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Life-history, ecology and fisheries of the Blackspot shark (*Carcharhinus sealei*) and Bluespotted maskray (*Neotrygon* spp.) in Southeast Asia: implications for conservation and management

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Other features

She's wild about conserving sharks and rays (2021) Straits Times:

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24 hours with shark and ray researcher Naomi Clark-Shen (2022) Eco-Business:

<https://www.eco-business.com/news/24-hours-with-shark-and-ray-researcher-naomi-clark-shen>

Three National Geographic Explorers reveal what it's like protecting the Earth (2022)
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What Is A Good Shark Documentary? We Ask A Marine Scientist (Who Is Also A Pro Wrestler) (2022) 8 days: <https://www.8days.sg/seeanddo/streamit/naomi-clark-shen-sharkfest-national-geographic-668516>

Shark's fin soup isn't the only threat to sharks (2023) Today Commentary: [TODAY Naomi shark fin soup](#)

Abstract

Due to their popularity and increasing vulnerability, elasmobranchs have received considerable research and conservation attention over the last few decades. However, much of this attention has focused on the larger, more charismatic species, leaving small-bodied species lacking in sufficient research and protection. There is also comparatively less research focusing on species that are endemic to Southeast Asia, and conservation solutions that are appropriate to this region. To address this gap, this thesis focused on small-bodied species that are commonly captured and traded within Southeast Asia; the blackspot shark (*Carcharhinus sealei*) and species in the bluespotted maskray complex (*Neotrygon* spp.). To learn about these animals, a multidisciplinary approach was used, studying the animals' life-history (age-growth and reproduction), ecology (diet), genetics, and fisheries (through social interviews).

To better understand the status of elasmobranchs and their management in Southeast Asia in general, a literature review was first conducted. Analysis revealed that 59% of assessed species in the region are threatened with extinction, with rays more threatened than sharks (69.3% vs 51.3%). The multi-species nature of fisheries (which blurs the line between 'target' and 'bycatch'), limited regulation around fishing gear, general overcapacity in the fishing sector, and lack of marine protected areas, threatens elasmobranchs. Geopolitical issues in the South China Sea, and resulting territorial disputes, exacerbate these issues, and limit cooperation between countries.

A total of 103 shark specimens matching the description of the blackspot shark and Indonesian whaler shark, and 251 stingray specimens matching the description of the bluespotted maskray, were obtained by traders in Singapore. The perspective of stakeholders is vital to understanding the fishing industry, species themselves, and the feasibility of management measures. The private supplier of 92 of the blackspot sharks, and 225 of the bluespotted maskrays, was interviewed through a semi-structured interview, for insights on the animals that they supplied for this research. All of the blackspot sharks were caught in Indonesian waters, and of the bluespotted maskrays, 206 were caught in Indonesian waters, 20 in Malaysia, and 25 in Singapore waters. The blackspot

sharks were caught using handlines, while the bluespotted maskrays were caught in traps and a small number on longlines. The private supplier shared that both animals are incidentally caught but still have a market, and are sold for their low-value meat in Singapore. The private supplier perceived that blackspot sharks have declined 50-70% and bluespotted maskrays about 50% over his 45 years in the industry, highlighting the need for science-based management measures.

Genetic analysis of the mitochondrial gene revealed all 103 sharks to be the blackspot shark (*C. sealei*), 146 of the stingrays to be oriental bluespotted maskray (*N. orientalis*) and 69 to be the mahogany maskray (*N. varidens*), while the remaining 36 stingray specimens yielded no result. However, for some of the bluespotted maskrays, the mitochondrial result and morphology of some animals did not always align. To further understand population genetics of bluespotted maskrays, 92 samples were sent for analysis of the nuclear gene using SNP genotyping. PCA plots of these results suggest potential introgression or hybridisation between bluespotted maskrays (with individuals falling between oriental bluespotted maskrays and mahogany maskrays on the PCA plot), as well as a genetically distinct cluster of bluespotted maskrays from Singapore, which may represent an undescribed species. The lack of clarity surrounding bluespotted maskray species complicated life-history and diet analysis.

Stomachs were removed from all specimens and excised to understand diet and ecology. Prey items were sorted to the lowest taxonomic level possible, and Percent Frequency Occurrence (%FO), was used for all further analysis. Blackspot sharks and bluespotted maskrays had significantly different diets—with blackspot sharks consuming more bony fishes and cephalopods, and bluespotted maskrays consuming more marine worms, algae and crustaceans. Dietary differences we observed among blackspot sharks, with mature males consuming more bony fishes, mature females consuming more cephalopods, and immature sharks of both sexes consuming more crustaceans. Analysis was performed for bluespotted maskrays categorising animals by mitochondrial results, nuclear results, and by grouping all individuals together. While significant differences were observed among some bluespotted maskray categories (e.g. by source country, between immature and mature oriental bluespotted maskrays), the confusion surrounding species identification, as well as the large number of locations animals were sourced from, diluted comparable samples and made it challenging to draw conclusions. Regardless, even when categorising bluespotted maskrays by

both mitochondrial results and nuclear results, no significant difference was observed between species. Overall, the bluespotted maskrays exhibited a more specialised diet (of predominantly marine worms and crustaceans) than blackspot sharks, which makes them vulnerable to prey loss.

The vertebrae of blackspot sharks and bluespotted maskrays were processed for ageing, and observation of gonads and embryos helped to decipher maturity and reproduction. Male and female blackspot sharks have a late age-at-maturity ($A_{50 \text{ maturity}} = 6.1$ years), and a moderately rapid growth rate with a k-value of 0.37 year⁻¹.

When grouping all bluespotted maskray species together, males mature earlier than females ($A_{50 \text{ maturity}} \text{ males} = 4.3$ years, $A_{50 \text{ maturity}} \text{ females} = 3.10$ years), and when analysing species separately, mahogany maskrays mature earlier than oriental bluespotted maskrays ($A_{50 \text{ maturity}} \text{ mahogany maskrays} = 2.08 - 5.53$ years, $A_{50 \text{ maturity}} \text{ oriental bluespotted maskray} = 3.69 - 7.16$). Bluespotted maskrays also have a moderately rapid growth rate with a k-value ranging between 0.17-0.75 year⁻¹ depending on how analysis is performed (e.g. male, female, mitochondrial species categories, nuclear species categories, all together).

For both blackspot sharks and bluespotted maskrays, females obtained larger sizes, heavier weights, and older ages - the maximum age recorded for female blackspot sharks and female bluespotted maskrays was 11 years. The maximum age for a male blackspot shark was nine, and the maximum age for a male bluespotted maskray was eight. Both blackspot sharks and bluespotted maskrays exhibit asynchronous reproduction, and have a low fecundity: two pups for blackspot sharks, and one to two pups for bluespotted maskrays. Both species compensate for this low fecundity with a large size-at-birth; the largest blackspot shark embryo was 43% the size of the mother, and the largest bluespotted maskray embryo was 44.3% the size of the mother.

Aside from bluespotted maskrays attaining maturity earlier than blackspot sharks, both species exhibit a similar life-history; with a relatively fast growth rate, sexual dimorphism, asynchronous breeding, low fecundity, and a large size-at-birth.

Knowledge from the literature review and interview with the private supplier were combined with insights from life-history, diet and genetic analysis, to consider feasible management solutions. The life-history of the species in this study, particularly their low fecundity, makes them vulnerable to fishing pressure. Population declines reported by the private supplier, as well as the IUCN, suggests

they cannot withstand current levels of exploitation. Both blackspot sharks and bluespotted maskrays are mainly caught incidentally by general fisheries, and depend on food sources (cephalopod, bony fishes, crustaceans), that are under threat from general fisheries in Southeast Asia. Improved regulations in the general fishery sector are warranted, although difficult to achieve due to a variety of factors, including lack of alternative livelihoods, poverty, and limited funds for reform. However, a region-wide trawl ban (or reform) could be considered to preserve benthic animals—such as shrimp—which are vital to the diets of bluespotted maskrays, juvenile blackspot sharks, and other species of shark and ray. Trawls also account for a large portion of shark and ray bycatch in Southeast Asia. While blackspot sharks will join other requiem sharks in Appendix II of CITES, the private supplier highlighted that this species is often already deceased when hauled in by fisheries (particularly longlines), and so mortality will not necessarily reduce as a result of this listing. Therefore, conservation efforts could focus on fishing gear modification to reduce capture. Although bluespotted maskrays are mainly caught incidentally, they have a huge market once caught, and so efforts to reduce consumer demand are needed. Additionally, the large trade in stingrays (for meat and leather), particularly those in the *Dasyatis* family (which includes bluespotted maskrays), makes them a potentially suitable candidate for a CITES Appendix II listing.

