Genetic studies of New Zealand's protected fish species

Prepared for Department of Conservation

June 2016

Prepared by:
Malcolm Francis, National Institute of Water and Atmospheric Research
Peter Ritchie, Victoria University of Wellington

For any information regarding this report please contact:
Malcolm Francis
Principal Scientist
Coastal
+64-4-386 0377
m.francis@niwa.co.nz

National Institute of Water & Atmospheric Research Ltd
Private Bag 14901
Kilbirnie
Wellington 6241

Phone +64 4 386 0300

NIWA CLIENT REPORT No: WLG2016-31
Report date: June 2016
NIWA Project: DOC16301

Quality Assurance Statement		
	Reviewed by:	Dr Judith Sutherland
	Formatting checked by:	Pauline Allen
	Approved for release by:	Dr Rosemary Hurst

Contents

Tables

Executive summary

Nine fish species are currently protected in New Zealand fisheries waters (white shark, basking shark, whale shark, oceanic whitetip shark, deepwater nurse shark, spinetail devilray, giant manta ray, spotted black grouper and giant grouper). All nine species have low productivity, which in combination with fisheries threats make them vulnerable to over-exploitation. 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 them 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. The present study carries out a detailed investigation of the genetics of the nine species in order to (a) establish a repository for genetic samples of protected fish species, (b) conduct a stock take of complete, current and planned genetic analyses internationally, and (c) 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.

NIWA has been collecting tissue samples from white shark since 1991, from basking shark since 1997, and from spinetail devilray since 2013. Many of these tissue samples have been contributed to international studies on the population genetics of these species. We aggregated all of NIWA's tissue samples to form the nucleus of a new library of protected species tissue samples, and a database of worldwide tissue samples of New Zealand's protected fish species was compiled. The database contains good sample sizes of white shark (N=102) and basking shark (N=56) but small or no samples of the remaining seven species. Few of the tissues are held in the NIWA repository, with most being held elsewhere.

Genetic studies on the nine protected species found during a literature review and correspondence with geneticists worldwide are summarised and reviewed. Worldwide population genetics studies have been completed for white shark, basking shark, whale shark and spinetail devilray, although no studies on whale shark have included New Zealand material. The remaining species have been studied in only part of their range (spotted black grouper; no New Zealand material included) or not at all (oceanic whitetip shark, deepwater nurse shark, giant manta ray, giant grouper).

Most of the species covered in this review have widespread, often global, distributions but the samples sizes of many studies were limited. A key priority is to continue to gather samples and make them available to other researchers to complement samples collected from other locations. To increase the levels of genetic resolution, future studies should aim to build comprehensive reference genomes and single-nucleotide polymorphism databases, by using genotyping-by-sequencing or brute force population-scale genome sequencing. These approaches better resolve weak patterns of genetic variation and detect local-adaptive differences among populations. The ability to detect population hierarchies will enable reproductive units to be more clearly defined and improve the setting of conservation priorities. Specific recommendations are made for further study of white shark, basking shark, deepwater nurse shark and spotted black grouper.

1. Introduction

Nine fish species are currently protected in New Zealand fisheries waters under Schedule 7A of the Wildlife Act: spotted black grouper (*Epinephelus daemelii*) was protected in 1996, white shark (*Carcharodon carcharias*) in 2007, spinetail devilray (*Mobula japanica*), giant manta ray (*Manta birostris*), whale shark (*Rhincodon typus*), deepwater nurse shark (*Odontaspis ferox*), giant grouper (*Epinephelus lanceolatus*) and basking shark (*Cetorhinus maximus*) in 2010, and oceanic whitetip shark (*Carcharhinus longimanus*) in 2013.

All nine protected species are considered to have low productivity because of their slow growth rates, low fecundity, and (for most species) small population sizes. They are all actually or potentially caught by fisheries targeting other species in New Zealand. In combination, low productivity and fisheries threats make these species vulnerable to over-exploitation, which led to them being protected.

All nine species range beyond New Zealand's Exclusive Economic Zone (EEZ). Spotted black grouper has the smallest distribution, being restricted to the southwestern Pacific Ocean, including eastern Australia (Francis et al. 2016). Giant grouper occurs throughout the Indo-Pacific Ocean (Heemstra & Randall 1993) and the remaining seven species are found worldwide. 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.

Important tools for understanding population structure include tagging and genetics. Under previous contracts carried out for the Department of Conservation, we briefly reviewed the tagging and genetic evidence for fish movement and population interaction for all nine species (Francis & Lyon 2012, 2014). The present study carries out a more detailed investigation of the genetics of the nine protected species, with the following 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

2. Methods

2.1 Repository for genetic samples of protected fish species

NIWA (and formerly MAF Fisheries) has been collecting tissue samples from white shark since 1991 and from basking shark since 1997. In 2013, NIWA also began collecting tissue samples from spinetail devilray in conjunction with a Department of Conservation study on the bycatch of this species in purse seine fisheries. Many of these tissue samples have since been contributed to several international studies on the population genetics of the three species (see section 3.2). Sub-samples of the tissues are variously held at NIWA in Wellington and/or one or more overseas laboratories.

In this study, we aggregated all of NIWA's tissue samples from the three species mentioned above to 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 (in the form of an Excel spreadsheet). Vials were then deposited in a secure, fire-proof facility approved for ethanol storage at –20 °C (freezer box A10, NIWA Invertebrate Collection, Greta Point, Wellington).

We also canvassed New Zealand and overseas researchers and genetics laboratories 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. However, some tissues have been returned to NIWA and deposited in the new repository.

2.2 Genetic studies of New Zealand's protected fish species

NIWA has previously been involved in international population genetics studies on white shark, basking shark and spinetail devilray (Pardini et al. 2001, Hoelzel et al. 2006, Noble et al. 2006, Lieber et al. in review; M. Poortvliet, University of California Santa Cruz, unpubl. data), and also recently reviewed previous genetics studies of all nine species (Francis & Lyon 2012, 2014). Those studies provided many important contacts and sources of information that were used in the present study. We also carried out a new literature search to locate additional and recent published genetic studies on the nine species.

In order to identify ongoing and planned genetic studies on the nine species, we contacted our colleagues in our previous studies, plus other shark, ray and teleost geneticists worldwide. The principal shark genetics specialists that we consulted were Drs Les Noble and Cath Jones (University of Aberdeen, Scotland), Prof Mahmood Shivji (Nova Southeastern University, Florida), Prof Andrew Martin (University of Colorado, Colorado), and Dr Marloes Poortvliet (University of California Santa Cruz, California). We also consulted a scientist working on the population genetics of serranid groupers, Dr Lynne van Herwerden (James Cook University, Queensland). 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.

3. Results

3.1 Repository for genetic samples of protected fish species

A database of worldwide tissue samples of New Zealand's protected fish species has been compiled. It contains good sample sizes of white shark (N=102) and basking shark (N=56) but small or no samples of the remaining seven species (Table 1). Few of the tissues are held in the NIWA repository, with most of them being held elsewhere. In many cases, duplicates of the tissue samples in the NIWA repository are also held in overseas institutions. White shark tissues are mainly held in CSIRO (Hobart, Australia), University of Colorado (Colorado, USA), and University of Aberdeen (Aberdeen, Scotland). Basking shark tissues are mainly held in Durham University (Durham, England), University of Aberdeen (Aberdeen, Scotland), and Nova Southeastern University (Florida, USA). Spinetail devilray tissues are mainly held in the University of Queensland (Brisbane, Australia) and the University of California (Santa Cruz, California, USA) in addition to the NIWA repository. Spotted black grouper tissues are mainly held in the Museum of New Zealand (Te Papa).

Table 1: 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	56	26
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

3.2 Genetic studies of New Zealand's protected fish species

Genetic studies on the nine protected species found during our literature review and correspondence with geneticists worldwide are summarised in Appendices A–I and are reviewed in the sub-sections below. Worldwide population genetics studies have been completed for white shark, basking shark, whale shark and spinetail devilray, although no studies on whale shark have included New Zealand material. The remaining species have been studied in only part of their range (spotted black grouper; no New Zealand material included) or not at all (oceanic whitetip shark, deepwater nurse shark, giant manta ray, giant grouper). However, species-level phylogenetic studies and methodological studies (e.g. development of microsatellites, genome sequencing) have been completed for most species.

A list of the genetics studies known to have included tissue samples from New Zealand's protected fish species is given in Table 2. In some studies, actual tissues were processed and sequenced, whereas in others the gene sequences deposited in the genetics database GenBank were downloaded and used.

A list of ongoing or planned population genetics studies on New Zealand's protected fish species is given in Table 3.

Table 2: 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)

Table 3: 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	?	

