Zoological Studies **59**:49 (2020)
doi:10.6620/ZS.2020.59-49

Zoological Studies

Open Access

Accessing Multiple Paternity in the Shortfin Mako Shark (*Isurus oxyrinchus*)

Shang Yin Vanson Liu[1,]*, Wen-Pei Tsai[2], Mengshan Lee[3], and Hsiu-Wen Chien[4]

[1]*Department of Marin Biotechnology and Resources, National Sun Yat-sen University, No.70 Lien-hai Rd., Kaohsiung 80424, Taiwan. *Correspondence: E-mail: oceandiver6426@gmail.com. (Liu) Tel: +866-75252000#5024*
[2]*Department of Fisheries Production and Management, National Kaohsiung University of Science and Technology, Kaohsiung City 80778, aiwan. E-mail: wptsai@nkust.edu.tw (Tsai)*
[3]*Department of Safety, Health and Environmental Engineering, National Kaohsiung University of Science and Technology, Kaohsiung City 80778, Taiwan. E-mail: mlee@nkust.edu.tw (Lee)*
[4]*Department of Chemical and Material Engineering, National Kaohsiung University of Science and Technology, Kaohsiung City 80778, Taiwan. E-mail: hsiu-wen.chien@nkust.edu.tw (Chien)*

Received 10 March 2020 / Accepted 16 August 2020 / Published 29 October 2020
Communicated by Hin-Kiu Mok

Multiple paternity has been demonstrated in a variety of sharks with different reproductive modes (*i.e.*, viviparous, ovoviviparous, adelphophagy, oviparous), although the number of sires per litter varies considerably among species. To date, such analyses have focused mainly on coastal and nearshore shark species due to the difficulty in sampling oceanic sharks. In the present study, we observed multiple paternity in the oceanic shark *Isurus oxyrinchus* from seven polymorphic microsatellite loci and three litters collected from Nanfangao Fishing Port. Paternity tests showed that an average of 4.6 sires were assigned to each litter of *I. oxyrinchus* using COLONY software, and that the average number of sires dropped to 2.5 when using GERUD. These findings suggest that multiple paternity could be a common reproductive strategy used by the shortfin mako shark, and that this mating system should be integrated into a demographic model to make more accurate population projections and risk analyses in the future.

Key words: Microsatellite, Multiple-paternity, Oceanic shark, Mating system, Shortfin mako shark.

BACKGROUND

In the majority of vertebrate mating systems, females invest more energy and effort in ensuring their reproductive success and are thus expected to be the more 'choosy' sex in regards to mate selection. Males, on the contrary, are expected to exhibit less parental care, be more sexually competitive, and have less selective mating preference (Birkhead 1998; Pizzari and Birkhead 2002). However, increasing evidence suggests that females mating with more than one male (polyandry) and having a single brood sired by multiple males (multiple paternity) is a common strategy in diverse taxa, including invertebrates and vertebrates (Evans and Magurran 2000; Toonen 2004; Adams et al. 2005; Bretman and Tregenza 2005; Daly-Engel et al. 2006; Dean et al. 2006; Jensen et al. 2006). In elasmobranch fishes, multiple paternity has been assessed in 20 shark and two ray species from six orders using microsatellite loci (reviewed by Rossouw et al. 2016), and several other such studies (*Carcharhinus acronotus*: blacknose shark, *C. leucas*: blue shark, *Galeocerdo cuvier*: bull shark, and *C. limbatus*: black tip shark) have been published since then (Barker et al. 2019; Pirog et al. 2019; Bester-van der Merwe et al. 2019). Although most shark reproductive modes have been well studied (placental, or aplacental viviparity, and oviparous), the mating systems used in most sharks have still not been well studied (Neff and Pitcher 2002; Byrne and Avise 2012; Boomer et al. 2013).