Future research should use the life-history information from this thesis to run Population Viability Assessments (PVA) to inform which segment of the populations are most critical to protect (e.g. juveniles, mature individuals). Additionally, research on the home-range, habitat use, and movements of blackspot sharks and bluespotted maskrays, will inform spatial protection measures. With global efforts to increase marine protected areas to cover 30% of the Oceans by 2030, there is good incentive to protect coastal regions, where these small-bodied, often forgotten sharks and rays (and their prey) reside.

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Chapter 1: Introduction

Comprehension of a species' biology and ecology is critical to our understanding of how to manage and conserve them: insights to diet can indicate their habitat use and dependence on prey, which informs which ecosystems and resources should be preserved (Simpfendorfer et al, 2001), while a species' life-history determines their vulnerability to exploitation and thus extinction risk (Garcia et al, 2008; Hutchings, 2002). Species with slower life-histories (i.e. slow growth rates ($k < 0.2 \text{ year}^{-1}$; Liu et al. 2021), late sexual maturity, long interbirth interval and small litters), known as k-selected species, are less able to compensate for mortality as they cannot replace themselves quickly enough. In contrast, species with faster life-histories (i.e. fast growth rates ($k > 0.2 \text{ year}^{-1}$; Liu et al. 2021), early sexual maturity, short interbirth interval and large litters), known as r-selected species, are better able to compensate for mortality as they can replace themselves faster (Cailliet, 2015; Garcia et al, 2008).

In general, elasmobranchs exhibit slow life-histories which, coupled with high fishing pressure and other threats, has contributed to over a third of all species being declared as threatened with extinction (Dulvy et al, 2021). Information on a species' life-history, ecology and habitat is critical to developing effective conservation planning (Harry et al, 2013; Lucifora et al, 2009), yet for many species of elasmobranchs, there is a paucity in such information.

The Coral Triangle, which encompasses parts of Southeast Asia, has been highlighted as one of three main hotspots where elasmobranchs are particularly threatened (a.k.a Indo-Pacific Biodiversity Triangle as described in Dulvy et al (2014)). Most fisheries in the region use non-selective fishing gear which are multi-species in nature, utilising most of what is caught (even if incidental), including sharks and rays (Adriano, 2011; Salayo et al, 2008; SEAFDEC, 2017a). In general, these fisheries are largely unregulated, face overcapacity and are overfished: with coastal fish stocks in the South China Sea just 5-30% of their unexploited levels (Pomeroy et al, 2016; Silvestre et al, 2003 in Salayo et al, 2008). Improving management of elasmobranchs in Southeast Asia is challenging due to a suite of complex factors, including high poverty rates and overcapacity in the general fisheries sector (Pomeroy et al, 2016), over-efficient and destructive

fishing gear (Ariadno, 2011), limited funds, capacity and technology for research and monitoring (Pomeroy, 2012; SEAFDEC, 2017), limited biology and ecology data for many of the species (Ahmad et al, 2018; Arai & Azri, 2019), and taxonomic confusions over the numerous cryptic (look-alike) species found in the region which complicate species-specific management plans (Jabado, 2019; Last et al, 2016; White, 2012).

The blackspot shark (*Carcharhius sealei*) and Indonesian whaler shark (*C. tjutjot*) are cryptic, small-bodied sharks (95 cm ~ 1.15 m in length) found in Southeast Asia, with the Indonesian whaler shark also ranging to Taiwan (White, 2012; Rigby & Kyne, 2018). These species were part of a taxonomic revision of the *Carcharhinus sealei-dussumieri* complex, whereby the blackspot shark was re-described and the Indonesian whaler shark resurrected (White, 2012). The oriental bluespotted maskray (*Neotrygon orientalis*) and mahogany maskray (*N. varidens*) are two small-bodied (33 ~ 38 cm) species of bluespotted maskray which are found in Southeast Asia (Last et al, 2016). They are part of a cryptic species-complex containing more than 10 species (Borsa et al, 2016; Borsa et al, 2018; Last et al, 2016).

These species (the blackspot shark, Indonesian whaler shark, and bluespotted maskrays), are small-bodied, and inhabit restricted, coastal ranges in Southeast Asia, making them particularly vulnerable to the unregulated fisheries operating in this region (Borsa et al, 2016; White, 2012). Landings data reveal that they are commonly caught (incidentally and targeted) throughout Southeast Asia (SEAFDEC, 2017a). In Singapore, where this thesis is based, the blackspot shark was the second most imported species of shark to the country's fishery ports, and bluespotted maskrays were the second most imported species of rays; with both groups of animals used primarily for their meat (Clark-Shen et al, 2021). The blackspot shark and Indonesian whaler shark are both Listed as Vulnerable by the IUCN: the blackspot shark has undergone a suspected population reduction of 30-49% over the past 24 years, and the Indonesian whaler shark of over 30% over the past 12 years (Dulvy et al, 2021; Rigby & Kyne, 2019). Both the oriental bluespotted maskray and mahogany maskray are listed as Least Concern by the IUCN with reported declines in some areas (Sherman et al, 2022a, 2022b).

Despite their frequent occurrence at landing sites and fishery ports throughout the region, little is known about these species' biology and ecology. The aim of this thesis is to improve biological understanding of these commonly fished, cryptic species: the blackspot shark, Indonesian whaler shark, and bluespotted maskrays, to collect the data needed to inform their management and conservation by:

- (1) Interviewing a supplier of these species of shark and ray, to understand their fisheries, trade, trends (population and market demand), and potential solutions.*
- (2) Establishing their life-history so that we know how quickly they grow and mature, how long they live, and how fast they reproduce, so that we understand their vulnerability to exploitation.*
- (3) Analysing their diet to inform which species they depend on as prey and which habitats they frequent. This informs ecosystem conservation needs.*

Chapter two presents a literature review of the status of sharks and rays in Southeast Asia. This chapter highlights the complexity of shark and ray management and conservation in this region due to unselective fisheries, a paucity in research data, and social and political challenges. This literature review has been published in Conservation Biology.

Chapter three outlines the sampling and methods used for the collection of biological information (methods for dissections, stomach and vertebrae preparation, and genetic analysis to confirm species composition) for the sharks and rays sampled during this thesis. This chapter reveals that only blackspot sharks (*C. sealei*) were in the sample but no Indonesian whaler sharks (*C. tjutjot*). For the bluespotted maskrays, oriental bluespotted maskrays (*N. orientalis*), and mahogany maskrays (*N. varidens*) were present in the sample, but there is confusion surrounding the genetics and morphology of these animals.

Chapter four outlines the description of fisheries for the sharks and rays sampled in this thesis, through an interview with the supplier.

Chapter five examines the ecology of the blackspot shark and the bluespotted maskrays through dietary analysis. Examination of stomach contents gives insights into feeding patterns, dietary preferences, and which habitats the species inhabit.

Chapter six is a life-history analysis of the blackspot shark. The vertebrae are analysed to estimate individual ages; gonads are examined to establish maturity; the presence of embryos used to determine reproductive patterns and litter size; and length-at-age data and growth models used to determine age and growth parameters.

Chapter seven is a life-history analysis of the bluespotted maskray. The vertebrae of these species are used to establish age; examination of gonads to establish maturity; presence of embryos to determine reproductive patterns and litter size; and length-at-age data to determine growth rates.

Chapter eight is a general discussion to synthesise the findings from each chapter and their significance and implications for real-world management and conservation in Southeast Asia.

Chapter 2: Literature review

Status of Southeast Asia's marine sharks and rays

2.1. Introduction

Over one third of chondrichthyans (sharks, rays, skates, and chimaeras) are threatened with extinction (Dulvy et al, 2021). Their slow life histories make them susceptible to overexploitation (Dulvy et al, 2021). Only 9% of global elasmobranch catches are biologically sustainable; 4% are managed for sustainability (Simpfendorfer & Dulvy, 2017).

