites) has arguably lacked sufficient discriminating power to adequately test assumptions surrounding panmictic population structure of many marine pelagic species [2, 3, 4, 5]. However, developments in next generation sequencing (NGS)

[†] United Nations Convention on the Law of the Sea (UNCLOS) and UN Fish Stocks Agreement (UNFSA). http://www.un.org/Depts/los/convention_agreements/Background%20paper%20on%20UNFSA.pdf

approaches have overcome limitations of previous technologies and delivered substantial increases in the power of genetic methods to detect population differentiation in marine species [6, 7, 8, 9, 10, 11]. Importantly, new high-throughput sequencing has low developmental costs and is capable of identifying co-dominant single nucleotide polymorphism (SNP) markers that can be used to distinguish among populations at scales relevant to fisheries assessment and management [7, 12]. These methods have the potential to test the "single stock" paradigm for highly migratory stocks and provide the technical foundation for global chain of custody and provenance systems necessary to improve accuracy of catch reporting and curb Illegal, Unregulated, and Unreported (IUU) fishing.

Here we outline genetic population structure investigations conducted by CSIRO Oceans and Atmosphere with a focus on yellowfin tuna (*Thunnus albacares*) in the Pacific, where it is the second-most important species in the world's largest tuna fishery[‡,13]. In addition, we summarize collaborative studies in the Indo-Pacific region examining bigeye, southern bluefin, and skipjack tunas. Results from these studies demonstrate the potential for these new genetic techniques to enhance sustainable management of these commercially important species at both basin and global scales.

Methods

Biological sampling

Current studies include a number of projects co-funded by CSIRO and various partner organizations that include broad regional sampling design for yellowfin, bigeye (*T. obesus*), and skipjack (*Katsuwonus pelamis*) tuna across the Indian Ocean and Pacific Ocean basins (Table 1). Partners include the Australian Centre for International Agricultural Research (ACIAR), Marine Stewardship Council (MSC), the Australian Department of Foreign Affairs and Trade (DFAT, Australia), the Indian Ocean Tuna Commission (IOTC), and the Australian Government Fisheries Research and Development Council (FRDC). The primary objectives of these studies were to investigate stock structure of tropical tuna (YFT, BET, ALB, and SKJ) across the Indo Pacific region (Table 1). Additional samples were also collected by CSIRO from "out-group" regions not specifically identified in each of these projects (Table 1). Most projects aimed to achieve minimum sample sizes of 25-50 individuals from each of the sites identified within each region, although in principal sample sizes 100 individuals were taken within strict time windows to minimize the potential for spatial and temporal bias. Standardized tissue sampling protocols developed by the CSIRO using purpose built sampling equipment ensured tissue integrity and avoided cross contamination issues. Tissue samples are either frozen on site or directly preserved in RNA-Later and then shipped to lab for subsequent DNA extraction.

DNA extraction and Population Analysis

DNA extractions were performed in lab at CSIRO Hobart facilities using magnetic bead based technology run on an Eppendorf liquid handling robot. Subsequent DNA genotyping was accomplished via ddRAD approach as developed at Diversity Arrays Technology Pty (DArT), Australia. Variation present at single nucleotide polymorphism (SNP) markers were examined for YFT, BET, and SKJ across regions listed in Table 1. Specific genomic analysis pipelines were developing at CSIRO was used to specifically detect the extent of stock structure present. These genomic pipelines also determined optimal provenance/stock identification marker panels and permitted examination of SNP markers to determine species identification among eight *Thunnus* species and skipjack tuna.

[‡] Western Central Pacific Fisheries Commission Year Book, https://www.wcpfc.int/statistical-bulletins

Results

The DArT sequencing technology combined with genomic pipelines developed for SNP marker analysis facilitated high throughput/low cost genotyping in a single genetic DNA profile assay. These DArT DNA profiles revealed three important types of information: firstly, verification of species identification[18] (Grewe, FSBI 2016); secondly, investigation of stock structure/provenance of ocean origin[14] (see also information paper, SA-IP-02); and thirdly, provision of individual ID markers for gene tagging and close-kin mark-recapture for estimation of year class strength and spawning stock biomass[17]. Next Generation Sequencing (NGS) provenance analysis has revealed strong evidence of stock structure in yellowfin tuna within the Pacific Ocean[14] (see also information papers SA-IP-02; Evans et al., SA-IP-15). Preliminary results from other current projects also indicate strong evidence for bigeye and skipjack tuna at similar spatial scales within ocean basins.

Table 1. Sampling coverage for current CSIRO projects for tropical tuna species across five broad regions of the Indo Pacific Oceans: Western Indian Ocean (WIO), eastern Indian Ocean (EIO), western Pacific Ocean (WPO), central Pacific Ocean (CPO), and eastern Pacific Ocean (EPO).

	Regional Sampling Effort by Project					
Project	WIO	EIO	WPO	CPO	EPO	years
CSIRO			✓	✓	✓	2011-2015
CSIRO-ACIAR	✓	✓	✓			2013/2014
CSIRO-MSC	✓		✓		✓	2014
CSIRO-DFAT	✓	✓	✓			2015/2016
CSIRO-IOTC	✓	✓				2015/2016
CSIRO-FRDC			✓	✓		2016/2019

Discussion

Summary

Technical advances in Next Gen Sequencing (NGS) platforms have provided powerful methods for extracting genetic data through DNA profiling in a way that has begun to reveal fine-scaled details of individuals and populations on an unprecedented scale. These projects are demonstrating that NGS DNA approaches are capable of resolving population structure at a scale that other techniques have had limited ability to resolve. The team at CSIRO Oceans & Atmosphere have developed an integrated suite of NGS methods and genetic analysis pipelines that incorporate the three main components required for genomic based testing: i) Sampling[15]; ii) DNA Profiling; and iii) Data Analysis. Key results provided to date demonstrate great potential for using a single 'illegal, unreported and unregulated' (IUU) fishing.

Next Steps in Pacific Ocean Stock Structure for Tropical Tunas

Results from recent experience demonstrates the importance of the following in the design and execution of large-scale sampling programs for population structure and provenance:

- a well-structured sampling design and quality control of tissue sampling to provide high quality data for analysis

- sampling design consideration in terms of spatial and temporal factors (e.g. impacts of monsoon, ocean currents, and seasonal movement of stocks)
- cooperation from member countries and collaboration across research labs
- focussed workshops/working group to ensure integrated high quality implementation of science plan, genotyping, and analysis for population structure
- consideration of needs with respect to mixed fishery and relevance to stock assessment and management needs.

Finally, we encourage the SC and members to consider necessary actions for: i) further development of a coordinated sampling strategy and analysis program for stock structure of tropical Pacific tuna species; ii) establishing a process for securing necessary partners for funding implementation.

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