SCIENTIFIC COMMITTEE
FOURTEENTH REGULAR SESSION

Busan, Republic of Korea
8-16 August 2018

Pacific stock structure of the Silky shark (Carcharhinus falciformis) resolved with next generation sequencing

WCPFC-SC14-2018/ EB-IP-04

Derek W. Kraft[1], Melanie Hutchinson[2,1], and Brian W. Bowen[1]

[1] Hawai'i Institute of Marine Biology, University of Hawai'i, Kaneohe, Hawai'i
[2] Joint Institute of Marine and Atmospheric Research, University of Hawaii, Honolulu, Hawaii. USA

Pacific stock structure of the Silky shark (*Carcharhinus falciformis*) resolved with next generation sequencing

Derek W. Kraft[1], Melanie Hutchinson[2,1], Brian W. Bowen[1]

[1]Hawai'i Institute of Marine Biology, University of Hawai'i, Kaneohe, Hawai'i
[2]Joint Institute of Marine and Atmospheric Research, University of Hawaii, Honolulu, Hawaii. USA

ABSTRACT
The silky shark (*Carcharhinus falciformis*) is subject to the second highest shark catch rates on the planet (Oliver et al. 2015) and stock assessments have found that some populations are overfished and that overfishing is still occurring (Rice and Harley 2013). Currently this species is managed as a single stock across the WCPFC jurisdiction due to a lack of information regarding stock structure. In this study, 298 silky sharks were sampled from across 5 regions of the Pacific Ocean, including off the coast of Taiwan, Papua New Guinea, the South Central Pacific, the North Central Pacific, and the Eastern Pacific. DNA sequence data included the entire mitochondrial genome as well as thousands of nuclear loci using pooled next generation sequencing. Nuclear markers yielded significant population structure between all five regions (significant F_{ST} = 0.0188 - 0.0421), and mitochondrial markers supported most of these differences (significant F_{ST} = 0.0117 - 0.0572). These analyses demonstrate multiple stocks within the WCPFC jurisdiction and provide a scientific foundation for formulating more appropriate management units for silky sharks across the Pacific.

Introduction

Silky sharks (*Carcharhinus falciformis*) are a coastal and pelagic species inhabiting tropical and sub-tropical waters across the globe. Their habitat overlaps with the intensively targeted tuna stocks. Unfortunately juvenile silky sharks are often found in association with Fish Aggregating Devices (FADs) used in tropical tuna purse seine fisheries and account for >90% of the shark bycatch (Lawson 2011). Demographic analyses for sharks have shown that this juvenile mortality is the most detrimental to population trajectories (Beerkircher 2003). Another prominent threat to silky shark populations is the high demand for shark fins. According to data from Hong Kong's Census and Statistics Department, 83 countries or territories supplied more than 10.3 million kilograms of shark fin products to Hong Kong in 2011. This intense fishing pressure is unsustainable for most shark species, and silky sharks are one of the most abundant species in the fin market (Bonfil 1994; Clarke et al. 2006). The silky shark was once abundant in all tropical oceans, yet due to incidental capture in pelagic fisheries worldwide as well as targeted fisheries for the fin trade, this pelagic shark has experienced a population decline of 85% over the last 20 years (Rice and Harley 2013).

Low fecundity, late maturity, and long life spans make sharks susceptible to overharvest, and populations can take decades to recover (Dulvy et al. 2008). A stock assessment of silky sharks by the Western Central Pacific Fisheries Commission found that spawning biomass, total biomass, and recruitment have all declined- indicating that fishing mortality has surpassed the maximum sustainable yield (Rice and Harley 2013). Globally, populations are overfished, and overfishing is still occurring, yet it is still the second most harvested shark species on the planet (Rice and Harley 2013; Oliver et al. 2015).

Currently silky sharks are managed as a single stock within each regional fisheries management organization (RFMO), due primarily to a lack of information. Testing for population genetic structure can resolve dispersal boundaries and provide an appropriate foundation for defining socks (Carvalho and Hauser 1994). Genetic tools provide a powerful means to delineating management units for populations important for fisheries (Altukhov et al. 2000; Ablan 2006; Ovenden et al. 2015), a prerequisite for any successful wildlife management (Dizon et al. 1993; Waldman 2005).

