Supporting genetic analysis of protected fish species

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Nine protected New Zealand fish species


White shark (R. Armstrong)


Whale shark


Giant manta ray


Basking shark (J. Stafford-Dietsch)


Oceanic whitetip shark (W. White)


Spinetail devilray (W. White)


Deepwater nurse shark (K. Westerskov)


Spotted black grouper


Giant grouper (R. Quinlan)

Nine protected New Zealand fish species


White shark (R. Armstrong)


Whale shark


Giant manta ray


Basking shark (J. Stafford-Dietsch)

Residents


Oceanic whitetip shark (W. White)


Spinetail devilray (W. White)


Deepwater nurse shark (K. Westerskov)


Spotted black grouper


Giant grouper (R. Quinlan)

Nine protected New Zealand fish species


White shark (R. Armstrong)


Whale shark


Giant manta ray


Basking shark (J. Stafford-Dietsch)

Tropical migrants


Oceanic whitetip shark (W. White)


Spinetail devilray (W. White)


Deepwater nurse shark (K. Westerskov)


Spotted black grouper


Giant grouper (R. Quinlan)

POP2015-07 Objectives

- To establish a repository for genetic samples of protected fish species
- To conduct a stock take of complete, current and planned genetic analyses internationally, in relation to New Zealand's [nine protected] fish species
- To provide recommendations on the most appropriate methods of furthering genetic analyses in order to inform management of New Zealand's protected fish species in relation to fisheries bycatch

The wide distributions of most species, and the broad expanses of ocean between New Zealand and other population centres of all nine species, raise the possibility that some or all of the species may have multiple, isolated, geographic populations. Understanding population structure is important for managing the New Zealand populations of these nine species. Even though the species are protected within the New Zealand EEZ, they may be subjected to fishing and environmental impacts elsewhere if they form part of more extensive geographic populations.

Methods - Repository

- NIWA (and formerly MAF Fisheries) has been collecting tissue samples from white shark since 1991, from basking shark since 1997, and from spinetail devilray since 2013 (in conjunction with a DOC study on bycatch in purse seine fisheries).
- Many tissue samples have been contributed to international studies on the population genetics of the three species.
- Sub-samples of the tissues are held at NIWA in Wellington and/or one or more overseas laboratories.
- NIWA's tissue samples form the nucleus of a new library of protected species tissue samples.
- Tissues were transferred to fresh 95% ethanol in 2 ml vials with O-ring sealed caps, provided with new labels containing unique specimen numbers, and recorded on a database. Vials were deposited in a secure, frozen, fire-proof facility approved for ethanol storage (NIWA Invertebrate Collection, Greta Point, Wellington).
- New Zealand and overseas researchers and genetics laboratories were canvassed to identify New Zealand tissue samples of the nine species held by other organisations. In most cases, those tissues remain in their current location and their details were recorded on the new database.

Methods – Review of genetics studies

- Previous studies with international collaborators provided many important sources of information and contacts that provided information to feed into the current review
- We also carried out a new literature search to locate additional and recent published genetic studies on the nine species
- Personal contacts and international listservers were used to identify other researchers working on these species with the aim of compiling an exhaustive list of past, ongoing and planned genetics studies

Tissue repository

Number of New Zealand tissue samples of nine protected fish species held worldwide and in the NIWA tissue repository

Species	Tissues held worldwide	Tissues held in NIWA repository
White shark	102	18
Basking shark	53	23
Whale shark	0	0
Deepwater nurse shark	0	0
Oceanic whitetip shark	1*	0
Spinetail devilray	11	10
Giant manta ray	0	0
Spotted black grouper	9	1
Giant grouper	1	0

* Includes tissues from each of 6 embryos

Non-NIWA specimens (main holdings):

White shark: CSIRO, Australia; University of Colorado; University of Aberdeen

Basking shark: Durham University; University of Aberdeen; Nova Southeastern University, Florida

Spinetail devilray tissues: University of Queensland; University of California Santa Cruz

Spotted black grouper: Museum of New Zealand (Te Papa)

List of genetic studies known to have incorporated tissue samples from New Zealand protected species

Species	References
White shark	Pardini et al. (2001), Chapman et al. (2003), Jorgensen et al. (2010), Tanaka et al. (2011), Gubili et al. (2011, 2012), Blower et al. (2012), O'Leary et al. (2015), Oñate-González et al. (2015), Andreotti et al. (2016)
Basking shark	Hoelzel et al. (2001, 2006), Noble et al. (2006), Magnussen et al. (2007), Lieber et al. (2013), Hester et al. (2015)
Spinetail devilray	Poortvliet (unpubl. data)

List of known ongoing or planned genetic studies of New Zealand's protected fish species