3.2.1. White shark (Carcharodon carcharias)

The white shark has received a lot of attention from geneticists. Fifteen microsatellite loci have been identified (Pardini et al. 2000, O'Leary et al. 2013) and the entire mitochondrial genome has been sequenced and found to contain 16,744 base pairs (Chang et al. 2013). Zenger et al. (2006) described an amplified fragment length polymorphism (AFLP) method that can be applied universally to sharks to identify highly informative genome-wide polymorphisms. White sharks examined in that study displayed relatively high levels of allelic diversity. A nucleotide diagnostic (ND) method has been developed for uniquely identifying shark species, including white shark (Wong et al. 2009).

Genetic tools have been used to identify white sharks from detached fins or carcasses (Chapman et al. 2003, Shivji et al. 2005, Liu et al. 2013, Fields et al. 2015), and partial mitochondrial DNA CO1 sequences have been used to identify degraded white shark DNA in shark fin soup (Fields et al. 2015). Partial mitochondrial D-loop sequences have been successfully amplified from dried, historical samples of teeth and cartilage (Gubili et al. 2015). Genetic diversity at two mitochondrial DNA regions has been used to investigate the effect of the past glaciation cycles on population abundance of a range of shark species with different ecological characteristics, including white shark (O'Brien et al. 2013).

There have been multiple studies of white shark population genetic structure at a regional scale (Pardini et al. 2001, Jorgensen et al. 2010, Gubili et al. 2011, Tanaka et al. 2011, Naylor et al. 2012, Gubili et al. 2015, O'Leary et al. 2015, Andreotti et al. 2016). Those studies found major differentiation between two genetic clades that occur in (a) the northwestern Atlantic Ocean and South Africa, and (b) the Pacific Ocean and eastern Indian Ocean (western Australia). The depth of separation between those clades is such that it has been suggested that they may represent separate species (Naylor et al. 2012), although that suggestion has not been echoed by other authors. Further population differentiation has been found within these two clades, with separate populations occurring in the southwestern Pacific (New Zealand and Australia), the northwestern Pacific (Japan), the northeastern Pacific (California and Mexico), South Africa, the northwestern Atlantic Ocean (eastern USA), and the Mediterranean Sea. Intriguingly, multiple studies have shown that the Mediterranean population falls within the Indo-Pacific clade and not the geographically closer Atlantic/South Africa clade.

Fine-scale population structuring has also been demonstrated. White sharks in central California are genetically distinct from those in southern California and Mexico (Oñate-González et al. 2015). Distinct populations of white sharks have been identified in south-western Australia and eastern Australia/New Zealand, albeit with a low level of migration between them (Blower et al. 2012). Further research is required to clarify the relationship between white sharks in eastern Australia and New Zealand (Blower et al. 2012). Furthermore, tagging of white sharks in New Zealand waters has shown no direct migration between major centres of abundance at Stewart Island and Chatham Islands (although sharks from these areas do co-occur in tropical waters) (Duffy et al. 2012), suggesting that New Zealand may have at least two different populations. No population differentiation has been found within South Africa (Andreotti et al. 2016).

The fine-scale population structuring of white sharks in at least two regions seems to conflict with the observed large-scale migrations of white sharks (Bonfil et al. 2005, Bruce et al. 2006, Domeier & Nasby-Lucas 2008, Bonfil et al. 2010, Jorgensen et al. 2010, Domeier & Nasby-Lucas 2012, Duffy et al. 2012). Mixing between populations has been genetically detected with the discovery of sharks having South African haplotypes in eastern Australia (Pardini et al. 2001, Blower et al. 2012). This apparent paradox is usually explained by the presence of philopatry, in which pregnant female white sharks return to their natal area to give birth (Pardini et al. 2001, Jorgensen et al. 2010).

At an individual shark level, microsatellite identification has been used to validate photographic identification of South African sharks (Gubili et al. 2009).

3.2.2. Basking shark (Cetorhinus maximus)

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).

3.2.3. Whale shark (*Rhincodon typus*)

The population genetic structure of *Rhincodon typus* has been investigated using DNA sequences from the mtDNA D-loop (Castro et al. 2007). Large sample sizes have been difficult to obtain for this species and the sample numbers from some collection sites were low. The mtDNA data from an analysis of 70 samples from eight areas (including South Africa, Taiwan, Western Australia and Quintana Roo, Mexico) showed high levels of haplotype diversity. The authors compared haplotype frequencies, which led them to conclude that there was no genetic differentiation between the Indian and Pacific basins, but there were differences between Atlantic and Indo-Pacific locations. The low samples sizes at each site and the high haplotype diversity means there is limited confidence in these findings. The complete mtDNA sequence for *R. typus* was reported by Alam et al. (2014).

Eight polymorphic microsatellite DNA loci were developed and used to determine the levels of population genetic structure (Schmidt et al. 2009). The statistical power of the findings in this study was also limited by the small sample size of 68 individuals from a range of locations. Samples analysed by Schmidt et al. (2009) were taken from similar locations to those reported by Castro et al. (2007). It does not appear that samples were shared between the two studies, which could have doubled their sample sizes. Most of the eight microsatellite DNA loci reported by Schmidt et al. (2009) were compound dinucleotide repeats. The number of alleles ranged from 3-8 at seven of the loci, and one locus had 34 alleles and its expected heterozygosity was 1.00. The expected heterozygosity at the other seven loci ranged from 0.402-0.874. Based on the relatively small samples size and eight loci, few genetic differences were seen between geographically distinct sites. Schmidt et al. (2009) suggest that their finding of high gene flow was consistent with long-range migrations that had been detected using satellite tracking data.

The most comprehensive study was conducted by Vignaud et al. (2014). They used a combination of mtDNA D-loop sequencing and genotypes from 14 microsatellite DNA loci. The microsatellites were a mixture of loci previously reported and eight new loci. A large sample (n = 635) was used to determine the levels of genetic diversity and gene flow among sites that were similar to the areas previously sampled by Castro et al. (2007) and Schmidt et al. (2009). Haplotype diversity of the mtDNA sequences was high (> 0.9 at most sites) and heterozygosity (H_E) of microsatellite loci ranged between 0.5 and 0.67. The locations in the Indian and Pacific oceans appeared to comprise a single, large, panmictic population. Pairwise comparisons of the Indo-Pacific locations with the sample location in the Gulf of Mexico were all significantly different. The overall conclusion was that there appeared to be very little or no gene flow between the Indo-Pacific and the Atlantic Oceans. However, the conclusion by Vignaud et al. was based on a single Atlantic site, so sampling of further sites is required to confirm this result.

3.2.4. Oceanic whitetip shark (*Carcharhinus longimanus*)

Twelve polymorphic microsatellite DNA loci have been developed for *C. longimanus*, which were used in a pilot study and assessed in 28 individuals (Mendes et al. 2015). The *C. longimanus* samples were collected from an area in the northeastern tropical Atlantic. Nine of the loci were dinucleotide repeats and three were trinucleotide repeats. The number of alleles at each locus ranged from 4-8 and H_E from 0.472-0.818. Five of the 12 loci successfully cross-amplified on *C. acronotus*, *C. perezi* and *Galeocerdo cuvier*. The study of Mendes et al. (2015) established a set of genetic markers, however a more comprehensive population genetic study has not been published. A project is currently underway at Nova Southeastern University in Florida.

The complete mtDNA sequence for *C. longimanus* was reported by Li et al. (2014). It had the standard set of protein-coding genes, but differed from the typical fish mtDNA structure by having two D-loop sequences. That unusual sequence arrangement could make a phylogeographic study based on control region sequencing somewhat complicated. A range of DNA barcoding studies have been conducted (using a portion of the CO1 mitochondrial gene), which aimed to determine the species identity and origin of shark fins (Fields et al. 2015, Chuang et al. 2016). A more substantial phylogeographic study based on DNA sequences from the mtDNA D-loop was reported in a conference abstract by Camargo et al. (2012). That study used 126 samples from the Atlantic Ocean, and mentioned additional samples that were sourced from the Indian and Pacific oceans. A full report of the phylogeographic study has not been published.

3.2.5. Deepwater nurse shark (*Odontaspis ferox*)

There are no published population genetic studies of *Odontaspis ferox*. DNA sequences from the mtDNA CO1 gene have been reported in barcoding studies (Wong et al. 2009) and used to identify *O. ferox* from carcasses in the absence of key morphological characters (Santander-Neto et al. 2011). The only other mtDNA sequences reported for this species have been for a phylogenetic study of Carcharhiniformes sharks (Iglésias et al. 2005).