For the past two decades studies identifying population structure of elasmobranchs have focused on a handful of mitochondrial and microsatellites loci (Domingues et al. 2017). For silky sharks in particular there have been two studies examining genetic population structure across the Pacific Ocean. Both of these studies examined the mitochondrial control region (mtCR) and detected weak but significant population structure across the Indo-Pacific (Galván-Tirado et al. 2013; Clarke et al. 2015). Mitochondrial DNA evolves rapidly and because of its smaller size has been an efficient target for genetic studies. With increased DNA sequencing technologies however, it is now possible for genetic studies to investigate both the smaller mitochondrial genome and the much larger nuclear genome.

Next-generation DNA sequencing is a very powerful tool for examining genetic diversity across tens of thousands of single nucleotide polymorphisms (SNPs) throughout the genome. In recent years, SNPs have become the premium method to establish population structure for commercially important marine species (Hess et al. 2011; Albaina et al. 2013; Diopere et al. 2017; Puncher et al. 2018) Since determining population-level allele frequency estimates are key to population genetics, pooling individual DNA into a single samples before sequencing (Pool-seq) is an affordable and accurate method for large scale genetic analysis (Futschik and Schlötterer 2010; Rellstab et al. 2013; Schlötterer et al. 2014; Mimee et al. 2015; Nielsen et al. 2018).

This study examines population structure of the silky shark from five regions across the Pacific Ocean. Using a next-generation sequencing, we assess both the mitochondrial and nuclear genomes of silky sharks to provide the most extensive silky shark population genetic study to date. Our analyses reveal significant population structure between all five regions examined. This information provides the scientific basis for implementing a multiple stock management plan for silky sharks across the Pacific Ocean.

Methods

Sample collection

Fin clips or muscle sections were collected from 298 silky sharks from across the Pacific by scientists and fishery observers aboard commercial purse seine and longline tuna fishing vessels. Samples were immediately stored in 80% ethanol or 20% saturated salt (NaCl) buffer. Samples were assigned to one of five different regions: Taiwan, Papua New Guinea, South Central Pacific, North Central Pacific, and East Pacific (Figure 1).