Species	Institution	Researcher
White shark	Flinders University, Bedford Park, South Australia	Charlie Huveneers
White shark	CSIRO, Hobart, Australia	Barry Bruce
White shark	College of Charleston, South Carolina, USA	Gavin Naylor
Basking shark	University of Aberdeen, Aberdeen, Scotland	Lilian Lieber, Les Noble, Cath Jones
Whale shark	Marine Megafauna Foundation, Tofo Beach, Mozambique	Simon Pierce, Alex Watts
Deepwater nurse shark	College of Charleston, South Carolina, USA; Moss Landing Marine Laboratories, California, USA	Gavin Naylor, Dave Ebert
Oceanic whitetip shark	Nova Southeastern University, Florida, USA	Mahmood Shivji
Devil and manta rays	Bangor University, Wales	Jane Hosegood
Devil and manta rays	Center for Fisheries, Aquaculture, & Aquatic Sciences, Carbondale, Illinois, USA	Tom Kashiwagi
Devil and manta rays	Marine Megafauna Foundation, Tofo Beach, Mozambique	Andrea Marshall
Devil and manta rays	Charles Darwin University, Darwin, Australia	Peter Kyne
Devil and manta rays	University of Queensland, Brisbane, Australia	Mike Bennett, Jenny Ovenden
Spotted black grouper	?	
Giant grouper	?	

Genetic studies of basking shark (see report for other species)

Study	Genetic marker	Sample source	Region	No. of samples	Additional GenBank samples	Populations identified	GenBank sequence	Comments
Hoelzel (2001)	mtDNA (cytochrome b)	North Atlantic, Mediterranean Sea, New Zealand	NAO, MS, WPO	17				Describes method of identifying species in shark fin soup and cartilage pills. Basking shark identifed in the latter
Hoelzel at al. (2006)	mtDNA (control region)	New Zealand, Taiwan, Norway, Scotland, eastern USA/Canada, Mediterranean Sea, Caribbean, South Africa	NAO, MS, WPO, WIO	62		0	Not stated	Very low genetic diversity. No population structure identified
Noble et al. (2006)	mtDNA (cytochrome b and D-loop), microsatellites	United Kingdom, Norway, Italy, Portugal, South Africa, eastern USA, eastern Canada, Australia, New Zealand	NAO, MS, WIO, WPO	41		2		Identified 18 microsatellites. Sufficient variation found in mtDNA to allow population differentiation once adequate samples are obtained. Little gene flow between Southern and Northern Hemispheres, and Pacific and Atlantic populations tentatively distinguished. Developed species identification method for basking shark in small quantities of tissue.
Magnussen et al. (2007)	nuclear gene (ITS2)	Northeastern and northwestern Atlantic, Mediterranean Sea, Caribbean, Indian Ocean, southwestern and southeastern Pacific	NAO, MS,WIO, WPO, EPO	44			EF194106	Identification of basking shark fins
Wong et al. (2009)	mtDNA (COI)	Sample records are on the Barcode of Life Data System (BOLD) (at http://www.boldsystems.org) under project code EWSHK)		48			Some of FJ518910–FJ519800, FJ529802–FJ519955. Sequences are on the BOLD System (at http://www.boldsystems.org) under project code EWSHK	Developed nucleotide diagnostic (ND) method for uniquely identifying shark species
Lieber et al. (2013)	mtDNA (control region, COI), nuclear gene (ITS2)	Ireland compared with other global regions incl. New Zealand	NAO, MS, WPO, WIO	30	44			Identified basking sharks from mucus swabs. Little global population structure and low genetic variability
Fields et al. (2015)	mtDNA (COI)	Not stated		1				Partial COI sequences used to identify shark species
Hester et al. (2015)	mtDNA genome	New Zealand	WPO	1			KF597303	Mitochondrial genome sequenced

Example of review text accompanying database – basking shark

Eighteen microsatellites have been described for basking sharks (Noble et al. 2006), and the entire mitochondrial genome of 16,670 base pairs has been sequenced (Hester et al. 2015). DNA can be extracted from mucus swabs collected from free-swimming sharks (Lieber et al. 2013).

Identification of basking sharks from processed products has been reported (Hoelzel 2001, Magnussen et al. 2007, Fields et al. 2015). A nucleotide diagnostic (ND) method has been developed for uniquely identifying shark species, including basking shark (Wong et al. 2009).

Basking sharks have very low genetic diversity (Hoelzel et al. 2006, Lieber et al. 2013) and no clear population structuring has been found on a global scale (Hoelzel et al. 2006, Noble et al. 2006, Lieber et al. 2013). Nevertheless, gene flow between the Northern and Southern hemispheres, and between the Pacific and Atlantic oceans, is low (Noble et al. 2006).