3.2.6. Spinetail devilray (*Mobula japanica*)

A worldwide study of the molecular phylogeny of eight *Mobula* species (and two *Manta* species) using both mitochondrial and nuclear genes included samples from the Pacific, Atlantic and Indian oceans (Poortvliet et al. 2015). *Mobula japanica* samples came from Mexico, Taiwan, Sri Lanka and western Africa. Three clades were identified, one of them consisting of the two *Manta* species, *Mobula tarapacana*, *M. japanica* and *M. mobular*. The latter two taxa could not be distinguished

genetically, leading to the suggestion that they are conspecific, which was a finding consistent with morphological data (Aschliman 2014, Poortvliet et al. 2015). If the two taxa are one species, the distribution of that species would extend worldwide including the Mediterranean Sea, from which only *M. mobular* had previously been reported (Poortvliet et al. 2015).

Twelve polymorphic microsatellite loci have been characterised for *M. japanica*, and they are regarded as good candidate markers for population genetic studies (Poortvliet et al. 2011). The complete mitochondrial genome of *M. japanica* has also been sequenced and consists of 18,880 base pairs (Poortvliet & Hoarau 2013, Kollias et al. 2015).

A comparison of the mitochondrial gene NAD2 from two Pacific Ocean sites (Mexico and Vietnam) found no genetic differences (Naylor et al. 2012). More recently, a worldwide population study of *M. japanica* has been carried out using microsatellites, two mitochondrial genes (CO1, NAD5) and whole mitochondrial genome sequencing. Significant population structuring was found between the Atlantic and Pacific/Indian oceans. No significant regional differences were found within those two populations. However, mitochondrial genome analysis identified four geographic clades whose members came mainly from the Atlantic Ocean, the north-east Pacific Ocean, the south-east Pacific Ocean, and the remainder of the Pacific Ocean and Indian Ocean combined, respectively (Poortvliet et al. in review). Nevertheless there was significant geographic 'leakage' among these clades suggesting some migration among regions.

Mobula japanica tissues collected from six individuals caught in the New Zealand tuna purse seine fishery have recently been included in an enlarged global analysis for comparison, but only the mitochondrial genes (CO1, NAD5) have been tested so far (M. Poortvliet, unpubl. data). Although comparisons are difficult because of the small size of the New Zealand sample, the New Zealand rays did not differ genetically from samples from the Atlantic, Indian or Pacific oceans (M. Poortvliet, pers. comm.). The comparison between New Zealand and Atlantic samples was marginally significant, but was not significant after correction for multiple tests. A more detailed population genetic study is currently underway on these tissues by Dr Jane Hosegood (Bangor University, Wales) using Next Generation Sequencing of nuclear genomes.

3.2.7. Giant manta ray (*Manta birostris*)

Microsatellites have been developed for *Manta alfredi*, and they amplified in *M. birostris* suggesting they could be applied to the latter, but no mention was made of genetic variability (Kashiwagi et al. 2012a). DNA has been successfully retrieved from *M. birostris* mucus, making it possible to sample individuals easily, non-invasively, and underwater (Kashiwagi et al. 2014).

A worldwide study of the molecular phylogeny of the two *Manta* species (and eight *Mobula* species) using both mitochondrial and nuclear genes included samples from the Pacific, Atlantic and Indian oceans (Poortvliet et al. 2015). *Manta birostris* samples came from Mexico and Indonesia. Three clades were identified, one of them consisting of the two *Manta* species and three *Mobula* species (*Mobula tarapacana*, *M. japanica* and *M. mobular*). This indicated that the genus *Manta* may be a synonym of *Mobula* (Poortvliet et al. 2015), a suggestion also proposed earlier by Naylor et al. (2012) and Aschliman (2014) based on genetic studies. In spite of a number of morphological similarities among the two genera (Aschliman 2014, Poortvliet et al. 2015), there are still sufficient morphological differences (especially the location of the mouth (i.e. terminal in *Manta* and ventral in *Mobula*)) for continued recognition of the two genera.

The common 'bar-coding' gene locus, CO1, failed to distinguish between the two *Manta* species, but another mitochondrial gene (ND5) and a nuclear gene (RAG1) did distinguish them (Kashiwagi et al. 2012b).

There have been no population genetics studies on either *Manta* species.

3.2.8. Spotted black grouper (*Epinephelus daemelii*)

Appleyard & Ward (2007) investigated the population genetic structure of *E. daemelii* using three mtDNA sequence regions and three microsatellite DNA loci, analysed in 91 samples sourced from biopsies and dried scales. Samples came from the east coast of Australia, and Lord Howe Island and Elizabeth and Middleton reefs in the Tasman Sea. Only the sites at Elizabeth (n = 31) and Middleton (n = 47) reefs had sufficient samples for a population-level study. Most other sites only had a single sample. DNA sequence data from the cytochrome-b, ND2 and control region (D-loop) were combined for the analysis, but no significant population genetic differentiation was found between Elizabeth and Middleton reefs. The three microsatellite loci had moderate to high levels of diversity but no significant differentiation was found between sites. The small sample sizes, short distance between the two sites that did have reasonable samples, and too few microsatellite loci, all limited the conclusions that could be drawn.

Soon after the first report was published, another study investigated the population genetic structure of *E. daemelii* (Van Herwerden et al. 2009). Seventeen microsatellite loci sourced from genetic markers developed for closely related species were tested on *E. daemelii;* nine were successfully amplified, but only six were polymorphic. This study appears to have used the same sample set as that used by Appleyard & Ward (2007). The microsatellite loci data from the two studies were not combined for an overall analysis. Based on a test for genetic differentiation using six loci, Van Herwerden et al. (2009) could not find any evidence of significant differences between Elizabeth and Middleton reefs.

3.2.9. Giant grouper (*Epinephelus lanceolatus*)

A total of 78 microsatellite DNA loci have been developed for *Epinephelus lanceolatus* (Zeng et al. 2008, Rodrigues et al. 2011, Yang et al. 2011, Kim et al. 2016 online), which is by far the most comprehensive marker set used for the species we have reviewed here. A significant amount of research investment has been made into the genetics of *E. lanceolatus* because of its value to aquaculture in Taiwan and mainland China. Most of the microsatellite loci had relatively low levels of allelic diversity and heterozygosity (H_E), based on fish sampled from a test population of about 20 individuals. However, although the microsatellite DNA loci might have been tested on samples from wild populations, they have only been used on farmed populations. There is no known population genetic study of wild populations, even though there is an extensive resource of microsatellite DNA loci available.

Eleven complete *E. lanceolatus* mitochondrial genome sequences have been reported in Genbank and used to design broodstock markers (Cheng et al. 2015), but they have not been used to study the structure of wild populations. Chiu et al. (2012) used a combination of random amplification polymorphic DNA (RAPD), 17 microsatellite loci and mtDNA CO1 sequencing on 17 samples collected from aquaculture farms and local fish markets. The results were of limited value for estimates of levels of diversity and population differentiation.

4. Recommendations

4.1. General recommendations

The overall goal of population genetics studies is to estimate the levels of genetic variation and gene flow, and test whether there are significant genetic differences among areas. Traditional genetic markers (e.g. mitochondrial DNA and microsatellite DNA loci) sample a small number of points on the genome and use the information to determine the average levels of genetic variation in a population. The exchange of a few migrants each generation is enough to genetically homogenise populations; however, a larger number of migrants are required to be exchanged before two populations are considered demographically coupled. Three important limitations of data sets are the number of genetic loci used, the number of samples available from a collection site, and the spread of samples across the species' distribution. Most of the species covered in this review have widespread, often global, distributions but the samples sizes of many studies were limited. A key priority is to continue to gather samples (at least 20 individuals per site, with a goal of 50-100 per site) and make them available to other researchers to complement samples collected from other locations. To increase the levels of genetic resolution, future studies should aim to build comprehensive reference genomes and single-nucleotide polymorphism (SNP) databases, by using genotyping-by-sequencing (GBS) or brute force population-scale genome sequencing (Andrews et al. 2016). These approaches better resolve weak patterns of genetic variation and detect local-adaptive differences among populations. The ability to detect population hierarchies will enable reproductive units to be more clearly defined and improve the setting of conservation priorities.

Mitochondrial DNA and microsatellite DNA lack the level of resolution needed to detect adaptive variation, which is linked to reproductive success and natural selection in a particular environment, limiting their value to only estimating levels of neutral gene flow. Genome-wide markers will enable both the neutral and selectively important components of population genetic variation to be quantified. Reproductive units that form because of population isolation and genetic drift, and/or local adaptation, can be identified and used as the focus of conservation priorities. This will enable a more comprehensive picture to be formed about the genetic, evolutionary and ecological processes influencing populations. We should encourage studies that utilise genome-wide markers (e.g. genome sequencing, GBS or Restriction site Associated DNA (RAD) sequencing) because this will produce the highest level of genetic resolution and DNA sequence information that can be built on by other researchers. The end result of moving to a whole-genome sequencing approach will be the complete genetic resolution of a population. All DNA sequencing information should be made available in an open database (e.g. GenBank) whenever a study is published.