Pelagic sharks are threatened by over-exploitation due to the high value of and demand for their fins,

Citation: Liu SYV, Tsai WP, Lee M, Chien HW. 2020. Accessing multiple paternity in the shortfin mako shark (*Isurus oxyrinchus*). Zool Stud **59:**49. doi:10.6620/ZS.2020.59-49.

which is causing a subsequent collapse of their fishery (Worm et al. 2013). In general, reproductive features such as slow growth rates, low fecundity, and late maturity make them vulnerable to overfishing (Stevens et al. 2000; Byrne and Avise 2012; Worm et al. 2013). The shortfin mako shark (*Isurus oxyrinchus*) is widely distributed in tropical and temperate waters, and is one of the most heavily fished sharks in the world due to the high value of its flesh and fins. As a result, it is currently listed as Endangered (EN) on the IUCN's Red List (Rigby et al. 2019) and has been placed in Appendix II of the CITES CoP 18 meeting (CITES 2019). Given the increasing trend in global shark catches and landings, global *I. oxyrinchus* populations should be constantly monitored to ensure their sustainability. Mating systems (*e.g.*, monogamy, polyandry, etc.) and demographic stochasticity (*e.g.*, natural mortality, fecundity, etc.) affect the proportion of breeding females, and have significant impacts on assessing population status in demographic models (Tsai et al. 2014 2015). However, the level of paternity may be underestimated if consider only that one study, which documents the presence of multiple paternity in *I. oxyrinchus* in merely one litter and five polymorphic microsatellite loci (Corrigan et al. 2015). Therefore, the aim of this study is to reevaluate multiple paternity and its prevalence in *I. oxyrinchus* using more litters and microsatellites. Our results can be incorporated into demographic models in the future.

MATERIALS AND METHODS

Sampling

Isurus oxyrinchus muscle samples were obtained from three pregnant females with *in utero* litters of 13, 6, and 13 pups for multiple paternity assessment. These sharks were collected from the Nanfangao Fishing Port as bycatch from a longline fishery operating in the area between Taiwan and mainland Japan (Fig. 1). The total weights of No. 1, No. 2, and No. 3 females were 403 kg, 336 kg, and 288 kg, respectively. One of the three pregnant female sharks was collected on January 16, 2016, and the other two on March 4, 2016. Total body lengths of the litters were 62.5–72.5 cm. DNA was isolated with a Genomic DNA extraction kit (Genomics BioSci. and Tech. Co., Taiwan) from muscle tissue according to the manufacturer's recommendations.

Microsatellite genotyping and analyses

Seven published microsatellite loci (CA1XD, CATY9, A96NC, BIC01, IOX-01, IOX12, and IOX-30; Table 1) were used for genotyping. One primer pair was labeled with fluorescent dyes FAM or TAMRA (Genomics, Taiwan). Microsatellites were amplified in 25 µL reactions in a Labnet gradient thermocycler over an initial denaturation step at 95°C for 3 min, 30 cycles of denaturation at 94°C for 30 s, annealing at 50–60°C for 30 s, extension at 72°C for 1 min, and a final extension step at 72°C for 5 min. Each reaction contained 30 ng DNA, 12.5 ul Taq DNA Polymerase 2X master Mix RED (15 mM $MgCl_2$, 0.4 mM each dNTPs, 200 nM of each primer, and 0.2 unit of Ampliqon DNA polymerase) (Ampliqon, Denmark). Loci that were successfully amplified and at the target size were then sent for further genotyping via ABI 3730XL sequencer. Allele sizes and genotypes were analyzed in GeneMapper® Software v. 4.1 (Applied Biosystems, USA). For each microsatellite locus, the number of alleles, allele frequencies, observed heterozygosities (Ho), and expected heterozygosities (He) were determined using GENALEX 6.5 (Peakall and Smouse, 2012). Significance of deviation from Hardy-Weinberg equilibrium was estimated by the Markov Chain method

Fig. 1. Sampling area for this study and location of Nanfangao Fishing Port. The blue area represents the longline fishing area. This map is modified from Joung and Hsu (2005).

comprised of 10,000 dememorizations, 500 batches, and 10,000 iterations by Genepop web service v4.0.1035. Genotypes were checked for null alleles using Micro-Checker 2.2.3 (Van Oosterhout et al. 2004). Analysis of paternity was initially checked by visually inspecting multi-locus genotypes. The number of sires and paternal skew within litters were inferred using two programs: 1) GERUD v2.037 (Jones 2005), which identifies the minimum number of fathers through exclusion calculations. Under the maternal genotype known scenario with allele frequency data of each litter, the exhaustive search tries every possible combination of paternal genotypes until it finds a combination that could explain the offspring array. And 2) COLONY v2.0.4.538 (Jones and Wang 2010), which uses a maximum likelihood approach, assuming a polygamous mating system for both sexes, to allow for assignment of full and half-sibs under the 1% typing error rate.