Although humans have long consumed sharks and rays (Clarke, 2014; Kobak & Gutierrez, 2004), China's economic growth in the 1980s fueled demand for shark fin soup (Fowler & Seret, 2010), incentivizing fishers to intensively target sharks and retain those caught incidentally (Bonfil, 2002; Dent & Clarke, 2015). Shark fins are a high-value product, and the value of elasmobranch meat and other parts is increasing (Clarke et al, 2006; Dent & Clarke, 2015). Elasmobranchs in the Coral Triangle, encompassing Southeast Asia, are particularly threatened (Dulvy et al, 2014), and this region plays a large role in capture and trade of elasmobranchs (Dent & Clarke, 2015).

Brunei, Cambodia, Indonesia, Lao People's Democratic Republic, Malaysia, Myanmar, Philippines, Singapore, Timor-Leste, Thailand, and Vietnam comprise Southeast Asia. Their populations depend heavily on fishes as a main source of protein and income (Pomeroy et al, 2007, 2016). Regionally, coastal fish stocks are depleted to an estimated 5–30% of unexploited levels (Silvestre et al, 2003). There are at least 273 species of marine elasmobranch in this region (IUCN, 2021). Considering their importance to ecosystems and susceptibility to threats (Fowler et al, 2005), synthesis of regionally available information for elasmobranchs will help identify data, policy, and management needs

2.2. Methods

We used the following keywords in a literature search of Web of Science, Google Scholar, and OneSearch: *shark*, *stingray*, *batoid*, *elasmobranch*, *wedgefish*, *guitarfish*, *chondrichthyan*, *fish**,

Southeast Asia, Indonesia, Malaysia, Sabah, Sarawak, Borneo, Thailand, Vietnam, Timor, Lao*, Myanmar, Burma, Brunei, Singapore, Philippines, and Cambodia.* Irrelevant literature was excluded (e.g., freshwater research). A search of SEAFDEC (Southeast Asian Fisheries Development Centre), IUCN, and other grey literature was also conducted. There was little relevant literature on Brunei, Timor-Leste, and Lao, so they were excluded from references to Southeast Asia unless otherwise stated. *Elasmobranch* collectively refers to sharks, rays, and chimaeras.

2.3. Results

2.3.1. Elasmobranch fisheries

Southeast Asia contained three of the top 20 elasmobranch fishing nations from 2000 to 2011 (Indonesia, Malaysia, and Thailand (Dent & Clarke, 2015)) and two of the top 20 elasmobranch fishing nations from 2007 to 2017 (Indonesia and Malaysia) (Oakes & Sant, 2019). Total landings of elasmobranchs reported to the Food and Agriculture Organization (FAO) (Figure 2.1a) are likely 3–4 times lower than actual catches (Clarke et al, 2006; Worm et al, 2013); however, reconstructed data (Sea Around Us, 2021) can be used to make estimates (Figure 2.1b).

Indonesia, the Philippines, Vietnam, and Myanmar are the only countries with reported targeted elasmobranch fisheries (DoA, 2009; DoF/BOBLME/FFI, 2015; Fahmi & Dharmadi, 2015; SEAFDEC, 2006). Because fin value increases with size (Fields et al., 2018), shark-fin fisheries often target larger sharks; methods include longlines, hook and line, and gill-nets (Dharmadi et al, 2017; DoA, 2009; DoF/BOBLME/FFI, 2015). Hammerhead sharks (*Sphyrna* spp.), wedgefishes (*Rhynchobatus* spp.), silvertip sharks (*Carcharhinus falciformis*) and oceanic white-tip sharks (*Carcharhinus longimanus*) are considered valuable species (D’Alberto et al, 2019; Dent & Clarke, 2015; DoA, 2009; Jaiteh et al, 2017).

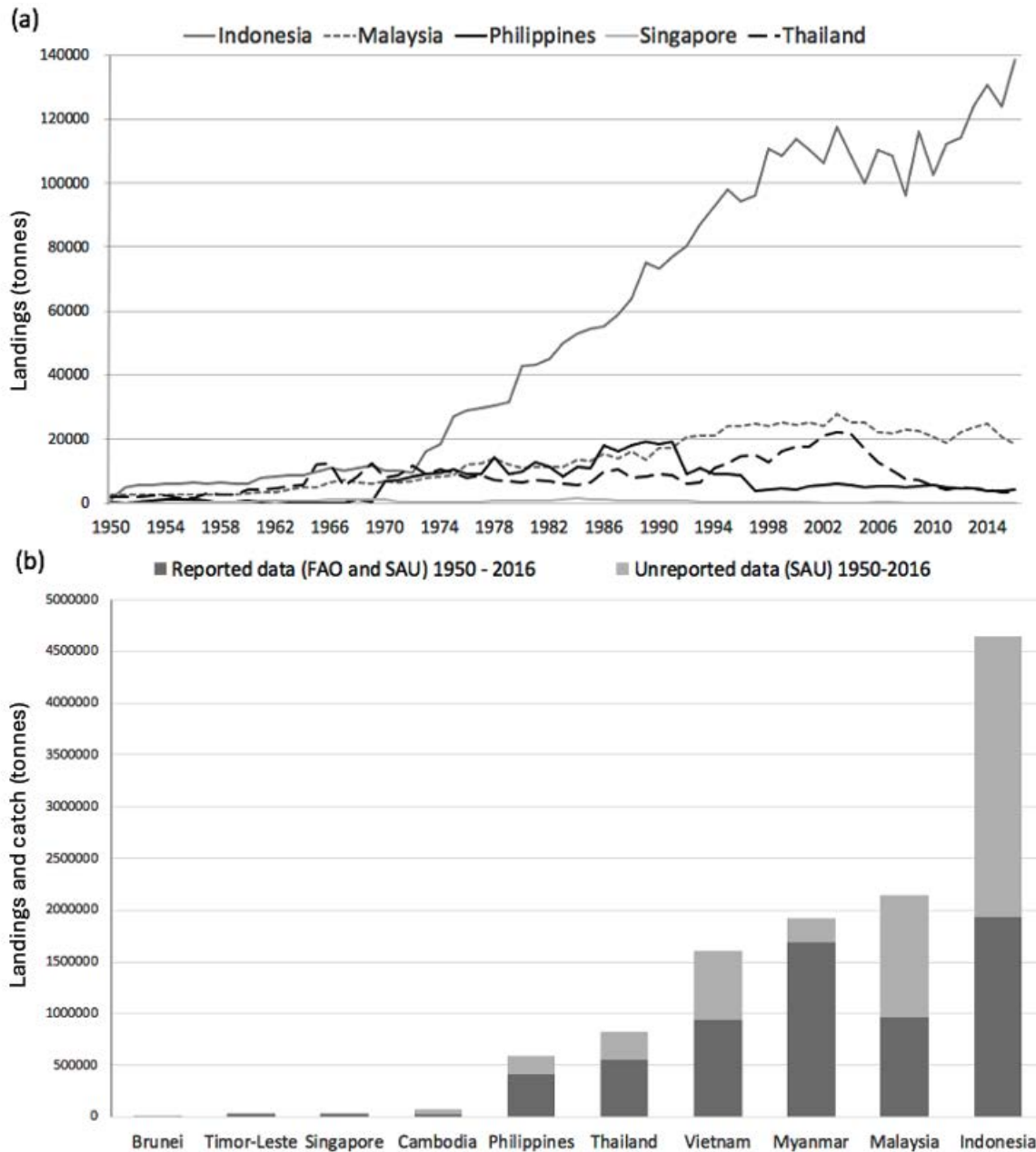


Fig 2.1. Elasmobranch (a) landings FishStatJ, 2016 (excluded jurisdictions: Cambodia, Myanmar, Timor-Leste, and Vietnam, lack reporting of specific elasmobranch data to FAO; Brunei, reporting to FAO started in 2015; Singapore, volumes too low to see clearly on the graph) and (b) total reported and unreported catch from Southeast Asia from 1950 to 2016 (data from FishStatJ [FAO, 2016] and Seas Around Us [SAU], 2021). Data from FishStatJ includes all reported elasmobranch landings whether caught within or outside of individual exclusive economic (EEZs). Data from SAU includes reported and reconstructed unreported elasmobranch catch within the countries' individual EEZs from their own local fleets and foreign fleets