4.2. Species-specific recommendations

4.2.1. White shark

Higher resolution genome-wide markers could reveal genetic differentiation between the New Zealand and eastern Australian white shark populations. If present, such differentiation must be maintained by reproductive isolation (possibly through female philopatry) despite the observed long-distance migrations of sharks from both regions and spatial overlap in tropical regions. Genetically distinct populations have been reported from eastern and south-western Australia (Blower et al. 2012), indicating the spatial scale over which such isolation can occur. Within New Zealand, white sharks tagged at Stewart Island and the Chatham Islands show no movement between those two sites, although they do overlap in tropical waters north of New Zealand, raising the possibility that

New Zealand supports two (or more) separate populations. Further collection of samples, particularly from the Chatham Islands and around mainland New Zealand, is a priority. These samples should be integrated with Australia-sourced samples in order to make more detailed comparisons.

4.2.2. Basking shark

Continue to collect samples for global-level studies.

4.2.3. Deepwater nurse shark

No population genetics studies have been conducted on this species. In New Zealand deepwater nurse sharks are restricted to the northern North Island (Francis & Lyon 2012), but its relationships with populations outside New Zealand are completely unknown. Furthermore, there have been no tagging studies to estimate the degree of movement in this species. In the absence of overseas studies, New Zealand cannot rely on other researchers to determine the regional population structure. Collection of tissue samples from New Zealand, and the development of collaborations with fisheries scientists and geneticists in other nearby countries, are priorities for establishing the basis for a future assessment of the genetic structure of this species.

4.2.4. Spotted black grouper

The most relevant population is that found around the Kermadec Islands, because the species is rare and the Kermadec population is potentially an isolated unit within a New Zealand territory. Genetic markers should be used to examine the relationship between the Kermadec population and those found in eastern Australia and the western Tasman Sea, with the aim of testing whether the Kermadecs are part of a larger Australasian group, or an isolated population. New samples should be collected from the Kermadecs and around North Island, and existing and new western Tasman Sea samples could be sourced from Australian researchers. The goal of a genetic study would be to estimate levels of variation and differentiation. Genome-wide markers should be used and the study conducted in a way that enables genetic information to be shared with other researchers and to form a basis for future work to build on.

4.2.5. Spinetail devilray, giant manta ray, oceanic whitetip shark, whale shark, giant grouper

These five species have their centres of population abundance in tropical regions north of New Zealand. They migrate seasonally (in summer) into northern New Zealand waters and are rarely caught by fishers. Consequently, access to tissue samples for genetics studies is difficult. However, individuals sampled in New Zealand could be valuable, as they represent the geographic limits of each species' range. New Zealand should actively seek to collect tissue samples from these species, and work with other range states, particularly Australia and the Pacific islands, to conduct regional population genetics studies. Many Pacific nations lack the infrastructure and expertise for conducting genetic studies. However, New Zealand has good research capabilities in genetics and fisheries and an appropriately funded work programme on the population genetics of these species could enable a range of Pacific nations to determine the population structure of the species, and thereby enhance management decisions or protection as required.

5. Acknowledgements

We are grateful to the many scientists who helped us track down tissues from New Zealand protected fish species, and references, including: James Andrew, Mike Bennett, Russ Bradford, Barry

Bruce, Andrew Christie, Clinton Duffy, Dave Ebert, Rus Hoelzel, Jane Hosegood, Charlie Huveneers, Cath Jones, Tom Kashiwagi, Peter Kyne, Andrea Marshall, Andrew Martin, Gavin Naylor, Les Noble, Jenny Ovenden, Vasiti Palavi, Simon Pierce, Marloes Poortvliet, Mahmood Shivji, Guy Stevens, Andrew Stewart, Tom Trnski, Lynne van Herwerden, Alex Watts. Warrick Lyon curated the tissue samples held by NIWA, and helped develop the tissue sample database. Sadie Mills arranged for the secure storage of the tissues in the NIWA Invertebrate Collection. We also thank Judy Sutherland for reviewing the draft report.

6. Glossary of abbreviations and terms

Allele. The DNA sequence of a gene. New alleles arise in a population by mutation. Vertebrates are typically diploid, which means there are two alleles (i.e. copies) of a gene in an individual. One copy was inherited from the mother and the other from the father. The two alleles of a gene are referred to as a genotype. If the two alleles in an individual are the same it is called a homozygote, and if they are different it is called a heterozygote. There are two ways of estimating the level of genetic diversity in a population; either (i) count the number of alleles found at a locus (allelic diversity), or (ii) count the number of heterozygotes in the population (heterozygosity).

Expected heterozygosity (H_E). A heterozygote is when there are different alleles at one or more genetic loci. The observed heterozygosity (H_O) is the number of heterozygous individuals per locus. The expected heterozygosity (also called gene diversity) is the heterozygosity estimated from calculations based on individual allele frequencies, and is a better estimate when there are different sample sizes among population samples.

Genotype. The pair of alleles that are at a locus. Sometimes it is used to describe the entire set of genes in an organism.

Haplotype diversity. A haplotype is the copy of a DNA sequence that is inherited from one parent. It is most often used to describe a mitochondrial DNA sequence because in vertebrates that genome is only inherited maternally. The diversity of haplotypes is estimated as the frequency of a DNA sequence type in a population weighted to the overall sample size.

Locus/loci. A particular position on the genome. It is often used to when referring to a gene.

Microsatellite DNA. A tract of DNA bases that are repeated to form certain motifs (ranging in length from 2-5 base pairs). Microsatellites are DNA loci that are common in most genomes and used in population genetic studies because of the high mutation rate and hence high levels of diversity. The high levels of diversity mean that the same microsatellite locus can be difficult to discover in other species. Microsatellite DNA are found in the nuclear genome, bi-parentally inherited, and most often in non-coding DNA regions. They are often neutral with respect to gene functions, but they can be located near genes that experience natural selection and appear to be non-neutral. They are also widely used for DNA profiling and kinship studies. Other names for these makers: Simple Sequence Repeats (SSR) and Short Tandem Repeats (STR).

Mitochondrial DNA (mtDNA). An extra-nuclear genome that is present in most vertebrate cells. Approximately 16,000 base pairs long, circular and maternally inherited. It is comprised of 13 protein coding genes (e.g. cytochrome oxidase 1 (CO1), cytochrome-b, and NAD5), two ribosomal genes, 22 tRNAs, and the control region (or D-loop). The latter is responsible for initiating transcription and replication of the genome. The CO1 mtDNA gene has been widely used for “DNA barcoding”.

7. References

Alam, M.T.; Petit, R.A.I.; Read, T.D.; Dove, A.D.M. (2014). The complete mitochondrial genome sequence of the world's largest fish, the whale shark (*Rhincodon typus*), and its comparison with those of related shark species. *Gene 539*: 44–49.

Andreotti, S.; Von der Heyden, S.; Henriques, R.; Rutzen, M.; Meyer, M.; Oosthuizen, H.; Matthee, C.A. (2016). New insights into the evolutionary history of white sharks, *Carcharodon carcharias*. *Journal of biogeography 43*: 328–339.

Andrews, K.R.; Good, J.M.; Miller, M.I.R.; Luikart, G.; Hohenlohe, P.A. (2016). Harnessing the power of RADseq for ecological and evolutionary genomics. *Nature reviews genetics 17*: 81–92.

Appleyard, S.; Ward, R. (2007). Genetic connectedness between black cod (*E. daemelii*) collections along the NSW coast and the Elizabeth & Middleton Reefs Reserve. *Final report to the Department of Environment and Water. CSIRO, Canberra.*

Aschliman, N.C. (2014). Interrelationships of the durophagous stingrays (Batoidea: Myliobatidae). *Environmental biology of fishes 97*: 967-979.

Blower, D.C.; Pandolfi, J.M.; Bruce, B.D.; Gomez-Cabrera, M.d.C.; Ovenden, J.R. (2012). Population genetics of Australian white sharks reveals fine-scale spatial structure, transoceanic dispersal events and low effective population sizes. *Marine ecology progress series 455*: 229–244.

Bonfil, R.; Francis, M.P.; Duffy, C.; Manning, M.J.; O'Brien, S. (2010). Large-scale tropical movements and diving behavior of white sharks *Carcharodon carcharias* tagged off New Zealand. *Aquatic biology 8*: 115-123.

