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Short Communication

Demographic history and the South Pacific dispersal barrier for school shark (*Galeorhinus galeus*) inferred by mitochondrial DNA and microsatellite DNA mark

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ABSTRACT

We used mitochondrial DNA (mtDNA) control region (CR) sequences and genotypes from eight microsatellite DNA (msatDNA) loci to determine the genetic structure of the school shark (*Galeorhinus galeus*) in New Zealand, Australia and Chile. The estimates of mtDNA haplotype and nucleotide diversity were very similar in New Zealand ($h = 0.735 \pm 0.032$, $\pi = 0.001 \pm 0.001$) and Australia ($h = 0.729 \pm 0.027$, $\pi = 0.001 \pm 0.001$), but in Chile they were higher ($h = 0.800 \pm 0.089$, $\pi = 0.002 \pm 0.001$). The haplotype genealogy showed evidence of two distinct clades, New Zealand and Australia combined (clade 1), and Chile (clade 2). A power analysis suggested that sample sizes were large enough to detect any significant differences within clade 1. Neutrality test, mismatch distribution, and demographic reconstructions based on a coalescence approach, suggested that the Oceania population (clade 1) went through a period of population expansion, whereas the population size of the Chile population (clade 2) has been relatively stable over the last 20,000 years. Data from microsatellite loci also supported the separation of the Oceania and Chile populations. Principal component analysis suggested that there might also be a separation of groups within clade 1, which was not statistically significant ($P = 0.434$). The genetic data reported in this study supported the model of a single *G. galeus* stock in New Zealand and Australia. Our findings were consistent with previous tagging data that showed individual *G. galeus* migrate across the Tasman Sea between Australia and New Zealand, and at least some of these migration events result in successful reproduction.

1. Introduction

Genetic markers are important tools for defining the reproductive units (stocks) within a fishery and monitoring the levels of genetic variation within an exploited species. From a biological perspective, a fishery stock is often defined as a group of individuals that is reproductively and demographically independent from other groups of the same species (Carvalho and Hauser, 1994; Waples et al., 2008). The genetic identification of fish stocks provides an indirect estimate of migration rates and the level of reproductive isolation (Nielsen et al., 2009). Furthermore, understanding and managing the genetic constituents of a fish stock is important for the long-term persistence and adaptability of a commercial species (Allendorf et al., 2008). Managing genetic diversity is particularly important for mitigating the risks of inbreeding and reduced adaptability that could be caused when stocks are fished down to small sizes. However, genetic information has been slowly integrated into stock assessment models and it is often used in combination with other approaches such as direct stock identification methods (e.g., tagging programmes) (Waples et al., 2008; Dichmont et al., 2012).

Sharks and rays are vulnerable to overfishing because they have low fecundity and many species are slow growers (Stevens et al.,

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2000). The sustainability of shark fisheries has been a concern for the last three decades (Baum et al., 2003). Many species have become overexploited because of the high demand for meat and fins for human consumption (Musick and Bonfil, 2005). Moreover, shark species often have low management priority when resources are limited and basic fisheries data are lacking. Effective fishery management relies on reliable stock assessments, which require a good understanding of a range of biological parameters such as the number and structure of reproductively independent of units (stocks) within a fishery (Carvalho and Hauser, 1994; Grant and Waples, 2000). Traditionally, large shark species are thought to form large and widely distributed populations due to their high mobility, whereas smaller species are thought to form more structured populations due to their lower dispersal power (Musick et al., 2004).

The school shark (*Galeorhinus galeus*) is a commercially fished shark species that is widely distributed in temperate waters (Last and Stevens, 2009). The limited reproductive potential and the worldwide trends of declining population size suggest that this species is overexploited in most areas so it has been listed as globally *Vulnerable* (Walker et al., 2006). In New Zealand, *G. galeus* occurs throughout its Exclusive Economic Zone (EEZ), but in Australia it is restricted to southern waters from Moreton Bay (southern Queensland) to Cape Leeuwin (Western Australia), including Lord Howe Island and Tasmania (Last and Stevens, 2009). In New Zealand, it is considered a sustainable fishery with the average annual catch of more than 3000 t (MPI, 2012). In Australia, *G. galeus* has been overfished and is now a protected species that is still taken as a bycatch of the gummy shark (*Mustelus antarcticus*) fishery; a 240 t total allowable catch has been set to limit the bycatch level (AFMA, 2010; MPI, 2012). The *G. galeus* stocks in New Zealand and Australia are assessed and managed independently. However, there is evidence of mixing between New Zealand and Australia stocks (Walker et al., 2008). Tagging studies have shown that the Australasian *G. galeus* are long-lived and highly mobile within and between New Zealand and Australian waters (Olsen, 1953, 1954; Stanley, 1988; Paul, 1988; Walker, 1989; Francis and Mulligan, 1998; Hurst et al., 1999; Walker et al., 2008; Francis, 2010).