RESULTS

The female total weights were recorded, and the No. 1 (403 kg), No. 2 (336 kg), and No. 3 (288 kg) females had 13, 13, and 6 pups, respectively. Thirty-five sharks comprising three pregnant females and their litters were genotyped by seven polymorphic loci. The multi-locus genotypes are shown in table S1. There was no significant deviation from Hardy-Weinberg equilibrium, and all locus pairs were in linkage equilibrium ($p < 0.05$). Mendelian inheritance of alleles at these loci was checked manually and further supported by the complete concordance of mother-offspring genotypes. Additionally, there was no evidence of null alleles present in these loci. The seven loci had 5–13 alleles and observed heterozygosities of 0.624–1.0 (Table 1). GERUD and COLONY were used to calculate multiple paternity under the 'mother genotype known' scenario on three litters, and the results are summarized in table 2. *Isurus oxyrinchus* had an average of 4.6 sires assigned to each litter by COLONY. The average number of sires dropped to 2.5 when using GERUD, but the program failed to estimate paternity in the No. 3 female shark's litter due to the complex genotypes of pups.

DISCUSSION

The total body lengths of the 32 pups were 62.5–72.5 cm, which fits the observation of Joung and Hsu (2005) that litters are mainly 65.1–74.0 cm long for females caught in January and March. A previous study conducted in Australia showed that the litter sizes of *Isurus oxyrinchus* vary from 4 to 16 (Stevens 1983), which is very close to what Joung and Hsu (2005) found in the Northwestern Pacific (4–15). A further study by Mollet et al. (2000) observed that litter size varies from 4 to 25, and increases with maternal size, based on 95 mature female *I. oxyrinchus* including 35 pregnant females plus data on 450 postnatal fish collected from around the world. However, in the present study, we

Table 1. Characteristics of microsatellite loci in three litters of *Isurus oxyrinchus*

Locus name	Repeat motif	#Allele	Allele range	Ho	HW-*p*	Reference
CA1XD	AC	13	362-404	0.857	0.9907	Taguchi et al. 2013
CATY9	TCAC	7	228-250	0.667	0.9939	Taguchi et al. 2013
A96NC	AC	7	265-302	0.524	0.934	Taguchi et al. 2013
BIC01	AC	5	151-161	0.786	0.9675	Taguchi et al. 2013
IOX-30	(CA)14 A(AC)6	9	142-161	0.857	0.9975	Schrey and Heist 2002
IOX12	(GT)8 GAGT(GA)4	7	297-310	0.643	0.9803	Schrey and Heist 2002
IOX-01	(GA)8 (GT)19	10	126-170	1	1	Schrey and Heist 2002

Ho: observed heterozygosity, HW-p: *p* value of Hardy-Weinberg test.

Table 2. Summary of multiple paternity assessment of litters using GERUD and COLONY software

Species	Sample No.	Sampling date	Female weight	Litter Size	Size Range of pups	#Sires(COLONY)	#Sires(GERUD)
Isurus oxyrinchus	No.1	2016/1/16	403 kg	13	62.5–72.5	6	3
Isurus oxyrinchus	No.2	2016/3/4	336 kg	6	64–67	3	2
Isurus oxyrinchus	No.3	2016/3/4	288 kg	13	63–72	5	N/A

N/A: not available.

did not observe litter size increasing with maternal size, which is congruent with what Joung and Hsu (2005) found when examining 24 pregnant females of *I. oxyrinchus*. The incongruence in the correlation between litter number and female size between the studies could be due to bias caused by abortion during capture (Mollet et al. 2000; Whitney and Crow 2007).