Bonfil, R.; Meÿer, M.; Scholl, M.C.; Johnson, R.; O'Brien, S.; Oosthuizen, H.; Swanson, S.; Kotze, D.; Paterson, M. (2005). Transoceanic migration, spatial dynamics, and population linkages of white sharks. *Science 310*: 100-103.

Bruce, B.D.; Stevens, J.D.; Malcolm, H. (2006). Movements and swimming behaviour of white sharks (*Carcharodon carcharias*) in Australian waters. *Marine biology 150*: 161-172.

Camargo, S.M.; Coelho, R.; Santos, M.N.; Oliveira, C.; Foresti, F.; Mendonca, F.F. (2012). Phylogeography of the oceanic whitetip shark *Carcharhinus longimanus* in the Atlantic using mitochondrial DNA markers. *In*: Foz do Iguaçu, pp. 210. Brasil.

Castro, A.L.F.; Stewart, B.S.; Wilson, S.G.; Hueter, R.E.; Meekan, M.G.; Motta, P.J.; Bowen, B.W.; Karl, S.A. (2007). Population genetic structure of Earth's largest fish, the whale shark (*Rhincodon typus*). *Molecular ecology 16*: 5183–5192.

Chang, C.-H.; Shao, K.-T.; Lin, Y.-S.; Fang, Y.-C.; Ho, H.-C. (2013). The complete mitochondrial genome of the great white shark, *Carcharodon carcharias* (Chondrichthyes, Lamnidae). *Mitochondrial DNA Online DOI: 10.3109/19401736.2013.803092*.

Chapman, D.D.; Abercrombie, D.L.; Douady, C.J.; Pikitch, E.K.; Stanhope, M.J.; Shivji, M.S. (2003). A streamlined, bi-organelle, multiplex PCR approach to species identification: Application to global conservation and trade monitoring of the greatwhite shark, *Carcharodon carcharias*. *Conservation genetics 4*: 415-425.

Cheng, S.S.; Senoo, S.; Siddiquee, S.; Rodrigues, K.F. (2015). Genetic variation in the mitochondrial genome of the giant grouper *Epinephelus lanceolatus* (Bloch, 1790) and its application for the identification of broodstock. *Aquaculture reports 2*: 139–143.

Chiu, T.-H.; Su, Y.-C.; Pai, J.-Y.; Hung-Chia, C. (2012). Molecular markers for detection and diagnosis of the giant grouper (*Epinephelus lanceolatus*). *Food control 24*: 29-37.

Chuang, P.S.; Hung, T.C.; Chang, H.A.; Huang, C.K.; Shiao, J.C. (2016). The species and origin of shark fins in Taiwan's fishing ports, markets, and customs detention: a DNA barcoding analysis. *PLoS ONE 11:e0147290*.

Domeier, M.L.; Nasby-Lucas, N. (2008). Migration patterns of white sharks *Carcharodon carcharias* tagged at Guadalupe Island, Mexico, and identification of an eastern Pacific shared offshore foraging area. *Marine ecology progress series 370*: 221-237.

Domeier, M.L.; Nasby-Lucas, N. (2012). Sex-specific migration patterns and sexual segregation of adult white sharks, *Carcharodon carcharias*, in the northeastern Pacific. *In*: Domeier, M.L. (ed.). Global perspectives on the biology and life history of the white shark, pp. 133-146. CRC Press, Boca Raton, USA.

Duffy, C.A.J.; Francis, M.P.; Manning, M.; Bonfil, R. (2012). Regional population connectivity, oceanic habitat, and return migration revealed by satellite tagging of white sharks, *Carcharodon carcharias*, at New Zealand aggregation sites. *In*: Domeier, M.L. (ed.). Global perspectives on the biology and life history of the white shark, pp. 301-318. CRC Press, Boca Raton, USA.

Fields, A.T.; Abercrombie, D.L.; Eng, R.; Feldheim, K.; Chapman, D.D. (2015). A novel mini-DNA barcoding assay to identify processed fins from internationally protected shark species. *PLoS ONE 10(2): e0114844*.

Francis, M.P.; Harasti, D.; Malcolm, H.A. (2016). Surviving under pressure and protection: a review of the biology, ecology and population status of the highly vulnerable grouper, *Epinephelus daemelii*. *Marine and freshwater research*.

Francis, M.P.; Lyon, W.S. (2012). Review of commercial fishery interactions and population information for eight New Zealand protected fish species. *NIWA client report No. WLG2012-64*. 67 p.

Francis, M.P.; Lyon, W.S. (2014). Review of commercial fishery interactions and population information for the oceanic whitetip shark, a protected New Zealand species. *NIWA client report No. WLG2014-40*. 15 p.

Gubili, C.; Bilgin, R.; Kalkan, E.; Karhan, S.U.; Jones, C.S.; Sims, D.W.; Kabasakal, H.; Martin, A.P.; Noble, L.R. (2011). Antipodean white sharks on a Mediterranean walkabout? Historical dispersal leads to genetic discontinuity and an endangered anomalous population. *Proceedings of the Royal Society. B Biological sciences 278*: 1679−1686.

Gubili, C.; Johnson, R.; Gennari, E.; Oosthuizen, W.H.; Kotze, D.; Meÿer, M.; Sims, D.W.; Jones, C.S.; Noble, L.R. (2009). Concordance of genetic and fin photo identication in the great white shark, *Carcharodon carcharias*, off Mossel Bay, South Africa. *Marine biology 156*: 2199–2207.

Gubili, C.; Robinson, C.E.C.; Cliff, G.; Wintner, S.P.; de Sabata, E.; De Innocentiis, S.; Canese, S.; Sims, D.W.; Martin, A.P.; Noble, L.R.; Jones, C.S. (2015). DNA from historical and trophy samples provides insights into white shark population origins and genetic diversity. *Endangered species research 27*: 233–241.

Heemstra, P.C.; Randall, J.E. (1993). Groupers of the world (Family Serranidae, Subfamily Epinephelinae). An annotated and illustrated catalogue of the grouper, rockcod, hind, coral grouper and lyretail species known to date. *FAO fisheries synopsis No. 125 vol. 16*. 382 p.

Hester, J.; Atwater, K.; Bernard, A.; Francis, M.; Shivji, M.S. (2015). The complete mitochondrial genome of the basking shark *Cetorhinus maximus* (Chondrichthyes, Cetorhinidae). *Mitochondrial DNA 26*: 730-731.

Hoelzel, A.R. (2001). Shark fishing in fin soup. *Conservation genetics 2*: 69-72.

Hoelzel, A.R.; Shivji, M.S.; Magnussen, J.; Francis, M.P. (2006). Low worldwide genetic diversity in the basking shark (*Cetorhinus maximus*). *Biology letters 2*: 639-642.

Iglésias, S.P.; Lecointre, G.; Sellos, D.Y. (2005). Extensive paraphylies within sharks of the order Carcharhiniformes inferred from nuclear and mitochondrial genes. *Molecular phylogenetics and evolution 34*: 569–583.

Jorgensen, S.J.; Reeb, C.A.; Chapple, T.K.; Anderson, S.; Perle, C.; Van Sommeran, S.R.; Fritz-Cope, C.; Brown, A.C.; Klimley, A.P.; Block, B.A. (2010). Philopatry and migration of Pacific white sharks. *Proceedings of the Royal Society B 277*: 679-688.

Kashiwagi, T.; Broderick, D.; Lance, S.L.; Bennett, M.B.; Ovenden, J.R. (2012a). Development and characterization of ten microsatellite loci for the reef manta ray *Manta alfredi*. *Conservation genetics resources 4*: 1055–1058.

Kashiwagi, T.; Marshall, A.D.; Bennett, M.B.; Ovenden, J.R. (2012b). The genetic signature of recent speciation in manta rays (*Manta alfredi* and *M. birostris*). *Molecular phylogenetics and evolution 64*: 212-218.

Kashiwagi, T.; Maxwell, E.A.; Marshall, A.D.; Christensen, A.B. (2014). Evaluating manta ray mucus as an alternative DNA source for population genetics study: underwater-sampling, dry-storage and PCR success. *PeerJ 3:e1188; DOI 10.7717/peerj.1188*.

Kim, K.-S.; Noh, C.H.; Moon, S.-J.; Han, S.-H.; Bang, I.-C. (2016 online). Development of novel tetra- and trinucleotide microsatellite markers for giant grouper *Epinephelus lanceolatus* using 454 pyrosequencing. *Molecular biology reports*.

Kollias, S.; Poortvliet, M.; Smolina, I.; Hoarau, G. (2015). Low cost sequencing of mitogenomes from museum samples using baits capture and Ion Torrent. *Conservation genetics resources 7*: 345-348.