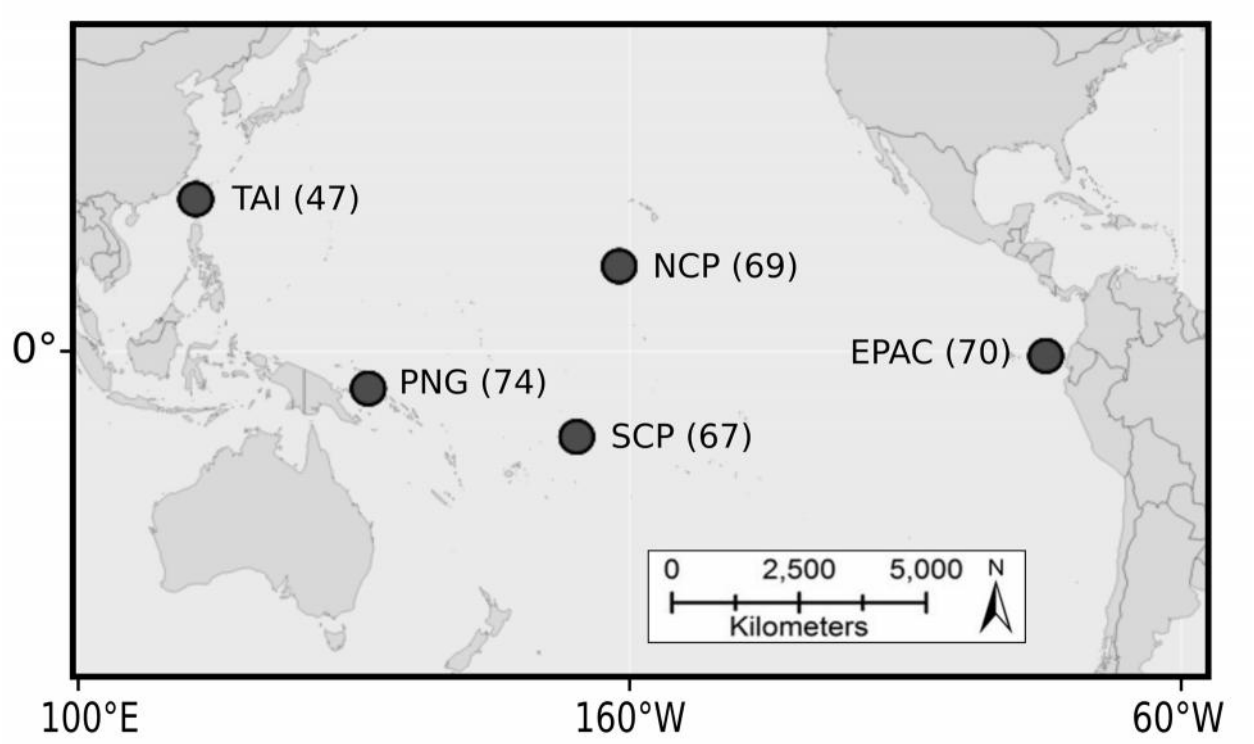

Figure 1. Collection sites for silky sharks (*C. falciformis*) and sample size. Abbreviations are as follows TAI = Taiwan, PNG = Papua New Guinea, SCP = South Central Pacific, NCP = North Central Pacific, and EPAC = Eastern Pacific.

DNA Sequencing

DNA was extracted using Qiagen DNeasy Blood & Tissue kit (Qiagen, Mississauga, ON, Canada), following manufactures instructions. Extracted DNA was then passed through electrophoresis gel and imaged using Gel Doc E-Z System (BIO RAD, Hercules, California, USA) to ensure intact, high quality DNA. The extracted DNA was prepared for quantification using an AccuClear Ultra High Sensitivity dsDNA Quantitation Kit (Biotium, Fremont, CA, USA) and quantified on a SpectroMax M2 (Molecular Devices, Sunnyvale, CA, USA). Equal amounts of DNA (ng/µl) per individual were added to regional pools to minimize individual contribution bias. The rest of the library preparation followed the ToBo laboratory ezRAD library preparation protocol (Toonen et al. 2013; Knapp et al. 2016), except no libraries were amplified by PCR in order to prevent PCR bias between samples, and maintain equal contributions of DNA from individuals across each library. Libraries were sequenced using Illumina MiSeq with paired end, 300 bp runs (performed by the Hawai‘i Institute of Marine Biology EPSCoR Core sequencing facility)

Genetic analyses

Sequence libraries were first examined with Multiqc V1.2 (Ewels et al. 2018) to assess sequence quality scores, sequence length distributions, duplication levels, overrepresented sequences, etc. To analyze the mitochondrial genome, a previously published silky shark mitochondrial genome was used as a reference (GeneBank accession number KF801102). Raw paired-end reads were trimmed, mapped to the mitochondrial genome reference, and SNPs were identified using an edited dDocent bioinformatics pipeline (Puritz et al. 2014).

The dDocent pipeline was also used to analyze the nuclear dataset. A de novo assembly was constructed and optimized utilizing the reference optimization steps following standard dDocent assembly protocols

(http://ddocent.com/assembly/). Once assembled sequences were mapped and SNPs were identified. To remove mitochondrial SNPs from the nuclear data, any contigs that aligned to the mitochondrial genome were removed from the nuclear dataset.

Due to differences between individual library analyses and pooled library analyses, the SNP calling portion of both pipelines, Freebayes v1.0.2 (Garrison and Marth 2012), was optimized with the addition of 'pooled continuous' option of the program and minor allele frequency was set to .05. SNPs were analyzed with the pool-seq specific bioinformatics pipeline assessPool (github.com/ToBoDev/assessPool). This pipeline uses VCFtools v0.1.14 to filter SNPs (Danecek et al. 2011) and Popoolation2 v1.2.2 to compare allele frequencies between populations by calculating pairwise F_{ST} values and significance in the form of a p-values (Kofler et al. 2011). assessPool then organizes, summarizes, and creates visualizations of the data using Rstudio (RStudio Team 2015).