Species with population genetics studies

- Only 4 out of 9 species have population genetics studies - white shark, basking shark, whale shark, spinetail devilray
- No population genetics studies (yet) for oceanic whitetip shark, deepwater nurse shark, giant manta ray, spotted black grouper, giant grouper

Populations distinguished worldwide – white shark

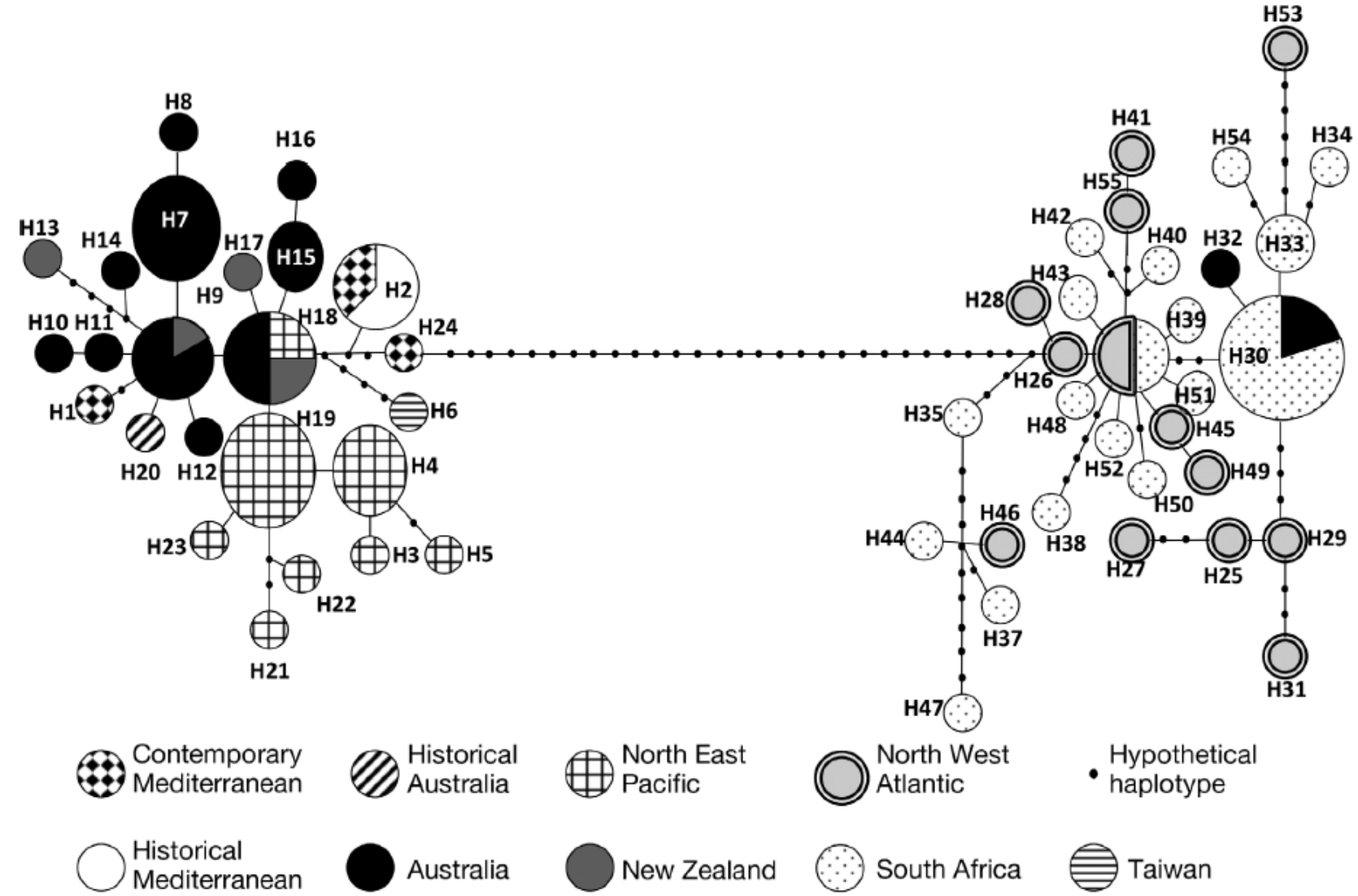

Fig. 2. *Carcharodon carcharias*. Median-joining network from a 749 bp partial mtDNA D-loop sequence consisting of 55 haplotypes derived from 6 historical and 96 contemporary white shark sequences, showing the low genetic differentiation of contemporary (H2) and historical (H1, H2, and H24) Mediterranean samples from Pacific (North East Pacific, Australia, and New Zealand) sharks. Circle size is proportional to the frequency of each haplotype; shading represents capture locality; small black circles represent hypothetical haplotypes; single mutational steps are assumed between haplotypes

Gubili et al. (2015)