Li, W.; Dai, X.; Xu, Q.; Wu, F.; Gao, C.; Zhang, Y. (2014). The complete mitochondrial genome sequence of oceanic whitetip shark , *Carcharhinus longimanus* (Carcharhiniformes: Carcharhinidae). *Mitochondrial DNA 27*: 1775–1776.

Lieber, L.; Berrow, S.; Johnston, E.; Hall, G.; Hall, J.; Gubili, C.; Sims, D.W.; Jones, C.S.; Noble, L.R. (2013). Mucus: aiding elasmobranch conservation through non-invasive genetic sampling. *Endangered species research 21*: 215–222.

Lieber, L.; Hall, G.; Hall, J.; Berrow, S.; Johnston, E.; Francis, M.; Duffy, C.; Wintner, S.P.; Gubili C; Sarginson, J.; Witt, M.J.; Hawkes, L.A.; Doherty, P.D.; Godley, B.J.; Henderson, S.; de Sabata, E.; Shivji, M.S.; Dawson, D.A.; Burke, T.; Sims, D.W.; Jones, C.S.; Noble, L.R. (in review). Basking without borders: Global gene flow in the basking shark despite first evidence of inter-annual site-fidelity in the Northeast Atlantic. *Molecular ecology*.

Liu, S.-Y.V.; Chan, C.-L.C.; Lin, O.; Hu, C.-S.; Chen, C.A. (2013). DNA barcoding of shark meats identify species composition and CITES-listed species from the markets in Taiwan. *PLoS ONE 8(11): e79373*.

Magnussen, J.E.; Pikitch, E.K.; Clarke, S.C.; Nicholson, C.; Hoelzel, A.R.; Shivji, M.S. (2007). Genetic tracking of basking shark products in international trade. *Animal conservation 10*: 199-207.

Mendes, N.J.; Cruz, V.P.; Mendonça, F.F.; Pardo, B.G.; Coelho, R.; Ashikaga, F.Y.; Camargo, S.M.; Martínez, P.; Oliveira, C.; Santos, M.N.; Foresti, F. (2015). Microsatellite loci in the oceanic whitetip shark and cross-species amplification using pyrosequencing technology. *Conservation genetics resources 7*: 585-589.

Naylor, G.J.P.; Caira, J.N.; Jensen, K.; Rosana, K.A.M.; White, W.T.; Last, P.R. (2012). A DNA sequence–based approach to the identification of shark and ray species and its implications for global elasmobranch diversity and parasitology. *Bulletin of the American Museum of Natural History No. 367*. 263 p.

Noble, L.R.; Jones, C.S.; Sarginson, J.; Sims, D.W.; Metcalfe, J.D. (2006). Conservation genetics of basking shark. *Final project report to the Global Wildlife Division. Department for Environment, Food and Rural Affairs, London*. 42 p.

O'Brien, S.M.; Gallucci, V.F.; Hauser, L. (2013). Effects of species biology on the historical demography of sharks and their implications for likely consequences of contemporary climate change. *Conservation genetics 14*: 125–144.

O'Leary, S.J.; Feldheim, K.A.; Chapman, D.D. (2013). Novel microsatellite loci for white, *Carcharodon carcharias* and sandtiger sharks, *Carcharias taurus* (order Lamniformes). *Conservation genetics resources 5*: 627-629.

O'Leary, S.J.; Feldheim, K.A.; Fields, A.T.; Natanson, L.J.; Wintner, S.; Hussey, N.; Shivji, M.S.; Chapman, D.D. (2015). Genetic diversity of white sharks, *Carcharodon carcharias*, in the Northwest Atlantic and Southern Africa. *Journal of heredity 106*: 258-265

Oñate-González, E.C.; Rocha-Olivares, A.; Saavedra-Sotelo, N.C.; Sosa-Nishizaki, O. (2015). Mitochondrial genetic structure and matrilineal origin of white sharks, *Carcharodon carcharias*, in the northeastern Pacific: implications for their conservation. *Journal of heredity 106*: 347-354.

Pardini, A.T.; Jones, C.S.; Noble, L.R.; Kreiser, B.; Malcolm, H.; Bruce, B.D.; Stevens, J.D.; Cliff, G.; Scholl, M.C.; Francis, M.; Duffy, C.A.J.; Martin, A.P. (2001). Sex-biased dispersal of great white sharks. *Nature 412*: 139-140.

Pardini, A.T.; Jones, C.S.; Scholl, M.C.; Noble, L.R. (2000). Isolation and characterization of dinucleotide microsatellite loci in the great white shark, *Carcharodon carcharias*. *Molecular ecology 9*: 1171–1193.

Poortvliet, M.; Galván-Magaña, F.; Bernardi, G.; Croll, D.A.; Olsen, J. (2011). Isolation and characterization of twelve microsatellite loci for the Japanese devilray (*Mobula japanica*). *Conservation genetics resources 3*: 733–735.

Poortvliet, M.; Hoarau, G. (2013). The complete mitochondrial genome of the spinetail devilray, *Mobula japanica*. *Mitochondrial DNA 24*: 28-30.

Poortvliet, M.; Hoarau, G.; Croll, D.A.; Bernardi, G.; Newton, K.M.; O'Sullivan, J.; Fernando, D.; Stevens, G.; Galván Magaña, F.; Seret, B.; Olsen, J.L. (in review). Influences of past climatic change on historical population structure and demography of the spinetail devilray, *Mobula japanica*, based on mitogenomes and nuclear DNA. *Molecular ecology*.

Poortvliet, M.; Olsen, J.L.; Croll, D.A.; Bernardi, G.; Newton, K.; Kollias, S.; O'Sullivan, J.; Fernando, D.; Stevens, G.; Galván Magaña, F.; Seret, B.; Wintner, S.; Hoarau, G. (2015). A dated molecular phylogeny of manta and devil rays (Mobulidae) based on mitogenome and nuclear sequences. *Molecular phylogenetics and evolution 83*: 72-85.

Rodrigues, K.F.; Shigeharu, S.; Ch'ng, C.L. (2011). Microsatellite markers for the identification of commercially important groupers *Epinephelus lanceolatus*, *Cromileptes altivelis* and *Epinephelus fuscoguttatus*. *Pertanika journal of tropical agricultural science 34*: 311–315.

Santander-Neto, J.; Faria, V.V.; Castro, A.L.F.; Burgess, G.H. (2011). New record of the rare ragged-tooth shark, *Odontaspis ferox* (Chondrichthyes: Odontaspidae) from the south-west Atlantic identified using DNA bar coding. *Marine biodiversity records 4: e75*.

Schmidt, J.V.; Schmidt, C.L.; Ozer, F.; Ernst, R.E.; Feldheim, K.A. (2009). Low genetic differentiation across three major ocean populations of the whale shark, *Rhincodon typus*. *PLoS ONE 4(4): e4988*.

Shivji, M.S.; Chapman, D.D.; Pikitch, E.K.; Raymond, P.W. (2005). Genetic profiling reveals illegal international trade in fins of the great white shark, *Carcharodon carcharias*. *Conservation genetics 6*: 1035–1039.

Tanaka, S.; Kitamura, T.; Mochizuki, T.; Kofuji, K. (2011). Age, growth and genetic status of the white shark (*Carcharodon carcharias*) from Kashima-nada, Japan. *Marine and freshwater research 62*: 548–556.

Van Herwerden, L.; Klanten, O.S.; Choat, J.H.; Jerry, D.R.; Robbins, W.D. (2009). Connectivity of black cod *Epinephelus daemelii* between Elizabeth and Middleton Reefs (as measured by population genetic structure based on microsatellites). *Final report to the Department of the Environment, Water, Heritage and the Arts*.

Vignaud, T.M.; Maynard, J.A.; Leblois, R.; Meekan, M.G.; Vázquez-Juárez, R.; Ramírez-Macías, D.; Pierce, S.J.; Rowat, D.; Berumen, M.L.; Beeravolu, C.; Baksay, S.; Planes, S. (2014). Genetic structure of populations of whale sharks among ocean basins and evidence for their historic rise and recent decline. *Molecular ecology 23*: 2590–2601.

Wong, E.H.-K.; Shivji, M.S.; Hanner, R.H. (2009). Identifying sharks with DNA barcodes: assessing the utility of a nucleotide diagnostic approach. *Molecular ecology resources 9 (suppl. 1)*: 243–256.

Yang, S.; Wang, L.; Zhang, Y.; Liu, X.C.; Lin, H.R.; Meng, Z.N. (2011). Development and characterization of 32 microsatellite loci in the giant grouper *Epinephelus lanceolatus* (Serranidae). *Genetics and molecular research 10*: 4006–4011.