Indonesia and the Philippines had the largest targeted elasmobranch fisheries. Their large, archipelagic, Exclusive Economic Zones (EEZ) allow access to large, pelagic species with valuable fins (SEAFDEC, 2006). They also have shark liver oil and meat fisheries (DoA, 2009; Jaiteh et al, 2017; Varkey et al, 2010). Indonesia has ray meat and skin (e.g., *Maculabatis gerrardi*) fisheries (Clark-Shen et al, 2021; D’Alberto et al, 2019). Shark fisheries developed in Vietnam in the 1980s for fins, skin, cartilage, and liver oil; catches peaked in the late 1980s before declining (SEAFDEC, 2006). It is unclear whether these fisheries persist. In Myanmar, shark fishing was banned in 2009 yet persists, and the fisheries remain unmanaged (DoF/BOBLME/FFI, 2015; MacKeracher et al, 2021). *Mobula* rays are targeted for gill rakers and meat in Myanmar (DoF/BOBLME/FFI, 2015), and a thriving ray fishery (WCS Myanmar, 2018) exists, largely driven by local consumption (MacKeracher et al, 2021). Fishers in Myanmar and Indonesia illegally use dynamite to kill fish and attract scavenging sharks (DoF/BOBLME/FFI, 2015). These sharks are a bonus in Myanmar but compensate for decreased shark catches in Indonesia (DoF/BOBLME/FFI, 2015; Jaiteh et al, 2017). Although Thailand reports they have no shark fisheries (Krajangdara, 2019; SEAFDEC, 2006, 2017a), there is contradictory literature (Stevens et al, 2005; WildAid, 2017), and some artisanal fishers report occasional, seasonal fishing for sharks (S.A, personal observation). Malaysia also claims to have no shark fisheries (Ahmad et al, 2018; Arai & Azri, 2019); however, phrases, such as the following, occur in the literature: “sharks and rays are *mostly* caught as bycatch” (Aswani et al, 2018) and “74.3% of [fishers who catch sharks during the tuna off-season] argue that sharks are not the target species” (Ahmad et al, 2018). These inconsistencies could be due to the multispecies nature of the region’s fisheries, whereby captured elasmobranchs are used, which obscures target and bycatch.

When fin values increased in the 1980s, many fishers engaged in “finning” (Jaiteh et al, 2017): cutting off fins and discarding bodies in the sea (Bonfil, 2002; Dent & Clarke, 2015). From the 1990s and into the 2000s, countries and regional fisheries management organisations (RFMOs) introduced anti-finning regulations, including requiring landing of whole sharks with fins attached. All Southeast Asian countries are prohibited from finning in waters under the Indian Ocean Tuna Commission (IOTC) and Western and Central Pacific Fisheries Commission (WCPFC) (Table 1). The increasing number of sharks landed whole due to anti-finning regulations is believed to be partly responsible for expanding shark meat markets. From 2000 to 2011, global meat import volumes

increased ~40% and value rose >60% (Dent & Clarke, 2015). Preliminary information suggests that even if fin value declines, shark fishing for meat will persist (Jaiteh et al, 2017).

Elasmobranchs in regional fisheries are largely reported as landed whole and fully used with finning described as “not rationale” by many fishers (Ahmad et al, 2019; SEAFDEC, 2006). However, it still occurs. For example, in North Maluku, Indonesia, fishers fin sharks at sea because locals do not eat the meat and boats have limited storage (Ichsan et al, 2019; Jaiteh et al, 2017).

2.3.2. Elasmobranch incidental catch

Most elasmobranchs captured in Southeast Asian fisheries are reportedly bycatch (Dharmadi et al, 2017; SEAFDEC, 2017a), which is similar globally (Dulvy et al, 2017; Simpfendorfer & Dulvy, 2017). However, many elasmobranchs are not discarded and are considered by-product because they are landed and used, making distinctions between bycatch and targeted ambiguous (Ahmad et al, 2018; SEAFDEC, 2006). Elasmobranchs are commonly caught incidentally by near-shore gillnets, trawlers, and pelagic longlines and gillnets targeting other species (Appendix S1) (Ahmad et al, 2018; DoF/BOBLME/FFI, 2015; Fahmi & Dharmadi, 2015; Jaiteh et al, 2017).

Incidental capture of sharks in pelagic tuna longline fisheries is high (Blaber et al, 2009; Sulaiman et al, 2018). Reported shark catches in Indonesia tuna fisheries vary: ~11% in 2009, <7% in 2012, and 8.5% from 2013 to 2017. Stingrays (Batoidea) are also incidentally caught (Setyadji & Nugraha, 2012; Sulaiman et al, 2018). In the Philippines, sharks accounted for 24% of total volume in Filipino fisheries (Guadiano, 2007 in DoA, 2009). Because tuna longline fisheries are often pelagic, incidental catches commonly include larger pelagic species (e.g., blue sharks (*Prionace glauca*), Mako sharks (*Isurus* spp.), and silky sharks (*Carcharhinus falciformis*) (Blaber et al, 2009; Sulaiman et al, 2018)

Nearshore fisheries—which are often multispecies and use a variety of fishing gear—catch (incidentally and targeted) mostly small-bodied elasmobranchs or immature individuals of large species (Arai & Azri, 2019; Ariadno, 2011; Arunrugstichai et al, 2018; SEAFDEC, 2017a). This suggests nearshore fishing grounds overlap with nursery habitats of some large-bodied species (Arunrugstichai et al, 2018; Knip et al, 2012). Trawl nets accounted for 87.9% and 96.57% of incidental elasmobranch catch in Malaysia and Thailand, respectively (SEAFDEC, 2006).

Elasmobranchs caught in nearshore fisheries account for a relatively small proportion of total marine catch in select regional fisheries: sharks, 1.4%; rays, 0.9%; and skates, 0.1% (SEAFDEC, 2017a). But, considering the size of fishing fleets and volumes of seafood caught, this is still substantial (SEAFDEC, 2017a).

2.3.3. Markets for elasmobranch products

Regionally, most shark parts are used and traded (Appendix S2). Stingrays are primarily used for their meat and skin (SEAFDEC, 2006, 2017a). Stingrays and small-bodied and juvenile sharks caught in nearshore fisheries are often sold fresh and whole at local markets for meat (SEAFDEC, 2017a). Prices vary with species, size, processing level, season, and country (SEAFDEC, 2017a). In Singapore, a premium for *Maculabatis* species was attributed to the higher quality meat for barbequed stingray, and more fresh stingrays are imported for domestic meat consumption than sharks (Clark-Shen et al, 2021). In Malaysia, stingray is preferentially ranked above shark for consumption (Ahmad et al, 2016). In Indonesia, the bluespotted maskray (*Neotrygon* spp.) and *Telatryon* spp. are the most common rays in supermarkets and restaurants because of taste, abundance, and low price (Mardijah & Pralampita, 2004; B.S., personal observation). In the Philippines, thresher shark meat is favoured and has high market value (A. Ponzo, personal communication). Regional trade in fresh, whole elasmobranchs is widespread (SEAFDEC, 2006, 2017a) but poorly documented, with multiple landing and aggregation sites and transport routes (Clark-Shen et al, 2021). Although fins are typically exported regionally, they are also consumed locally mainly among Chinese communities (Dent & Clarke, 2015; SEAFDEC, 2006).

Elasmobranch fins, meat, cartilage, and skin dominate the region's export market (Dent & Clarke, 2015; SEAFDEC, 2017a). Singapore, Malaysia, Indonesia, and Thailand are major global trade hubs for the import and export of elasmobranch meat and fins (Appendix S2). Large fins, of high value (Fields et al, 2018), are the primary export product, typically traded to China, Hong Kong, and Singapore (Dent & Clarke, 2015; SEAFDEC, 2006) (Appendix S2). *Manta* and *Mobula* gill rakers were primarily traded to China from Indonesia and Vietnam (O'Malley et al, 2016), but these species have since been listed on the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) Appendix II. Undocumented and illegal trade of CITES-listed species still occurs regionally (Choo et al, 2021; Clark-Shen et al, 2021; Friedman et al, 2018).