The first case demonstrating polyandry in the shortfin mako *I. oxyrinchus* was carried out using five polymorphic loci on only one litter of eight pups (Corrigan et al. 2015), and showed that 2–3 males contributed to its multiple paternity. From an examination of only one litter, it is impossible to conclude that multiple paternity is a common reproductive strategy of *I. oxyrinchus*. Herein, we genotyped three litters of *I. oxyrinchus* with 7 microsatellite loci. In general, the results of paternity tests showed multiple paternity among the three litters that we examined. The number of sires that were detected from these three litters differed slightly depending on the software used (average of 4.6 by COLONY v.s. average 2.5 by GERUD) (Table 2). This trend could be due to an exhaustive search algorithm GERUD adopted to determining the minimum number of sires (Rossouw et al. 2016). This exhaustive search algorithm may also prevent us from estimating paternity with pups from the No. 3 female shark due to the presence of complex multilocus genotypes. Based on these findings, we suggest that polyandry could be a common strategy in the shortfin mako shark.

To date, the majority of studies assessing elasmobranch multiple paternity have been conducted on coastal and nearshore species, as there is a lack of research on pelagic sharks almost certainly due to low sample accessibility (Corrigan et al. 2015). Although multiple mating has previously been considered less common in pelagic species because mating encounters are probably rare in the open ocean, fertilizing multiple ova during any single mating event would maximize reproductive fitness (Gilmore 1993). With accumulating molecular evidence, pelagic sharks such as the tiger shark show no evidence of multiple paternity (Holmes et al. 2018), nor does the whale shark (*Rhincodon typus*) (Schmidt et al. 2010). On the other hand, the great white shark (Gubili 2008) and shortfin mako shark (Corrigan et al. 2015; present study) have been documented as using multiple paternity as a mating strategy. Although a global population connectivity study showed that *I. oxyrinchus* should be considered a unistock on the global scale (Corrigan et al. 2018), recent tagging studies showed that *I. oxyrinchus* displayed region-specific movements and thus may not be a nomadic wanderer as previously considered (Vaudo et al. 2017; Francis et al. 2019). This region-specific movement behavior suggests that *I. oxyrinchus* may have a greater opportunity to encounter mating partners than previously thought, and might explain the polyandry that we found.

According to FAO global catch production statistics (1981–2016), total landings of *Isurus oxyrinchus* increased by 69% from 2004–2009 to 2010–2016 (FAO 2018). And the proportion of *I. oxyrinchus* of global shark fins in international trade has declined from 2.7% (Clarke et al. 2006) to 0.2%–1.2% (Fields et al. 2018) over the past two decades, along with a historical decline (first 10 years with data vs. last 10 years) of 16.4% to 96% in different regions (CoP18 Proposal 42). Therefore, due to its global decline, *I. oxyrinchus* was further listed in CITES appendix II in August 2019 by CITES CoP18. In previous predictions based on sex-specific models under a polyandry mating system, the Northwest Pacific Ocean *I. oxyrinchus* population was predicted to decline in all three tested scenarios (A: under current fishing pressure; B: under current fishing pressure, but without fishing pressure on female neonates or juveniles; and C: under current fishing pressure, but without fishing pressure on female subadults and adults) (Tsai et al. 2015). Based on the present study, we suggest that polyandry could be a common mating strategy of shortfin mako sharks; however, other possibilities, including polygynous or mixed polygamous and monogamous, cannot be ruled out. Although it has been listed in CITES Appendix II, there is an urgent need to establish a fishery management plan regionally. Based on the current population status and demographic model predictions, we suggest that size-specific management be implemented regionally, specifically targeting female neonates and juveniles as Tsai et al. (2015) proposed, to achieve sustainable utilization of the Northwest Pacific Ocean *I. oxyrinchus* population.

CONCLUSIONS

We successfully reevaluated the mating system of and multiple paternity in *I. oxyrinchus.* Our results suggest that polyandry could be a common strategy in the shortfin mako shark. The sire number obtained through our study could be incorporated into a demographic model established previously (Tsai et al. 2014 2015) to achieve more precise results around population projections and risk analyses of the shortfin mako shark in the future.

Acknowledgments: We would like to thank Chi Cheng for her assistance in microsatellite genotyping and Chi-Ju Yu for her assistance in sample collection.