Zeng, H.-S.; Ding, S.-X.; Wang, J.; Su, Y.-Q. (2008). Characterization of eight polymorphic microsatellite loci for the giant grouper (*Epinephelus lanceolatus* Bloch). *Molecular ecology resources 8*: 805–807.

Zenger, K.R.; Stow, A.J.; Peddemors, V.; Briscoe, D.A.; Harcourt, R.G. (2006). Widespread utility of highly informative AFLP molecular markers across divergent shark species. *Journal of heredity 97*: 607–611.

Appendix A Summary of known genetic studies of white shark

EPO, Eastern Pacific Ocean; EAO, Eastern Atlantic ocean; WPO, Western Pacific Ocean; WIO, Western Indian Ocean (mainly South Africa); MS, Mediterranean Sea; NAO, North Atlantic Ocean; SAO, South Atlantic Ocean

Study	Genetic marker	Sample source	Region	No. of samples	Additional GenBank samples	Populations identified	GenBank sequence	Comments
Pardini et al. (2000)	microsatellites	South Africa	WIO	20			AF184087, AF184089, AF216864–AF216866	Five polymorphic microsatellites identified
Pardini et al. (2001)	mtDNA (control region), microsatellites	South Africa, Australia, New Zealand	WIO, WPO	95		2	AY026196–AY026224, AF184085	Identified different populations in South Africa and Australia/NZ using mtDNA but not microsats. Suggests female philopatry.
Chapman et al. (2003)	nuclear gene (ITS2)	Japan, Taiwan, California, South Africa, Australia, New Zealand, Argentina, eastern USA	WIO, WPO, EPO, NAO, SAO	53				Species identification from partial carcasses or fins
Shivji et al. (2005)	nuclear gene (ITS2) and mtDNA (cytochrome b)	Eastern USA	NAO	21				Identified white shark fins in traded products
Zenger et al. (2006)	multiple loci	Eastern Australia	WPO	7				Described an amplified fragment length polymorphism method that can be applied universally to sharks to identify highly informative genome-wide polymorphisms. White sharks displayed relatively high levels of allelic diversity
Gubili et al. (2009)	microsatellites	South Africa	WIO	110			AF184085, AF184087, AF216864–AF216866, AF426735	Validation of photo identification
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)		6			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
Jorgensen et al. (2010)	mtDNA (control region)	California, South Africa, Australia, New Zealand	EPO, WPO, WIO	59	29	3	GU002302-GU002321	California population distinct from those in Australia/NZ and South Africa
Tanaka et al. (2011)	mtDNA (control region)	Japan, California, South Africa, Australia, New Zealand	EPO, WPO, WIO	7	49	4	AB598391–AB598397	Japanese population distinct from those in USA, Australia/NZ and South Africa
Gubili et al. (2011)	mtDNA (control region)	Mediterranean, Florida, Japan, California, South Africa, Australia, New Zealand	EPO, WPO, WIO, MS, NAO	5	49	5	HQ540294–HQ540298	Mediterranean population differs from North-west Atlantic sharks, and is most similar to Indo-Pacific sharks
Gubili et al. (2012)	mtDNA (COI)	Mediterranean, South Africa, Australia, New Zealand, California	EPO, WPO, WIO, MS	11	Not stated		DQ108328, DQ884985, EU398646, FJ518939-44, GU440260, HQ167639	No geographically plausible stocks evident

White shark (continued)

Study	Genetic marker	Sample source	Region	No. of samples	Additional GenBank samples	Populations identified	GenBank sequence	Comments
Naylor et al. (2012)	mtDNA (NADH2)	South Africa, eastern USA, California, South Australia	WPO, EPO, NAO, WIO	17		2	JQ518732	Two clades recognised, South Africa/western North Atlantic and California/South Australia
Blower et al. (2012)	mtDNA (control region), microsatellites	Australia, New Zealand	WPO	97	54	2	HQ414073–HQ414086	Distinguished eastern Aust/NZ population from SW Aust population with both markers. Five WIO individuals also identified suggesting that transoceanic dispersal, or migration resulting in breeding, may occur sporadically. Given the potential for philopatric reproductive behaviour and restricted gene flow, further research is needed to test for white shark genetic population structure between Australia and New Zealand
Liu et al. (2013)	mtDNA (COI)	Taiwan	WPO	1	3			One individual white shark identified in Taiwan landings
Chang et al. (2013)	mtDNA genome	Taiwan	WPO	1			KC914387	Mitochondrial genome sequenced
O'Leary et al. (2013)	microsatellites	Eastern USA	NAO	31			KC154203-KC154212	10 new microsatellites identified
O'Brien et al. (2013)	mtDNA (control region, ND2)	California	EPO	0	59			White shark used in a multi-species comparison of ecological characteristics and climate change effects
O'Leary et al. (2015)	mtDNA (control region), microsatellites	Eastern USA, South Africa, Mediterranean, California, Australia, New Zealand	NAO, WIO, WPO, MS, EPO	166	Not stated	2		Distinct populations in Northwest Atlantic and southern Africa
Fields et al. (2015)	mtDNA (COI)	Not stated		10				Partial COI sequences used to identify shark species. Reported a GenBank error: white shark JQ654702.1 has a 99% sequence identity to blue shark and only 83% to closest white shark
Gubili et al. (2015)	mtDNA (D-loop)	South Africa, Mediterranean Sea	WIO, MS	9	95	2		Extracted DNA from dried teeth and cartilage. Mediterranean sharks fell within Pacific clade, not North Atlantic/South Africa clade
Oñate-González et al. (2015)	mtDNA (control region)	California, western Mexico, eastern USA, South Africa, Mediterranean, Australia, New Zealand	NAO, WIO, WPO, MS, EPO	127	59 from California plus others from the rest of the world	2	KM014766-KM014781	Separate populations in central California and southern California/Mexico
Andreotti et al. (2016)	mtDNA (control region), microsatellites	Eastern USA, South Africa, Mediterranean, California, Australia, New Zealand	NAO, WIO, WPO, MS, EPO	302	58		KP058665–KP058902	No population sub-structure within South Africa. Identified potential haplotype errors due to manual sequencing by Pardini et al. (2001). Three mtDNA clades confined to (1) the Mediterranean and Indo-Pacific Ocean, (2) the North West Atlantic and South Africa, and (3) a single divergent haplotype restricted to South Africa.

Appendix B Summary of known genetic studies of basking shark

EPO, Eastern Pacific Ocean; EAO, Eastern Atlantic ocean; WPO, Western Pacific Ocean; WIO, Western Indian Ocean (mainly South Africa); MS, Mediterranean Sea; NAO, North Atlantic Ocean; SAO, South Atlantic Ocean

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

Appendix C Summary of known genetic studies of whale shark

EPO, Eastern Pacific Ocean; EAO, Eastern Atlantic ocean; WPO, Western Pacific Ocean; WIO, Western Indian Ocean (mainly South Africa); MS, Mediterranean Sea; NAO, North Atlantic Ocean; SAO, South Atlantic Ocean

Study	Genetic marker	Sample source	Region	No. of samples	Populations identified	GenBank sequence	Comments
Castro et al. (2007)	mtDNA (D-loop)	Australia, Mexico, Philippines, Taiwan, South Africa, Mozambique, Kenya, Maldives, eastern USA	WPO, EPO, WIO, NAO	70	2	EU182401-EU182444	Differentiation between samples from Atlantic and Indo-Pacific populations
Schmidt et al. (2009)	microsatellites	India, Honduras, Ecuador, Florida, South Africa, Seychelles, Costa Rica, Western Australia, Djibouti, Mexico, Maldives	EPO, WIO, NAO, EPO	68			Eight polymorphic microsatellite loci. Most sample locations had small sample sizes. Low levels of genetic differentiation between geographically distinct locations
Vignaud et al. (2014)	mtDNA (D-loop) and microsatellites	Red Sea, Djibouti, Seychelles, Maldives, Mozambique, Australia, Taiwan, Philippines, western Mexico, eastern Mexico	EPO, WIO, NAO, EPO, WPO	msat 406, mtDNA 574			14 microsatellite loci; developed eight and three sourced from each of Schmidt et al. (2009) and Ramırez-Macıas et al. (2009). High level of genetic differentiation between eastern Mexico and Indo-Pacific
Alam et al. (2014)	mtDNA genome	Taiwan	WPO	1		KF679782	DNA extracted from liver tissue of a specimen from Taiwan

Appendix D Summary of known genetic studies of oceanic whitetip shark

EPO, Eastern Pacific Ocean; EAO, Eastern Atlantic ocean; WPO, Western Pacific Ocean; WIO, Western Indian Ocean (mainly South Africa); MS, Mediterranean Sea; NAO, North Atlantic Ocean; SAO, South Atlantic Ocean