Results

Mitochondrial Genome

Analysis of the mitochondrial genome identified 180 variable sites (SNPs) and after considering minimum coverage of 20x, read quality, and mapping quality; 34 SNPs were selected for further analyses. Almost all F_{ST} comparisons within the mitochondrial genome were statistically significant except between Taiwan and the North Central Pacific (Table 1). The highest F_{ST} values were between the Eastern Pacific to the South Central Pacific (F_{ST} = 0.0572 and p-value <0.0001), and the two next highest were the Eastern Pacific to Taiwan and Taiwan to Papua New Guinea respectively (Table 1). The lowest significant difference was found between South Central Pacific and the North Central Pacific (F_{ST} = 0.0117 and p-value 0.0002)

Table 1. Mitochondrial genome pairwise F_{ST} values (below diagonal) and p-values (above diagonal) between 5 regionally pooled samples. Statistically significant F_{ST} and respective p-values are in bold.

	TAI	PNG	SCP	NCP	EPAC
TAI	*	**<0.0001**	**<0.0001**	0.1523	**<0.0001**
PNG	**0.0502**	*	**<0.0001**	**0.0001**	**0.0120**
SCP	**0.0159**	**0.0451**	*	**0.0002**	**<0.0001**
NCP	0.0103	**0.0282**	**0.0117**	*	**0.0006**
EPAC	**0.0546**	**0.0361**	**0.0572**	**0.0401**	*

Nuclear Genome

A total of 30,570 candidate SNPs were identified across 26,522 nuclear contigs (average length 272 base pairs). 1,078 SNPs were selected after considering a minimum coverage of 20x, read quality, and mapping quality. All pairwise F_{ST} values were significant (Table 2). The highest F_{ST} was again between the Eastern Pacific and the South Central Pacific (F_{ST} =0.0421 and p-value >0.0001). The next two highest were the Eastern Pacific to the North Central Pacific and Papua New Guinea respectively (Table 2). The lowest nuclear F_{ST} observed was Taiwan to the North Central Pacific (F_{ST} = 0.0188 and p-value >0.0001)

Table 2. Nuclear pairwise F_{ST} values (below diagonal) and p-values (above diagonal) between 5 regional pooled samples. Statistically significant F_{ST} and respective p-values are in bold.

	TAI	PNG	SCP	NCP	EPAC
TAI	*	**>0.0001**	**>0.0001**	**>0.0001**	**>0.0001**
PNG	**0.0212**	*	**>0.0001**	**>0.0001**	**>0.0001**
SCP	**0.0277**	**0.0295**	*	**>0.0001**	**>0.0001**
NCP	**0.0188**	**0.0205**	**0.0245**	*	**>0.0001**
EPAC	**0.0297**	**0.0260**	**0.0421**	**0.0316**	*

Discussion

Given the lack of obvious dispersal barriers for marine species, especially for large mobile ones, it is expected and often observed that there tends to be low population genetic structure between locations within oceans (Ely et al. 2005; Daly-Engel et al. 2012). When considering truly pelagic sharks, such as the whale shark, basking shark, and blue shark, global F_{ST} values rarely rise above 0.01 (Hoelzel et al. 2006; Castro et al. 2007; Vignaud et al. 2014; Taguchi et al. 2015). Most analyses of these pelagic species are performed on small regions of the mitochondria or over a few microsatellites (Domingues et al. 2017). In comparison, the genetic signal between silky shark populations observed in this study were relatively high. Given our large sample size and the high number of SNP markers analyzed, 1,112 in total, this is a powerful assessment of silky shark genetic population structure.