Trade in small, low-value fins (used for inexpensive shark-fin soup) is growing (US\$1–2/processed fin) in Thailand, Malaysia, Vietnam, Hong Kong, and Japan (Cardenosa et al, 2020; Dent & Clarke, 2015; Fields et al, 2018). In dried-seafood stalls in Hong Kong in 2014–2015, 48% of fins came from small-bodied sharks and chimaeras (despite large fins historically dominating the market). These are believed to have come from Southeast Asia’s nearshore, multispecies fisheries (Fields et al, 2018) that catch small-bodied sharks, often incidentally (SEAFDEC, 2017a). It is unclear whether the increase in traded small fins is due to large sharks declining or demand for more affordable fins.

The market for ray skins (e.g., whiprays, family *Dasyatidae*) for products, including wallets and belts, is increasing (D’Alberto et al, 2019; Save Sharks Network Philippines, 2017). Thailand is a common destination for skins from Singapore and Indonesia (B.S., personal observation, N.C.-S., personal observation). Stingray skins were the second most important product after wedgefish (*Rhinidae* spp.) fins in a tangle-net fishery in Indonesia (D’Alberto et al, 2019). Now that wedgefishes are listed on CITES Appendix II and should not be traded internationally by CITES signatories without a nondetriment finding (CITES, 2021), stingrays may be increasingly targeted. Wedgefish snout usage in shark head soup is a delicacy in Singapore and Malaysia (Clark-Shen et al, 2021; Kyne et al, 2020).

2.3.4. Status of elasmobranch populations

Of 273 assessed marine elasmobranchs (117 rays, 152 sharks, 4 chimaera) in 11 countries, ~59% are considered threatened with extinction (6.6% data deficient, 19.8% least concern, 15% near threatened, 25.6% vulnerable, 22.7% endangered, and 10.3% critically endangered) (Figure 2.2) (IUCN, 2021). Additionally, 72.5% of species have declining populations, 9.5% of species are stable, 0.7% are increasing (crocodile shark [*Pseudocarcharias kamoharai*], bluespotted lagoon ray [*Taeniura lymma*] only), and status of 17.2% is unknown. More rays are threatened with extinction (69.3%) than sharks (51.3%) (IUCN, 2021). Fisheries mechanisation, destructive fishing methods (e.g., trawlers), and overfishing are the main causes for regional population declines (Arunrugstichai et al, 2018; Howard et al., 2015; Jaiteh et al, 2017).

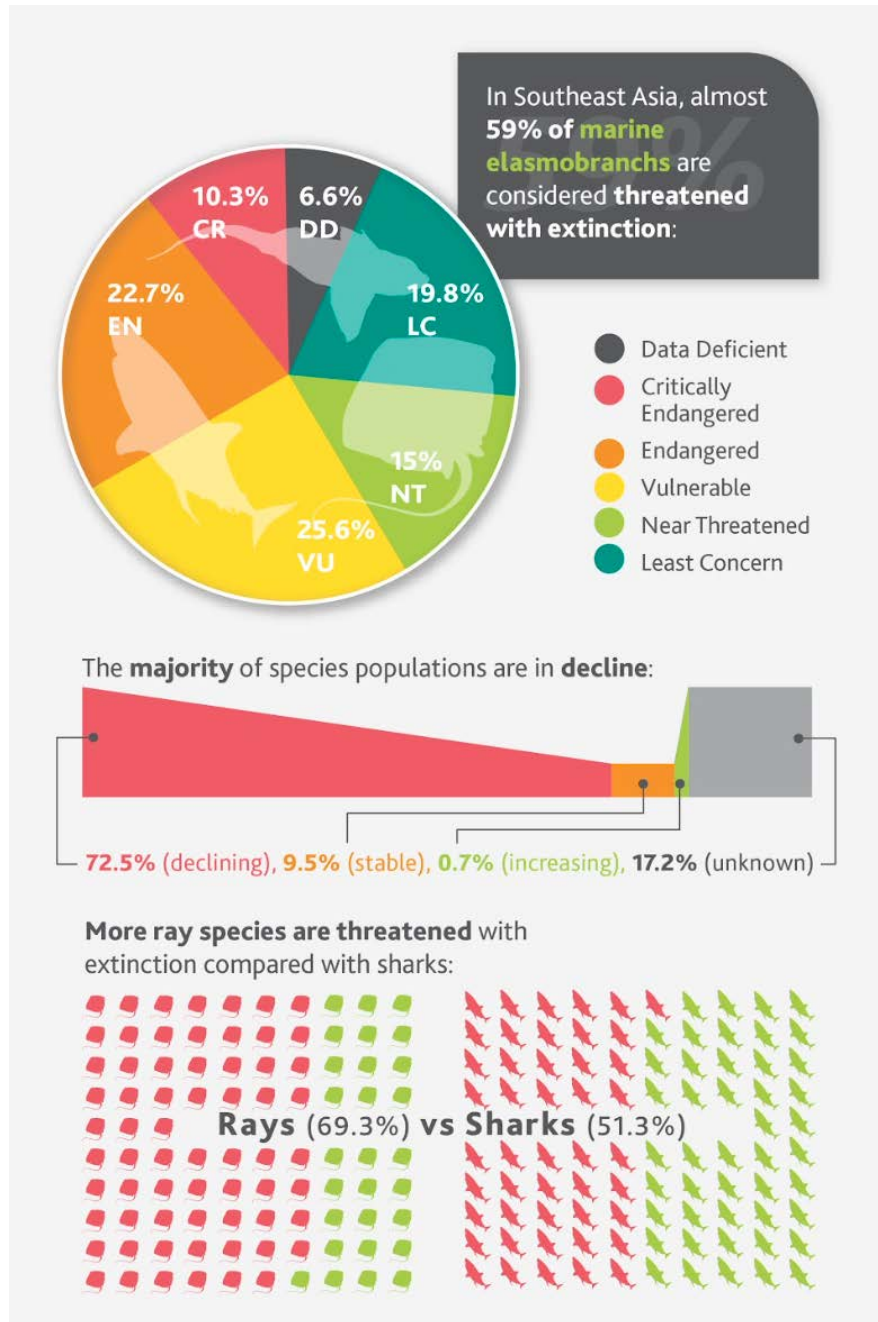


Fig 2.2. Status of sharks and rays in Southeast Asia. Threat categories are from International Union for the Conservation of Nature Red List (IUCN, 2021)

2.3.5. Catch and landing trends

In Myanmar, over 50% of “household heads” report declines of elasmobranch catches over the past 5 years (Howard et al, 2015). In the Philippines, fishers reported catch declines of *Mobula* ray (Acebes, 2012). Indonesian fishers report declines in the number of sharks caught, primarily in the last 5–10 years (Jaiteh et al, 2017). In Vietnam and Thailand, targeted fishing effort reportedly declined because of depleted shark numbers (SEAFDEC, 2006; WildAid, 2017).

These reported declines are mirrored in landings data. In the Philippines, landings and catch per unit effort declined (DoA, 2009). In Indonesia, wedgefish landings declined ~90% from 2005 to 2008 (D’Alberto et al, 2019). From 1996 to 1997, elasmobranch catch in the Java Sea declined by one order of magnitude (Blaber et al, 2009). In the Philippines, whale shark landings had decreased by 1997 (Alava et al., 2002). Shifting fishing grounds suggest local depletions. In Indonesia, shark fishing effort shifted from west to east (Bonfil, 2002). In Thailand, buyers report sharks sourced from ever-more-distant fishing grounds (Arunrugstichai et al, 2018). In the Philippines, manta ray were fished farther offshore by the 1980s (Acebes, 2012).

2.3.6. Changes in species catch composition

Fishers in eastern Indonesia report declines in large sharks caught (Jaiteh et al, 2017), and surveys of Thailand’s nearshore fisheries show declines in landings of large sphyrnid and carcharhinid species (Arunrugstichai et al, 2018). In contrast, landings surveys of nearshore, multispecies fisheries in Thailand, Malaysia, Indonesia, and the Philippines reveal bamboo sharks (*Chiloscyllium* spp.) are the most abundant species (Arai & Azri, 2019; Arunrugstichai et al, 2018; Dharmadi & Satria, 2015; DoA, 2009; SEAFDEC, 2017a). In Ranong province in Thailand, proportions of landed bamboo sharks increased from 26% in 2004 to 65% in 2016 (Arunrugstichai et al, 2018; Krajangdara, 2005). This may be due to their relatively high fecundity, which makes them more able to withstand fisheries and proliferate, whereas larger, more vulnerable sharks become depleted, known as mesopredator release (Sherman et al, 2020b), which may be responsible for a regional increase in the bluespotted lagoon ray as well (Sherman et al, 2020b).