We gratefully acknowledge funding from the Ministry of Science and Technology, Taiwan (MOST) under MOST 106-2611-M-110-009 to SYV Liu and Ministry of Education, Taiwan under 180E9010P02 to WP Tsai, M Lee and HW Chien.

Authors' contributions: SYV Liu is the leading author and was in charge of data production and analyses. WP Tsai, M Lee, and HW Chien were in charge of sample collection and commented on the first draft.

Competing interests: The authors declare that they have no conflicts of interest.

Availability of data and materials: Multi-locus genotypes used in this study are included in Table S1 as the appendix.

Consent for publication: Not applicable.

Ethics approval consent to participate: Not applicable.

REFERENCES

Adams EM, Jones AG, Arnold SJ. 2005. Multiple paternity in a natural population of a salamander with long-term sperm storage. Mol Ecol **14:**1803–1810. doi:10.1111/j.1365-294X.2005.02539.x.

Barker AM, Frazier BS, Gelsleichter J, Grubbs RD, Hollenbeck CM, Portnoy DS. 2019. High rates of genetic polyandry in the Blacknose Shark, *Carcharhinus acronotus*. Copeia **107(3):**502–508. doi:10.1643/CG-19-180.

Bester-van der Merwe AE, Maduna SN, Hull KL, Bell J, Rossouw C, Wintner SP. 2019. Evidence for multiple paternity and confirmation of an Indo-Pacific origin of blacktip shark *Carcharhinus limbatus* occurring in South Africa. Afr J Mar Sci **41(3):**281–289. doi:10.2989/1814232X.2019.1653991.

Birkhead TR. 1998. Cryptic female choice: criteria for establishing female sperm choice. Evolution **52(4):**1212–1218. doi:10.1111/j.1558-5646.1998.tb01848.x.

Boomer JJ, Harcourt RG, Francis MP, Walker TI, Braccini JM, Stow AJ. 2013. Frequency of multiple paternity in gummy shark, *Mustelus antarcticus*, and rig, *Mustelus lenticulatus*, and the implications of mate encounter rate, postcopulatory influences, and reproductive mode. J Hered **104(3):**371–379. doi:10.1093/jhered/est010.

Bretman A, Tregenza T. 2005. Measuring polyandry in wild populations: a case study using promiscuous crickets. Mol Ecol **14:**2169–2179. doi:10.1111/j.1365-294X.2005.02556.x.

Byrne RJ, Avise JC. 2012. Genetic mating system of the brown smoothhound shark (*Mustelus henlei*), including a literature review of multiple paternity in other elasmobranch species. Mar Bio **159(4):**749–756. doi:10.1007/s00227-011-1851-z.

CITES. 2019. Consideration of Proposals for Amendment of Appendices I and II. CoP18 Prop. 42. Available at https://cites.org/sites/default/files/eng/cop/18/prop/060319/E-CoP18-Prop-42.pdf, Accessed 1st July 2020.

Clarke SC, McAllister MK, Milner-Gulland EJ, Kirkwood GP, Michielsens CG, Agnew DJ, Pikitch EK, Nakano H, Shivji MS. 2006. Global estimates of shark catches using trade records from commercial markets. Ecol lett **9(10):**1115–1126. doi:10.1111/j.1461-0248.2006.00968.x.

Corrigan S, Kacev D, Werry J. 2015. A case of genetic polyandry in the shortfin mako *Isurus oxyrinchus*. J Fish Bio **87:**794–798. doi:10.1111/jfb.12743.

Corrigan S, Lowther AD, Beheregaray LB, Bruce BD, Cliff G, Duffy CA, Foulis A, FrancisMP, Goldsworthy SD, Hyde JR, Jabado RW, Kacev D, Marshall L, Mucientes GR, Naylor GJP, Pepperell JG, Queiroz N, White WT, Wintner SP, Rogers PJ. 2018. Population connectivity of the highly migratory shortfin mako (*Isurus oxyrinchus* Rafinesque 1810) and implications for management in the Southern Hemisphere. Front Ecol Evol **6:**187. doi:10.3389/fevo.2018.00187.