All five sample regions across the Pacific showed significant genetic differentiation among them. The comparisons between the Eastern Pacific and all other regions demonstrated the highest F_{ST} values, indicating that this region is the most isolated from the rest of the Pacific. Therefore, individuals are not often crossing the oceanic barrier between eastern and central Pacific, a result which has been observed in several marine taxa (Lessios and Robertson 2006; Toonen et al. 2016). The lowest observed F_{ST} value in both the mitochondrial and nuclear genome were between Taiwan and the North Central Pacific. However, even the lowest F_{ST} values recorded in this study were higher than those observed for truly pelagic sharks mentioned above. Further, life history studies corroborate the finding of stock structure, showing demographic differences between populations in Taiwan, Papuan New Guinea, and the Central Pacific (Oshitani et al. 2003; Joung et al. 2008; Grant et al. 2018), and therefore lend further evidence in support of multiple management units across the Pacific.

When it comes to managing harvestable stocks, up to 10% exchange between populations could still justify two separate management units (Kasapidis et al. 2008). However, it only takes a handful of effective migrants between regions per generation to homogenize genetic population structure (Mills and Allendorf 1996; Hartl and Clark 1997; Vucetich and Waite 2000; Wang 2004). Given the long generation time of silky sharks (7-10 years), a handful of migrants per generation would certainly not sustain populations under current harvesting practices. Therefore, our significant F_{ST} values indicate that depleted stocks will need to recover via local recruitment rather than relying on adjacent populations to bolster numbers.

To properly manage this highly harvested marine resource the accurate identification of stock structure is essential. This study supplies the scientific basis to distinguish at least five genetically distinct stocks existing across the Western and Central Pacific Ocean, challenging the single population per RMFO

default management plan currently in place. These delineations need to be implemented into more effective conservation management strategies for this over-exploited and key species.

References

Ablan CA (2006) Genetics and the study of fisheries connectivity in Asian developing countries. Fish Res 78:158–168.

Albaina A, Iriondo M, Velado I, et al (2013) Single nucleotide polymorphism discovery in albacore and Atlantic bluefin tuna provides insights into worldwide population structure. Anim G. doi: 10.1111/age.12051

Altukhov YP, Salmenkova EA, Omelchenko, Vladimir T (2000) Salmonid Fishes: Population Biology, Genetics and Managment. Wiley-Blackwell, Oxford

Beerkircher LR, Shivji MS, Cortes E (2003) A Monte Carlo Demographic Analysis of the silky Shark (*Carcharhinus falciformis*): Implications of Gear Selectivity A Monte Cario demographic analysis of the silky shark CCorchorhinus fo / cifolJllis): implications of gear selectivity. 174:168–174.

Bonfil R (1994) Overview of world elasmobranch fisheries. FAO Fisheries Techincal Paper No. 341. Rome. 119 pp.

Carvalho GR, Hauser L (1994) Molecular genetics and the stock concept in fisheries. Rev Fish Biol Fish 4:326–350. doi: 10.1007/BF00042908

Castro ALF, Stewart BS, Wilson SG, et al (2007) Population genetic structure of Earth's largest fish, the whale shark (*Rhincodon typus*). Mol Ecol 16:5183–5192. doi: 10.1111/j.1365-294X.2007.03597.x

Clarke CR, Karl SA, Horn RL, et al (2015) Global mitochondrial DNA phylogeography and population structure of the silky shark, Carcharhinus falciformis. Mar Biol 162:945–955. doi: 10.1007/s00227-015-2636-6

Clarke SC, Magnussen JE, Abercrombie DL, et al (2006) Identification of Shark Species Composition and Proportion in the Hong Kong Shark Fin Market Based on Molecular Genetics and Trade Records Identificación de la Composición y Proporción de Especies de Tiburón en el Mercado de Aletas de Tiburón en Hong Kong. Conserv Biol 20:201–211. doi: 10.1111/j.1523-1739.2005.00247.x

Daly-Engel TS, Randall JE, Bowen BW (2012) Is the Great Barracuda (*Sphyraena barracuda*) a reef fish or a pelagic fish? The phylogeographic perspective. Mar Biol 159:975–985. doi: 10.1007/s00227-012-1878-9