2.3.7. Lost and rare species

Dwarf sawfish (*Pristis clavata*) have not been recorded regionally for over a century (Kyne et al, 2013); sawfishes appear to be gone from Thailand and Indonesian (IUCN Shark Specialist Group, 2021); and the lost shark (*Carcharhinus obsoletus*) and Java stingaree (*Urolophus javanicus*) are likely extinct (Dulvy et al, 2021; Kyne et al, 2021). However, because countries have limited monitoring and face challenges identifying elasmobranchs to species level (DoA, 2009; DoF/BOBLME/FFI, 2015; Krajangdara, 2019; Nijman, 2015), undetected remnant populations may persist. For example, the clown wedgefish (*Rhynchobatus cooki*) was undocumented for over 20 years until found at a fishery port in 2019 (Clark-Shen, Venkatesh, et al, 2019). A subsequent search of social media revealed sightings of this species in Indonesia between 2015 and 2020 (McDavitt & Kyne, 2020).

2.3.8. Elasmobranch management in Southeast Asia

Numerous regional management initiatives explicitly relate to elasmobranchs (Table 2.1). Countries must adhere to RFMO regulations while fishing in the Indian Ocean and the Western Pacific Ocean, but the South China Sea is not subject to RFMOs (Zhang, 2018). Therefore, SEAFDEC (2021) and the Coral Triangle Initiative (2021) play important roles in establishing management and conservation of regional resources. Elasmobranch-specific national laws focus primarily on CITES-listed species, and elasmobranch sanctuaries often occur where tourism is high (Table 2.2) (Topelko & Dearden, 2005).

Table 2.1. Regional Initiatives in Southeast Asia with relevance to elasmobranch management and conservation

Country	CITES^a	CMS^b	SEAFDEC member^c	WCPFC^d	IOTC^e	CTI-CFF^f
Brunei	✓		✓			
Cambodia	✓		✓			
Indonesia	✓		✓	✓	✓	✓
Malaysia	✓		✓		✓	✓
Myanmar	✓		✓			
Philippines	✓	✓	✓	✓	✓	✓
Singapore	✓		✓			
Timor-Leste						✓
Thailand	✓		✓	✓*	✓	
Vietnam	✓		✓	✓*		

**Vietnam and Thailand are co-operating non-members of the WCPFC.*

^a *CITES (The Convention on the International Trade of Endangered Species) is a legally binding treaty that aims to ensure that international trade does not threaten the survival of wild plants and animals.*

^b *CMS (The Convention on the Conservation of migratory Species of Wild Animals) uses legally binding treaties and less formal instruments to coordinate conservation measures throughout a species' migratory range. There are 40 species of elasmobranch that are included under the CMS.*

^c *SEAFDEC (Southeast Asian Development Centre) is an autonomous inter-governmental body that 'promote[s] and facilitate[s] concerted actions among the Member Countries to ensure the sustainability of fisheries and aquaculture in Southeast Asia' specifically in the ASEAN region. There are several initiatives relating to elasmobranchs including the development of Standard Operating Procedures (SOP) for elasmobranch data collection and data collection at landing sites throughout Southeast Asia.*

^d *WCPFC (The Western and Central Pacific Fisheries Commission) is a legally binding convention which sets provisions of fishing in the Western and Central Pacific Ocean (not including the South China Sea). There are several management measures related to elasmobranchs including the live releases of whale sharks, silky sharks and oceanic white-tips, and the development of Total Allowable Catch (TAC) for targeted shark fisheries. Shark finning is also prohibited.*

^E *IOTC (Indian Ocean Tuna Commission) has legally binding and non-binding measures relating to the management of tuna and tuna-like species in the Indian Ocean. There are several management measures related to elasmobranchs including the live release of thresher sharks and the recording of species-specific catch data. Shark finning is also prohibited.*

^F *CTI-CFF (Coral Triangle Initiative on Coral Reefs, Fisheries, and Food Security) is a non-legally binding initiative with numerous goals relating to the preservation of the coral triangle marine region in the Western Pacific Ocean. Species ID training, Regional Assessments and National Conservation Plans are underway for sharks and rays.*

Table 2.2. National laws, national plans of action (NPOA), and marine protected areas in Southeast Asian countries that were created specifically for marine elasmobranchs

Country	Nationally protected marine species	Fishing gear ban	NPOA	Spatial protection for elasmobranchs *indicates the presence of elasmobranch tourism sites
Brunei	Ban on the catch, landing, sale, import and trade of all shark species from 2013 (OCEANA 2013)			Shark fishing prohibited in Brunei's waters from 2013 (OCEANA 2013)
Cambodia	(1) Whale shark <i>Rhincodon typus</i> (FAO FIRMS 2020)			
Indonesia	(1) Whale shark <i>Rhincodon typus</i> (2) Giant oceanic manta ray <i>Manta birostris</i> (3) Reef manta <i>Mobula alfredi</i> (4) Sawfish spp. (Ministerial Decree 18/2013; Ministerial Decree 14/2014) National export bans for: (1) Scalloped hammerhead <i>Sphyrna lewini</i> (2) Great hammerhead <i>Sphyrna mokarran</i> (3) Smooth hammerhead <i>Sphyrna zygaena</i> (4) Oceanic white tip shark <i>Carcharhinus longimanus</i> (Ministerial Decree 5/2018) and catch quota for sharks listed on CITES (Ministerial Decree 10/2021).	Minimum mesh size for wedgefish gillnets (Ministerial Decree number 18/2021)	✓	Raja Ampat*, West Manggarai* and Komodo National Park* are elasmobranch sanctuaries where fishing of them is prohibited. Whale shark sanctuary to open in 2020 in Cendrawasi Bay* (Jaiteh et al. 2017a; Erdmann 2014; Langenheim 2017) Wedgefish and hammerhead shark sanctuary in Aceh (Ministerial Decree 76/2020 and Ministerial Decree 55/20)
Malaysia	(1) Whale shark <i>Rhincodon typus</i> (2) Sawfish spp. (3) Great hammerhead shark <i>Sphyrna mokarran</i> (4) Smooth hammerhead shark <i>Sphyrna zygaena</i> (5) Winghead shark <i>Eusphyra blochii</i> (6) Oceanic white-tip shark <i>Carcharhinus longimanus</i> (7) Giant oceanic manta ray <i>Manta birostris</i> (8) Reef manta ray <i>Mobula alfredi</i> (Control of Endangered Species of Fish Regulation 1999 and Malaysia Fisheries Act 1998)	'Pukat pari' drift nets with large mesh size to target large sharks and rays banned since 1990 (Ahmad et al. 2018)	✓	Marine parks in Sabah* were to be declared shark sanctuaries where fishing of sharks is prohibited. Unclear if this has yet been signed into law (Sabah Parks 2020)