Populations distinguished worldwide – white shark sub-structuring

Eastern and southwestern Australia

Table 5. *Carcharodon carcharias*. Genetic population structure and significance level (F_{ST}/p) for pairwise comparisons between Australian regions. F_{ST} values below diagonal are based on mitochondrial DNA (mtDNA), and above diagonal, microsatellite loci (nDNA). N and n represent mtDNA and nDNA sample sizes, respectively. *: significant at $p \leq 0.05$; ^: comparison not significant after Bonferroni correction, $\alpha = 0.0125$

n/N	Region	Eastern Australia (EA)	Southwestern Australia (SWA)
(a) All animals			
62/61	EA		0.00927/0.03186*,^
32/30	SWA	0.14174/0.00000*	
(b) Juvenile animals only			
55/54	EA		0.00140/0.35181
13/12	SWA	0.17348/0.00003*	

Blower et al. (2012)

California and Mexico

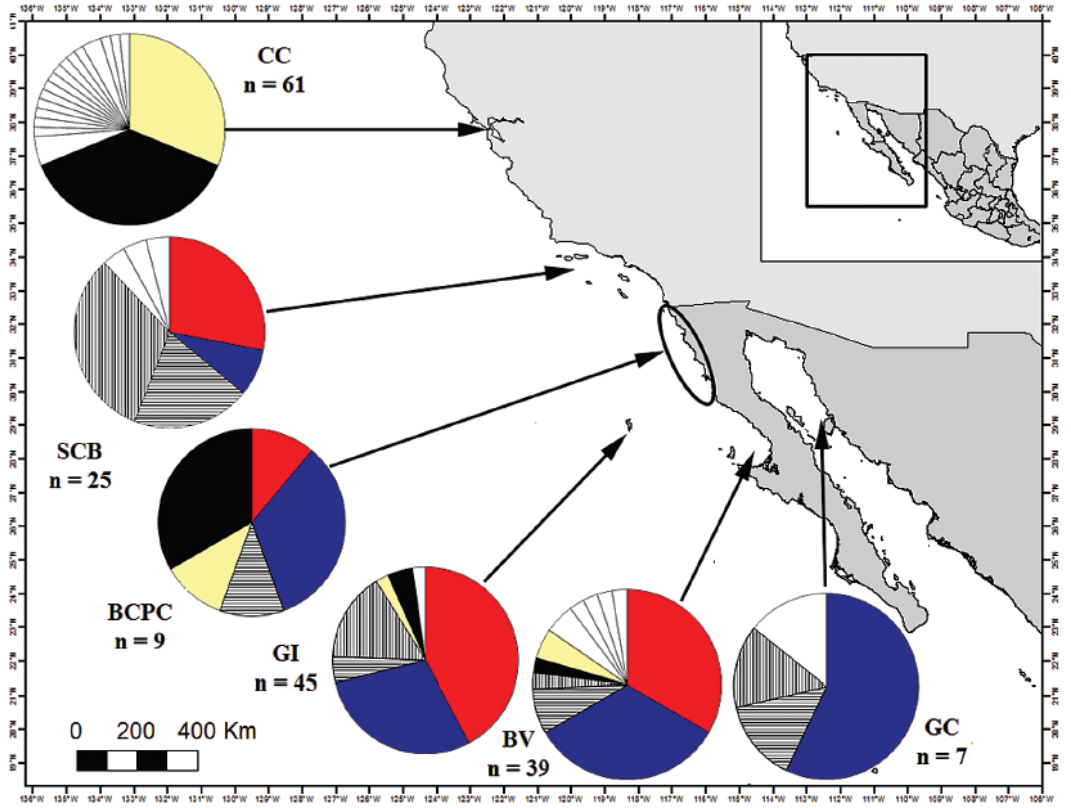

Figure 1. Mitochondrial variation in haplotype frequencies from Northeastern Pacific white shark samples. Each filled slice represents a shared haplotype, whereas white haplotypes are private. Studied areas are: central California (CC), Southern California Bight (SCB), Baja California Pacific coast (BCPC), Guadalupe Island (GI), Bahía Vizcaíno (BV), and the Gulf of California (GC).

Oñate-González et al. (2015)

Populations distinguished worldwide – spinetail devilray

Statistical parsimony network for 50 mtDNA (COX1 and NADH5) haplotypes of *Mobula japanica*. Colors refer to sampling locations, and are explained in the legend. Circle size is proportional to the number of sampled individuals with a given haplotype. Lines between haplotypes represent one mutational step. New Zealand haplotypes are indicated with dashed lines, and the number of individuals is indicated (1X or 3X).

East Atlantic Ocean significantly different from most other sites. Others not different. The comparison between New Zealand (n= 6) and Atlantic samples was marginally significant, but not after correction for multiple tests.

Poortvliet (unpubl. data)