G. galeus in Chilean waters is found from Arica to Valdivia (Lamilla et al., 2005). It is also common bycatch in Chilean fisheries, such as the artisanal fishery targeting in cusk-eel (*Genypterus* sp.) using longlines. In 2001, about 1 t of *G. galeus* was reported landed, however, there has been a scarcity of catch data since that record (Lamilla et al., 2005; SERNAPESCA, 2011). The fisheries data are complicated because *G. galeus* catches are usually lumped together with *Mustelus mento*, *M. whitney* and *Squalus acanthias* as a single category called "tollo". The combined shark meat is typically sold for human consumption in the domestic Chilean market, whereas dried fins are exported to Asian markets (Hernández et al., 2008).

The current Australian stock assessment treats the New Zealand and Australian stocks separately based on genetic differences (Punt et al., 2000). Genetic differentiation between stocks has been previously reported based on allozyme and restriction fragment length polymorphisms (RFLPs) markers (Ward and Gardner, 1997), but the research had several limitations. First, both types of genetic markers had low levels of polymorphism. As a consequence their potential to detect genetic differences were limited. Second, sampling limitations meant the sample sizes were uneven among regions, and the eastern Pacific was not sampled. Third, the differences reported between New Zealand and Australia were based on allele frequencies only, rather than alleles or haplotypes unique to a region. Fourthly, though differences between regions were identified, their statistical significance was only slight ($P = 0.04–0.052$) and only simple null models were tested.

Overall, the combined results of tagging and genetic studies to date have indicated that mating by school sharks did not occur at random between New Zealand and Australia (Olsen, 1953, 1954; Stanley, 1988; Paul, 1988; Walker, 1989; Francis and Mulligan, 1998; Hurst et al., 1999; Walker et al., 2008; Francis, 2010). However, the lack of a geographically comprehensive sample limited the ability of previous genetic studies to compare any localised genetic differences to broader distribution of the species. In this study we determined the genetic structure of the school shark (*G. galeus*) in New Zealand, Australia and Chile by measuring the level of genetic diversity in the mtDNA control region (CR) and at eight msatDNA markers. We included samples from Chile to provide a more comprehensive coverage of the South Pacific, which compliments the previous study of Chabot and Allen (2009). Possible contraction or expansion of the historical effective population sizes was also examined.

2. Materials and methods

2.1. Sample collection

Muscle tissue samples or fin clips were dissected from *G. galeus* specimens captured in three geographic regions: New Zealand (NZ), Australia (AUS), and Chile (CHI) (Fig. 1). The regional samples comprise: four NZ sites, which are East of North Island (ENI), Kaikoura (KAI), Chatham Rise (CHA) and Solander Islands (SOL); six AUS sites, which are Shoulder (SHO), Great Australian Bight (GAB), Eyre (EYR), King (KIN), northeastern Tasmania (TAS) and Frederick Henry Bay (FRE); and one CHI site, which is Santiago (SAN).

2.2. Mitochondrial DNA sequences

Genomic DNA was extracted from tissue samples using phenol–chloroform protocols (Sambrook and Russell, 2001). The mtDNA CR was amplified using species-specific primers designed using Primer 3 (Rozen and Skalersky, 2000) and implemented in Geneious Pro v5.5.2 (Biomatters Ltd.). Primer sequences were: GGCR_F 5′-CGAACCTAGCCCTTGGCCCAC-3′ and GGCR_R 5′-ACGTCGGTCCTCGTTTTAGGGG-3′. PCR amplifications were carried out in 25 μL volumes containing 67 mM Tris–HCl pH 8.8, 16 mM $(NH_4)_2SO_4$, 2.0 mM $MgCl_2$, 20 mM dNTPs, 1.5 mM $MgCl_2$, 10 μM of each primer, 0.4 units of BIOTAQ™ DNA Polymerase (Bioline) and 1 μL of DNA (20–40 ng DNA). PCR was performed in a TGradient Thermal Block (Biometra, Goettingen, Germany) with an initial denaturation at 95 °C for 5 min, followed by 30 cycles of 95 °C for 30 s, 60 °C for 30 s and 72 °C for 60 s and a final cycle of 72 °C for 10 min. PCR products were purified using ExoSAP-IT kits (GE Healthcare Biosciences). DNA sequences were determined at the Massey University Genome Service using an ABI 3730 Genetic Analyzer. Control region (CR) sequences were edited in Geneious and aligned using the Geneious Alignment option defined by default parameters (Drummond et al., 2011). In order to resolve any ambiguities and confirm the nucleotide calling, the chromatograms were thoroughly checked by eye, and both ends of the sequences were trimmed to obtain overlapping sequences for dataset.

2.3. Microsatellite DNA and genotyping

The samples were genotyped using eight microsatellite loci. One new locus, Ggal15 was isolated and develop by a genomic libraries enriched for repetitive sequence from *G. galeus* genomic DNA. The genomic DNA was processed with the GS FLX emulsion PCR, resulting in several reading where the msatDNA were found using the software QDD 1.0 (Meglécz et al., 2010). The other seven loci (Gg20, Gg15, Gg22, Gg23, Gg12, Gg07, and Gg11) were taken from Chabot and Nigenda (2011). Microsatellite loci were PCR amplified separately in 10 μL volumes containing 67 mM Tris–HCl pH 8.8, 16 mM $(NH_4)_2SO_4$, 2.0 mM $MgCl_2$, 0.05 μM of