Myanmar	(1) Whale shark <i>Rhincodon typus</i> There was a national ban on targeted shark fishing through a declaration made by the Department of Fisheries however this is reportedly not formalised in law (Howard et al. 2015). Other sources say fishing ban on CITES-listed species only (Friedman et al. 2018)		In progress	Two shark reserves in Myeik Archipelago where targeting of sharks is prohibited (not including rays) but with no management plan or enforcement. This also contradicts with the national ban on targeting of sharks in the entire country (DoF/BOBLME/FFI 2015)
Philippines	(1) Whale shark <i>Rhincodon typus</i> (2) Giant oceanic manta ray <i>Manta birostris</i> (3) Reef manta ray <i>Mobula alfredi</i> (Friedman et al. 2018) (4) All sawfishes <i>Pristidae</i> spp. (SEAFDEC 2020). Thresher sharks protected in Batangas City (Batangas City ordinance resolution 95 s-2008). Fishing and selling of sharks prohibited in Cebu (RP Provincial Board Ordinance No. 2015-05). Palawan protects all elasmobranchs listed in CITES Appendices or Critically Endangered, Endangered or Vulnerable by the IUCN (RP RA 7611 PCSD Resolution 19-682, PCSD Resolution 15-521). Take and trade of CITES-II and III species prohibited until NDF (RP RA 8550, as amended by RA 10654)		✓	Donsol, Sorsogon* municipal waters are a whale shark sanctuary (DoA 2009); two seamounts* in Malapascua are shark and ray sanctuaries. (RP Executive Order 16-2015)
Singapore	(1) Devil rays <i>Mobula</i> spp. (2) Sawfishes <i>Pristidae</i> spp. (Wildlife (Protected Wildlife Species) Rules 2020)			
Timor-Leste	All sharks used to be protected but this was reduced to 12 threatened species (species not listed) sometime in or after 2018 (Lopez-Angarita et al. 2019)			
Thailand	(1) Whale shark <i>Rhincodon typus</i> (2) Sawfish spp. (<i>A. cuspidate</i> , <i>P. pristis</i> , <i>P. zijsron</i>) (3) Shark ray <i>Rhina ancylostoma</i> (4) Giant oceanic manta ray <i>Manta birostris</i> (5) Reef manta ray <i>Mobula alfredi</i> (6) <i>Mobula</i> spp. (<i>M. mobular</i> , <i>M. kuhlii</i> , <i>M. thurstoni</i>) (Krajandara 2019)		✓	
Vietnam	Fishing ban on CITES-listed species (Friedman et al. 2018)			

Brunei and Myanmar have banned shark fishing. We found no information on the effectiveness of Brunei's ban, prior to which 12.7% of sharks were taken as bycatch in selected fisheries (SEAFDEC, 2006), and a recent study reports sharks caught as bycatch (Azri et al, 2020). Myanmar's regulations seem unenforced (Howard et al, 2015; MacKeracher et al, 2021), and there are no clear regulations on retaining or selling shark bycatch, which authorities appear to tolerate (Howard et al, 2015). Only 49% of surveyed fishers in Myanmar were aware of the shark fishing ban, citing food and income as motivations for not complying (MacKeracher et al, 2021).

2.3.9. Complex regional management

Regional challenges to elasmobranch management relate to systemic issues of general fisheries (Dharmadi et al, 2017; SEAFDEC, 2006, 2017a). Overcapacity is a leading cause of regional overfishing (Pomeroy et al, 2016) that arises from open access to the resource, poverty rates, subsidies, and lack of alternative livelihoods (Pomeroy, 2012; SEAFDEC, 2018). Other problems include absence of an RFMO to regulate activity (Zhang, 2018); overefficient and destructive fishing (Ariadno, 2011); and multispecies nature of many fisheries that complicates species-specific management (Ariadno, 2011; Salayo et al, 2008). There are insufficient funds, capacity, technology, and human resources to monitor fisheries and collect data (Pomeroy, 2012; SEAFDEC, 2017a); enforcement of fisheries regulations and protected areas is weak and there is corruption and illegal, unreported, and unregulated fishing (Kamil et al, 2017; Pomeroy et al, 2015; Pomeroy et al, 2016).

2.3.10. Presence of China

Although China is not part of Southeast Asia, it claims sovereignty over the South China Sea and fishes there (Fravel, 2011). These territorial disputes cause conflict and complicate cooperative management of transboundary populations (Dharmadi et al, 2015; Zhang, 2018). China is a main importer and consumer of shark fins (Dent & Clarke, 2015; Oakes & Sant, 2019), but their reports to the FAO do not provide true volumes or locations of catch (Dent & Clarke, 2015; FishStatJ). Targeted shark fisheries in southern China collapsed between the 1970s and 1990s (Lam & de Mitcheson, 2011), and reconstructed elasmobranch catches suggest a decline of 67% since the 1950s (Zeller & Pauly, 2016). Reported and reconstructed unreported elasmobranch catches near disputed

South China Sea islands in Southeast Asia from 1950–2016 were ~1.6 million t: 46% caught by Mainland China, 29% by Taiwan and Hong Kong, 19% by other Southeast Asian countries, and 6% by other nations (Sea Around Us, 2021). Timor-Leste (outside the South China Sea) protected all sharks, discovered them onboard a Chinese vessel, and reduced protection to 12 species (Lopez-Angarita, 2019).

2.3.11. Social and development contexts

Many fishers in Southeast Asia face poverty (Jaiteh et al, 2017; Jaiteh et al, 2017; Save Sharks Network Philippines, 2017). Therefore, even when caught in small amounts elasmobranchs provide important income (Ahmad et al, 2018; Aswani et al, 2018). Although some shark fishers may consider alternative livelihoods, they often live in areas with few options: land may be unsuitable for agriculture; regional markets distant; funds, infrastructure, and expertise to develop other income sources lacking; and tourism development difficult (Acebes et al, 2016; Jaiteh, 2017; Lestari et al, 2017; Mizrahi et al, 2019).

Some shark fishers resort to illegal livelihoods that use their skills (navigation) and resources (boats), such as human and petrol smuggling (Jaiteh et al, 2016; Jaiteh et al, 2017). Shark fishers in Myanmar and Indonesia switched to fishing of other species; however, this was less profitable and involved learning new fishing techniques (Howard et al, 2015; Jaiteh et al, 2017). In Indonesia, a shark-fishing community successfully switched to seaweed farming until there was an oil spill and no funds to restart the project (Jaiteh et al, 2017).

These situations demonstrate why harvesting of sharks, particularly for fins, is a viable livelihood: fins are valuable; dried fins can be stockpiled; fins are light and easily transported; and sharks can be harvested with simple gear (Jaiteh et al, 2017). Some shark and *Mobula* ray fishers are unwilling to adopt alternative livelihoods because of the tradition, culture, and identity associated with this work (Acebes et al, 2016; Jaiteh et al, 2017; Yulianto et al, 2018), and Western conservation initiatives may be rejected or incompatible with community contexts and needs (Clifton & Foale, 2017).

2.3.12. Limited landings data

Species-specific catch and landings data are limited and mostly aggregated into sharks or rays in national statistics and FAO reports (Appendix S3) (FishStatJ, 2016). Cambodia, Myanmar, Timor-Leste, and Vietnam do not report elasmobranch data to the FAO, although it may be reported under “marine fish” (Holmes et al, 2014). Fishing gear type, fishing ground location, and size and sex of specimens are rarely reported and typically do not come from long-term monitoring programs; there limited data hinder population assessments, identifying key habitat, and creating management plans (Arunrugstichai et al, 2018; Blaber et al, 2009; DoA, 2009; SEAFDEC, 2017a). The Sea Around Us database provides some detail (e.g., catch volumes by gear type), but their “unreported” data are reconstructed estimates.

Reasons for a lack of data include difficulties identifying elasmobranchs to species level and limited capacity and funds for monitoring (Dharmadi et al, 2015; DoA, 2009; DoF/BOBLME/FFI, 2015; Krajangdara, 2019). In countries with bans on shark fishing, fishers may be reluctant to share catch data out of fear (M.M, personal observation). In Thailand, citizen outrage and scoldings by authorities (even when landed sharks are legal) can make sellers hide sharks (S.A., personal observation). Because many elasmobranchs in Southeast Asia are caught incidentally and are of low value (SEAFDEC, 2017a), there may be less political will to invest in monitoring. For example, the National Stock Assessment Programme (NSAP) in Thailand only monitors landings of the 10 most commercially important species, which does not include elasmobranchs (Arunrugstichai et al, 2018). The SEAFDEC has implemented monitoring programs for elasmobranchs through-out Southeast Asia (SEAFDEC, 2017b), but continuity is not yet reported.

2.3.13. Limited biological data and taxonomic confusion

Life-history (e.g. age, growth, reproduction), behavioural, and habitat data on elasmobranchs are limited regionally (Ahmad et al., 2018; Arai & Azri, 2019; DoF/BOBLME/FFI, 2015), and information from one region may not be applicable to another. For example, male gray sharpnose sharks (*Rhizoprionodon oligolinx*) differ in size at maturity in India (Purushottama et al, 2017) and Indonesia (White, 2007).