Study	Genetic marker	Sample source	Region	No. of samples	Populations identified	GenBank sequence	Comments
Camargo et al. (2012)	mtDNA (D-loop)	Ivory Coast, Cameroon, Senegal, Brazil	EAO	126		KT160318-KT160329	Conference abstract
Li et al. (2014)	mtDNA genome	Not stated		1		KM434158	DNA extracted from muscle
Mendes et al. (2015)	microsatellites	Northeast tropical Atlantic	EAO	28			12 microsats identified. Five loci cross-amplified on *C. acronotus* , *C. perezi* and *Galeocerdo cuvier*
Fields et al. (2015)	mtDNA (COI)	Asian markets	WPO	10			DNA barcoding
Chuang et al. (2016)	mtDNA (COI)	Taiwan	WPO	4			DNA barcoding of market and port samples

Appendix E Summary of known genetic studies of deepwater nurse shark

EPO, Eastern Pacific Ocean; EAO, Eastern Atlantic ocean; WPO, Western Pacific Ocean; WIO, Western Indian Ocean (mainly South Africa); MS, Mediterranean Sea; NAO, North Atlantic Ocean; SAO, South Atlantic Ocean

Study	Genetic marker	Sample source	Region	No. of samples	Populations identified	GenBank sequence	Comments
Iglésias et al. (2005)	12S-16S rRNA mtDNA and nuclear gene (RAG1)	New Caledonia	WPO	1		AY462144, AY462145d	Phylogenetic study
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)		1		Sequences are at Barcode of Life Data System (BOLD at http://www.boldsystems.org) project code EWSHK	DNA barcoding study
Santander-Neto et al. (2011)	mtDNA (COI)	North-eastern Brazil	SAO	1			Identified carcasses

Appendix F Summary of known genetic studies of spinetail devilray

EPO, Eastern Pacific Ocean; EAO, Eastern Atlantic ocean; WPO, Western Pacific Ocean; WIO, Western Indian Ocean (mainly South Africa); MS, Mediterranean Sea; NAO, North Atlantic Ocean; SAO, South Atlantic Ocean

Study	Genetic marker	Sample source	Region	No. of samples	Additional GenBank samples	Populations identified	GenBank sequence	Comments
Poortvliet et al. (2011)	microsatellites	Mexico	EPO	1			JF800912-JF800923	12 microsats identified
Naylor et al. (2012)	mtDNA (NADH2)	Mexico, Vietnam	EPO, WPO	12			JQ519163	*M. japanica* and *M. birostris* cluster close to each other and in among other *Mobula*
Kashiwagi et al. (2012a)	microsatellites	Not stated		2				*Manta alfredi* microsatellites amplified in *M. japanica* but no variability mentioned
Kashiwagi et al. (2012b)	mtDNA (CO1, ND5), nuclear genes (RAG1)	Indonesia	WPO	2			FJ235624–FJ235631	*Mobula* species used as outgroups for *Manta* analysis
Poortvliet & Hoarau (2013)	mtDNA genome		EPO	1			JX392983	mtDNA genome sequenced
Aschliman (2014)	mtDNA (ND2, ND4), nuclear genes (RAG1, SCFD2)	Not stated						*M. japanica* and *M. birostris* cluster close to each other and in among other *Mobula*
Poortvliet et al. (2015)	mtDNA genome, mtDNA (COX1, NADH5), nuclear genes (HEMO, RAG1)	Mexico, Taiwan, Sri Lanka, Togo	EPO, WPO, WIO, EAO	4			KM364891, KM364916, KM364939-KM364942, KM364964-KM364967, KM364984, KM364988, KM435072	Three clades recognised in *Manta/Mobula*. *Mobula mobular* and *Mobula japanica* indistinguishable genetically and may be con-specific.
Poortvliet et al. (in review)	mtDNA genome, mtDNA (CO1, NADH5), microsatellites	Mexico, Costa Rica, Galapagos, Ecuador, Indonesia, Sri Lanka, Togo, Mediterranean	EPO, WPO, WIO, EAO, MS	283		2	Available on publication	Atlantic Ocean significantly different from Indian/Pacific oceans
Poortvliet (unpubl. data)	mtDNA (CO1, NADH5)	New Zealand compared with multiple locations elsewhere (see Poortvliet et al. (in review))	WPO and others	6				NZ samples not statistically different from all other regions after correction for multiple tests (but sample size very low). Most divergent from EAO, with negligible difference from Indian and Pacific ocean regions, indicating high gene flow

Appendix G Summary of known genetic studies of giant manta ray

EPO, Eastern Pacific Ocean; EAO, Eastern Atlantic ocean; WPO, Western Pacific Ocean; WIO, Western Indian Ocean (mainly South Africa); MS, Mediterranean Sea; NAO, North Atlantic Ocean; SAO, South Atlantic Ocean

Study	Genetic marker	Sample source	Region	No. of samples	Additional GenBank samples	Populations identified	GenBank sequence	Comments
Naylor et al. (2012)	mtDNA (NADH2)	Indonesia, Philippines	WPO	2			JQ519062	*M. japanica* and *M. birostris* cluster close to each other and in among other *Mobula*
Kashiwagi et al. (2012a)	microsatellites	Japan, Mozambique	WIO, WPO	2				*Manta alfredi* microsatellites amplified in *M. birostris* but no variability mentioned
Kashiwagi et al. (2012b)	mtDNA (CO1, ND5), nuclear genes (RAG1)	Mexico, Japan, Indonesia, Australia, South Africa	EPO, WPO, WIO	37			FJ235624–FJ235631	CO1 did not distinguish between two *Manta* species but other genes did
Kashiwagi et al. (2014)	mtDNA (ND5), nuclear gene (RAG1), microsatellites	Ecuador	EPO	18	Not stated		KR703234-KR703237	DNA extracted from mucus
Aschliman (2014)	mtDNA (ND2, ND4), nuclear genes (RAG1, SCFD2)	Not stated		Not stated	Not stated			*M. japanica* and *M. birostris* cluster close to each other and in among other *Mobula*
Poortvliet et al. (2015)	mtDNA genome, mtDNA (CO1, NADH5), nuclear genes (HEMO, RAG1)	Mexico, Indonesia	EPO, WPO	2			KM364883, KM364908, KM364933, KM364934, KM364958, KM364959, KM364991	Three clades recognised in *Manta/Mobula*
Kollias et al. (2015)	mtDNA genome	Not stated (probably Mexico)	Probably EPO	1				Recovered DNA from museum specimens (but not *M. birostris*)

Appendix H Summary of known genetic studies of spotted black grouper

EPO, Eastern Pacific Ocean; EAO, Eastern Atlantic ocean; WPO, Western Pacific Ocean; WIO, Western Indian Ocean (mainly South Africa); MS, Mediterranean Sea; NAO, North Atlantic Ocean; SAO, South Atlantic Ocean

Study	Genetic marker	Sample source	Region	No. of samples	Populations identified	GenBank sequence	Comments
Appleyard and Ward (2007)	mtDNA (16S rRNA, CO1, cytochrome-b, NAD2, control region) and 3 microsatellite loci	Elizabeth and Middleton Reefs, Australia	WPO	91			The two sampled location were approx. 35 km apart. No significant differentiation was detected
van Herwerden et al. (2009)	6 microsatellites	Elizabeth and Middleton Reefs, NSW and southern Queensland, Australia	WPO	82			No significant differentiation among sites

Appendix I Summary of known genetic studies of giant grouper

EPO, Eastern Pacific Ocean; EAO, Eastern Atlantic ocean; WPO, Western Pacific Ocean; WIO, Western Indian Ocean (mainly South Africa); MS, Mediterranean Sea; NAO, North Atlantic Ocean; SAO, South Atlantic Ocean

Study	Genetic marker	Sample source	Region	No. of samples	Populations identified	GenBank sequence	Comments
Zeng et al. (2008)	8 microsatellites	Malaysia, Taiwan	WPO	45			Primer development note
Rodrigues et al. (2011)	24 microsatellites	Malaysia	WPO	Not stated			Primer development note, for aquaculture
Yang et al. (2011)	32 microsatellites	China	WPO	31		JN185622-JN185653	Primer development note, for aquaculture
Chiu et al. (2012)	Random amplified polymorphic DNA (RAPD), 17 microsatellites, and mtDNA (CO1)	Taiwan	WPO	14			Distinguished between wild and cultured fish
Cheng et al. (2015)	mtDNA (CO1)	Malaysia	WPO	31			Identified maternal parent from hybrids with *E. fuscoguttatus*
Kim et al. (2016)	24 microsatellites	Malaysia	WPO	38		KT757324–KT757347	Primer development note, for aquaculture