Dean MD, Ardlie KG, Nachman MW. 2006. The frequency of multiple paternity suggests that sperm competition is common in house mice (*Mus domesticus*). Mol Ecol **15:**4141–4151. doi:10.1111/j.1365-294X.2006.03068.x.

Evans JP, Magurran AE. 2000. Multiple benefits of multiple mattings in guppies. Proc Natl Acad Sci USA **97:**10074–10076. doi:10.1073/pnas.180207297.

FAO. 2018. Fishery and Aquaculture Statistics. Global capture production 1950-2016 (FishstatJ). En: FAO Fisheries and Aquaculture Department [online]. Roma. Actualizado 2018. Available at www.fao.org/fishery/statistics/software/fishstatj/en. Accessed 1st July 2020.

Fields AT, Fischer GA, Shea SK, Zhang H, Abercrombie DL, Feldheim KA, Babcock EA, Chapman DD. 2018. Species composition of the international shark fin trade assessed through a retail-market survey in Hong Kong. Conserv Biol **32(2):**376–389. doi:10.1111/cobi.13043.

Francis MP, Shivji MS, Duffy CA, Rogers PJ, Byrne ME, Wetherbee BM, Tindale SC, Lyon WS, Meyers MM. 2019. Oceanic nomad or coastal resident? Behavioural switching in the shortfin mako shark (*Isurus oxyrinchus*). Mar Bio **166(1):**5. doi:10.1007/s00227-018-3453-5.

Gilmore R. 1993. Reproductive biology of lamnoid sharks. Environ Biol Fishes **38:**95–114. doi:10.1007/BF00842907.

Gubili C. 2008. Application of molecular genetics for conservation of the great white shark, *Carcharodon carcharias*. PhD dissertation, School of Biological Sciences, Aberdeen University, UK.

Holmes BJ, Pope LC, Williams SM, Tibbetts IR, Bennett MB, Ovenden JR. 2018. Lack of multiple paternity in the oceanodromous tiger shark (*Galeocerdo cuvier*). R Soc Open Sci **5(1):**171385. doi:10.1098/rsos.171385.

Jensen MP, Abreu-Grobois FA, Frydenberg J, Loeschcke V. 2006. Microsatellites provide insight into contrasting mating patterns in arribada vs. non-arribada olive ridley sea turtle rookeries. Mol Ecol **15:**2567–2575. doi:10.1111/j.1365-294X.2006.02951.x.

Jones AG. 2005. GERUD 2.0: a computer program for the reconstruction of parental genotypes from half-sib progeny arrays with known or unknown parents. Mol Ecol Notes **5:**708–711. doi:10.1111/j.1471-8286.2005.01029.x.

Jones OR, Wang J. 2010. COLONY: a program for parentage and sibship inference from multilocus genotype data. Mol Ecol Resour **10:**551–555. doi:10.1111/j.1755-0998.2009.02787.x.

Joung SJ, Hsu HH. 2005. Reproduction and embryonic development of the shortfin mako, *Isurus oxyrinchus* Rafinesque, 1810, in the northwestern Pacific. Zool Stud **44:**487–496.

Mollet HF, Cliff G, Pratt Jr HL, Stevens J. 2000. Reproductive biology of the female shortfin mako, *Isurus oxyrinchus* Rafinesque, 1810, with comments on the embryonic development of lamnoids. Fish Bull **98(2):**299–318.

Neff BD, Pitcher TE. 2002. Assessing the statistical power of genetic analyses to detect multiple mating in fish. J Fish Bio **61:**739–750. doi:10.1111/j.1095-8649.2002.tb00908.x.

Peakall PE, Smouse R. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics **28(19):**2537. doi:10.1093/bioinformatics/bts460.

Pirog A, Magalon H, Poirout T, Jaquemet S. 2019. Reproductive biology, multiple paternity and polyandry of the bull shark *Carcharhinus leucas*. J Fish Bio **95(5):**1195–1206. doi:10.1111/jfb.14118.

Pizzari T, Birkhead TR. 2002. The sexually-selected sperm hypothesis: sex-biased inheritance and sexual antagonism. Biol Rev **77(2):**183–209. doi:10.1017/S1464793101005863.