Danecek P, Auton A, Abecasis G, et al (2011) The variant call format and VCFtools. 27:2156–2158. doi: 10.1093/bioinformatics/btr330

Diopere E, Vandamme SG, Hablu PI, et al (2017) Seascape genetics of a flatfish reveals local selection under high levels of gene flow. ICES J Mar Sci. doi: 10.1093/icesjms/fsx160

Dizon AE, Lockyer C, Perrin WF, et al (1993) Rethinking the stock concept: a phylogeographic approach. Biol Conserv 64:176–177. doi: 10.1016/0006-3207(93)90670-V

Domingues RR, Hilsdorf AWS, Gadig OBF (2017) The importance of considering genetic diversity in shark and ray conservation policies. Conserv Genet 19:501–525. doi: 10.1007/s10592-017-1038-3

Dulvy NK, Baum JK, Clarke S, et al (2008) You can swim but you can't hide: The global status and conservation of oceanic pelagic sharks and rays. Aquat Conserv Mar Freshw Ecosyst 18:459–482. doi: 10.1002/aqc.975

Ely B, Viñas J, Bremer JRA, et al (2005) Consequences of the historical demography on the global population structure of two highly migratory cosmopolitan marine fishes: the yellowfin tuna (*Thunnus albacares*) and the skipjack tuna (Katsuwonus pelamis). BMC Evol Biol 5:1–9. doi: 10.1186/1471-2148-5-19

Ewels P, Lundin S, Max K (2018) Data and text mining MultiQC : summarize analysis results for multiple tools and samples in a single report. 32:3047–3048. doi: 10.1093/bioinformatics/btw354

Futschik A, Schlötterer C (2010) The next generation of molecular markers from massively parallel sequencing of pooled DNA samples. Genetics 186:207–218. doi: 10.1534/genetics.110.114397

Galván-Tirado C, Díaz-Jaimes P, García-de León FJ, et al (2013) Historical demography and genetic