Rigby CL, Barreto R, Carlson J, Fernando D, Fordham S, Francis MP, Jabado RW, Liu KM, Marshall A, Pacoureau N, Romanov E, Sherley RB, Winker H. 2019. *Isurus oxyrinchus*. The IUCN Red List of Threatened Species 2019: e.T39341A2903170.

Rossouw C, Wintner SP, Bester-Van Der Merwe AE. 2016. Assessing multiple paternity in three commercially exploited shark species: *Mustelus mustelus*, *Carcharhinus obscurus* and *Sphyrna lewini*. J Fish Bio **89(2):**1125–1141. doi:10.1111/jfb.12996.

Schmidt JV, Chen CC, Sheikh SI, Meekan MG, Norman BM, Joung SJ. 2010. Paternity analysis in a litter of whale shark embryos. Endanger Species Res **12(2):**117–124. doi:10.3354/esr00300.

Schrey AW, Heist EJ 2003. Microsatellite analysis of population structure in the shortfin mako (*Isurus oxyrinchus*). Can J Fish Aqua Sci **60(6):**670–675. doi:10.1139/f03-064.

Stevens JD. 1983. Observations on reproduction in the shortfin mako *Isurus oxyrinchus*. Copeia **1983(1):**126–130. doi:10.2307/1444706.

Stevens JD, Bonfil R, Dulvy NK, Walker PA. 2000. The effects of fishing on sharks, rays, and chimaeras (chondrichthyans), and the implications for marine ecosystems. ICES J Mar Sci **57:**476–494. doi:10.1006/jmsc.2000.0724.

Taguchi M, Kitamura T, Shigenobu Y, Ohkubo M, Yanagimoto T, Sugaya T, Nakamura Y, Saitoh K, Yokawa K 2013. Development of 15 polymorphic microsatellite markers for the shortfin mako, *Isurus oxyrinchus*, and cross-species amplification in lamniforme sharks. Conserv Genet Resour **5(3):**675–678. doi:10.1007/s12686-013-9880-1.

Toonen RJ. 2004. Genetic evidence of multiple paternity of broods in the intertidal crab *Petrolisthes cinctipes*. Mar Ecol Prog Ser **270:**259–263. doi:10.3354/meps270259.

Tsai WP, Liu KM, Punt AE, Sun CL. 2015. Assessing the potential biases of ignoring sexual dimorphism and mating mechanism in using a single-sex demographic model: the shortfin mako shark as a case study. ICES J Mar Sci **72(3):**793–803. doi:10.1093/icesjms/fsu210.

Tsai WP, Sun CL, Punt AE, Liu KM. 2014. Demographic analysis of the shortfin mako shark, *Isurus oxyrinchus*, in the Northwest Pacific using a two-sex stage-based matrix model. ICES J Mar Sci **71(7):**1604–1618. doi:10.1093/icesjms/fsu056.

Van Oosterhout CV, Hutchinson W, Wills D, Shipley P. 2004. MICRO-CHECKER: software for identifying and correcting genotype errors in microsatellite data. Mol Ecol Notes **4:**535–538. doi:10.1111/j.1471-8286.2004.00684.x.

Vaudo JJ, Byrne ME, Wetherbee BM, Harvey GM, Shivji MS. 2017. Long-term satellite tracking reveals region-specific movements of a large pelagic predator, the shortfin mako shark, in the western North Atlantic Ocean. J Appl Ecol **54(6):**1765–1775. doi:10.1111/1365-2664.12852.

Whitney NM, Crow GL. 2007 Reproductive biology of the tiger shark (*Galeocerdo cuvier*) in Hawaii. Mar Bio **151:**63–70. doi:10.1007/s00227-006-0476-0.

Worm B, Davis B, Kettemer L, Ward-Paige CA, Chapman D, Heithaus MR, Kesseld ST, Gruber SH. 2013. Global catches, exploitation rates, and rebuilding options for sharks. Mar Policy **40:**194–204. doi:10.1016/j.marpol.2012.12.034.

Supplementary materials

Table S1. Multi-locus genotypes of three litters of *Isurus oxyrinchus* examined in the present study. Sample name with M in the end indicates mother shark, with O indicates offspring. (download)