differentiation inferred from the mitochondrial DNA of the silky shark (*Carcharhinus falciformis*) in the Pacific Ocean. Fish Res 147:36–46. doi: 10.1016/j.fishres.2013.03.020
Garrison E, Marth G (2012) Haplotype-based variant detection from short-read sequencing. 1–9.
Grant MI, Smart JJ, White WT, et al (2018) Life history characteristics of the silky shark Carcharhinus falciformis from the central west Pacific. Mar Freshw Res 69:562–573.
Hartl DL, Clark AG (1997) Principles of Population Genetics. Third edition. Sinauer Associates, Inc. Sunderland, Massachusetts
Hess EJ, Matala PA, Narum RS (2011) Comparison of SNPs and microsatellites for fine-scale application of genetic stock identification of Chinook salmon in the Columbia River Basin. Mol Ecol Resour 11:137–149. doi: 10.1111/j.1755-0998.2010.02958.x
Hoelzel AR, Shivji MS, Magnussen JE, Francis MP (2006) Low worldwide genetic diversity in the basking shark (*Cetorhinus maximus*). Biol Lett 2:639–642. doi: 10.1098/rsbl.2006.0513
Joung SJ, Chen CT, Lee HH, Liu KM (2008) Age, growth, and reproduction of silky sharks, Carcharhinus falciformis, in northeastern Taiwan waters. Fish Res 90:78–85. doi: 10.1016/j.fishres.2007.09.025
Kasapidis P, Magoulas A, García-Cortés B 2, Mejuto J (2008) Stock Structure of swardfish (Xiphias gladius) in the Pacific Ocean using microsatellite DNA markers. WCPFC-SC4-2008/BI-WP-4 1–12.
Knapp ISS, Puritz JB, Bird CE, et al (2016) ezRAD- an accessible next-generation RAD sequencing protocol suitable for non-model organisms_v3.2.
Kofler R, Pandey RV, Schlötterer C (2011) PoPoolation2: Identifying differentiation between populations using sequencing of pooled DNA samples (Pool-Seq). Bioinformatics 27:3435–3436. doi: 10.1093/bioinformatics/btr589
Lawson T (2011) Estimation of catch rates and catches of key shark species in tuna fisheries of the Western and Central Pacific Ocean using observer data.
Lessios HA, Robertson DR (2006) Crossing the impassable : genetic connections in 20 reef fishes across the eastern Pacific barrier. Proc R Soc B Biol Sci 273:2201–2208. doi: 10.1098/rspb.2006.3543
Mills LS, Allendorf FW (1996) The One-Migrant-per-Generation Rule in Conservation and Management. Conserv Biol 10:1509–1518.
Mimee B, Duceppe MO, Véronneau PY, et al (2015) A new method for studying population genetics of cyst nematodes based on Pool-Seq and genomewide allele frequency analysis. Mol Ecol Resour 15:1356–1365. doi: 10.1111/1755-0998.12412
Nielsen ES, Henriques R, Toonen RJ, et al (2018) Complex signatures of genomic variation of two non-model marine species in a homogeneous environment. BMC Genomics 19:347. doi: 10.1186/s12864-018-4721-y
Oliver S, Braccini M, Newman SJ, Harvey ES (2015) Global patterns in the bycatch of sharks and rays. Mar Policy 54:86–97. doi: 10.1016/j.marpol.2014.12.017
Oshitani S, Nakano H, Tanaka S (2003) Age and growth of the silky shark Carcharhinus falciformis from the Pacific Ocean. Fish Sci 69:456–464.
Ovenden JR, Berry O, Welch DJ, et al (2015) Ocean ' s eleven : a critical evaluation of the role of population , evolutionary and molecular genetics in the management of wild fi sheries. Fish Fish. doi: 10.1111/faf.12052
Puncher GN, Cariani A, Maes GE, et al (2018) Spatial dynamics and mixing of bluefin tuna in the Atlantic Ocean and Mediterranean Sea revealed using next-generation sequencing. 18:620–638. doi: 10.1111/1755-0998.12764
Puritz JB, Hollenbeck CM, Gold JR (2014) *dDocent* : a RADseq, variant-calling pipeline designed for population genomics of non-model organisms. PeerJ 2:e431. doi: 10.7717/peerj.431
Rellstab C, Zoller S, Tedder A, et al (2013) Validation of SNP allele frequencies determined by pooled next-generation sequencing in natural populations of a non-model plant species. PLoS One. doi: 10.1371/journal.pone.0080422
Rice J, Harley S (2013) Updated stock assessment of the Silky sharks in the Western and Central Pacfific Ocean. WCPFC-SC9-2013/ SA-WP-03

RStudio Team (2015) RStudio: Integrated Development Environment for R.
Schlötterer C, Tobler R, Kofler R, Nolte V (2014) Sequencing pools of individuals-mining genome-wide polymorphism data without big funding. Nat Rev Genet 15:749–763. doi: 10.1038/nrg3803
Taguchi M, King JR, Wetklo M, et al (2015) Population genetic structure and demographic history of Pacific blue sharks (Prionace glauca) inferred from mitochondrial DNA analysis. Mar Freshw Res 66:267–275. doi: 10.1071/MF14075
Toonen RJ, Bowen BW, Iacchei M, Briggs JC (2016) Biogeography, Marine. Encycl Evol Biol 1:166–178. doi: 10.1016/B978-0-12-800049-6.00120-7
Toonen RJ, Puritz JB, Forsman ZH, et al (2013) ezRAD: a simplified method for genomic genotyping in non-model organisms. PeerJ 1:e203. doi: 10.7717/peerj.203
Vignaud TM, Maynard JA, Leblois R, et al (2014) Genetic structure of populations of whale sharks among ocean basins and evidence for their historic rise and recent decline. Mol Ecol 23:2590–2601. doi: 10.1111/mec.12754
Vucetich JA, Waite TA (2000) Is one migrant per generation sufficient for the genetic management of fluctuating populations? Anim Conserv 3:261–266.
Waldman JR (2005) Definition of Stocks. In: Stock Identification Methods. Elsevier, pp 7–16
Wang J (2004) Application of the one-migrant-per-generation rule to conservation and management. Conserv Biol 18:332–343.