Taxonomic confusion can lead to unsuitable management based on the incorrect identification of species' behaviour, biology, and range (Simpfendorfer et al, 2011; White & Last, 2012). Genetic tools have enabled distinctions between morphologically similar species historically grouped together (White & Last, 2012). For example, reevaluation of *Carcharhinus sealei-dussumieri* group resulted in resurrection of Indonesian whaler shark (*Carcharhinus tjutjot*) and redescription of the blackspot shark (*Carcharhinus sealei*) (White, 2012). Both species are still recorded occasionally as *Carcharhinus dussumieri* (believed to occur only in western Indian Ocean [White, 2012]) in regional landings data (Arunrugstichai et al, 2018; Krajangdara, 2019). The dwarf whiplay (*Brevitrygon walga*) is now considered to occur only outside Southeast Asia (Last et al, 2016), making it unclear what the species recorded as such in surveys (Appendix S1) actually is. Such ambiguities reduce confidence in landings data and species trends.

2.3.14. Future Management

Landings surveys should clarify whether elasmobranchs are targeted, bycatch, or by-product to guide management (Gupta et al, 2020) and collect biological information and catch locations to determine critical habitats during different life stages and seasons (Heupel et al, 2018; Ward-Paige et al, 2012). Analysis of DNA from tissue samples could help identify cryptic and “lost” species (Clark-Shen et al, 2021; Feitosa et al, 2018). Because a lack of capacity and funds affects monitoring (Dharmadi et al, 2015; DoA, 2009; DoF/BOBLME/FFI, 2015; Krajangdara, 2019), more could be done to engage fishers and traders and maximise input of local ecological knowledge, providing opportunities for collaboration, employment, research, and successful management (Acebes et al, 2016; Ahmad et al, 2018).

2.3.15. Responsible elasmobranch fisheries and trade

Making elasmobranch fisheries sustainable is critical (Simpfendorfer & Dulvy, 2017). Barriers include cost and complexity of certification in developing countries (Washington & Ababouch, 2011). Alternatively, tailored adjustments could make fisheries more responsible.

In Indonesia, the release of all bamboo sharks above 700 mm was recommended (Fahmi et al, 2021), and in a targeted shark fishery, spatiotemporal closures, restrictions on fishing effort, and incentives to control hook numbers were suggested (Yulianto et al, 2018). Catch and trade quotas for threatened

species not regulated by CITES should be considered. For example, whitespotted whipray (*Maculabatis gerrardi*) is endangered (Sherman, 2020a). Their suspected decline is up to 79% (Sherman, Ali, et al, 2020), but they are traded among Singapore, Indonesia, and Malaysia in large volumes (Clark-Shen et al, 2021).

2.3.16. Bycatch reduction

Bycatch release programs are underway in Thailand for trawlers (Krajangdara, 2019), and in Malaysia, shrimp trawlers are encouraged to release juvenile elasmobranchs, which fishers reportedly agree to because of their low value (Ahmad et al, 2018). Species' survival upon release needs consideration. Some studies indicate high levels of survival (Musyl & Gilman, 2018), whereas others indicate high mortality from capture stress (Gallagher et al, 2014). Some fishers in Sabah claim that sharks caught in gillnets are already dead, so discarding them would be wasteful (Ahmad et al, 2018).

Alternatively, bait restrictions, hook-type changes, and use of repellents can reduce sharks being caught, and is recommended under the Philippines' proposed shark law (Shark Conservation Act of the Philippines, 2019). Electric fields, tested on gillnets in Indonesia (Aristi et al, 2018), green LED lights on gillnets (Senko et al, 2022), and magnets on fish traps (Richards et al, 2018) decrease elasmobranch bycatch. The latter deterrents are effective on stationary fishing gear but not trawls, which are considered most hazardous to elasmobranchs in certain Southeast Asian countries (SEAFDEC, 2006). Turtle excluder devices (TEDs) used in multiple trawl fisheries in Malaysia (Marine Research Foundation, 2019) and Indonesia (where trawls were banned but mini trawls persist [Chong et al, 1987]) may also reduce bycatch of elasmobranchs (Brewer et al, 2006; Dharmadi et al, 2015). In Australia, TEDs used in prawn trawl fisheries reduce catch of larger elasmobranchs (Campbell et al, 2020).

Assessment of individual fisheries is essential (e.g., fishers in India favour release of elasmobranchs over net restrictions, fishery closures, and bycatch reduction devices because these were deemed to affect income too severely [Gupta et al, 2020]), but in general, catch-based regulations are harder to enforce than gear-based regulations (MacNeil et al, 2020).

2.3.17. Fisheries sector reform

Improvements to the general fishery sector is essential (Pomeroy et al, 2016) and will also ensure functioning ecosystems and prey supply. Reforms may include prohibiting subsidies that contribute to overcapacity (SEAFDEC, 2018) and creating alternative livelihoods (Asiedu & Nunoo, 2013). Because data are scarce in the region, the allowable biological catch (ABC) is a good tool for setting of catch species limits (Chumchuen & Chumchuen, 2019; Saleh et al, 2020). Restricting fisheries in critical habitats (e.g., nursery grounds) (Birkmanis et al, 2020; Di Lorenzo et al, 2020) and reducing or eliminating destructive fishing gear, such as trawlers, would reduce bycatch and protect habitats (Ariadno, 2011; Seafood Source, 2016; MacNeil et al, 2020). Countries should embrace remote electronic monitoring on vessels as a cost-effective and safe way to monitor catch and ensure legality (Van Helmond et al, 2019). Southeast Asian countries and China need to cooperate on marine resources in the South China Sea (Clark-Shen et al, 2019; Zhang, 2018). The growth of cell-based and plant-based foods could help alleviate demand on ocean resources (Good Food Institute, 2021).

2.3.18. Protected areas for elasmobranchs

Significantly higher abundances of sharks are recorded in Marine Protected Areas (MPAs); *‘a defined region designated and managed for the long-term conservation of marine resources, ecosystems services, or cultural heritage’* [NOAA]) in Raja Ampat, Indonesia, and Tubbataha Reefs Natural Park, the Philippines, than in adjacent unprotected areas (Jaiteh et al, 2016; Murray et al, 2019). Their success is attributed to their large sizes, high enforcement, and value to the local economy (Jaiteh et al, 2016; Murray et al, 2019). Southeast Asian countries committed, under the UN Convention on Biological Diversity (2020), to expand MPAs and should consider elasmobranchs in their designs. Many reefs in Southeast Asia have low elasmobranch abundance (MacNeil et al, 2020), but identification of hope spots for protection is possible and should focus on areas that would yield positive stakeholder involvement instead of displacement (Dwyer et al, 2020; Kamil et al, 2017; Murray et al, 2019; Musa, 2003). Where this criterion cannot be met, fisheries management or less strict area protection (e.g., no-take zones, closed seasons) could be effective (MacNeil et al, 2020). For site-attached coral reef sharks, MPAs should be >10 km² and for less site-attached species >50 km² (Dwyer et al, 2020). Although large MPAs provide better protection

for elasmobranchs, where enforcement is limited, small MPAs protecting critical habitats would enable better enforcement and overall success (MacKeracher et al, 2018). A network of MPAs for migratory elasmobranchs, similar to the Turtle Island Heritage Protected Area (which spans Malaysia and the Philippines) (ASEAN Centre for Biodiversity, 2010), could be considered. Only 14% of marine parks in Southeast Asia are effectively managed (Burke et al, 2002), so assessment of the likely success of MPAs is essential. Locally managed marine areas, which give fishers and communities the power to create and manage areas (Howard, 2017), could prove more successful.

Chapter 3:

Sampling of blackspot shark (*Carcharhinus sealei*) and bluespotted maskray (*Neotrygon* spp.), dissection protocol and genetics

3.1. General Methods

This chapter documents the sampling design and approach, and methods used to collect, process, and verify the identity of the sharks and rays sourced from regional fisheries.

3.1.1. Collection of specimens

One hundred and three sharks matching the description of cryptic species the blackspot shark (*C. sealei*) and Indonesian whaler shark (*C. tjutjot*), and 251 bluespotted maskrays (*Neotrygon* spp.) were collected from Jurong Fishery Port in Singapore as well as from a private seafood supplier between 2019 and 2021. Supply numbers by year and month are outlined in Figure 3.1. When animals were delivered, the seafood supplier and fish merchants at Singapore's fishery ports were asked where the animals were caught or imported from.

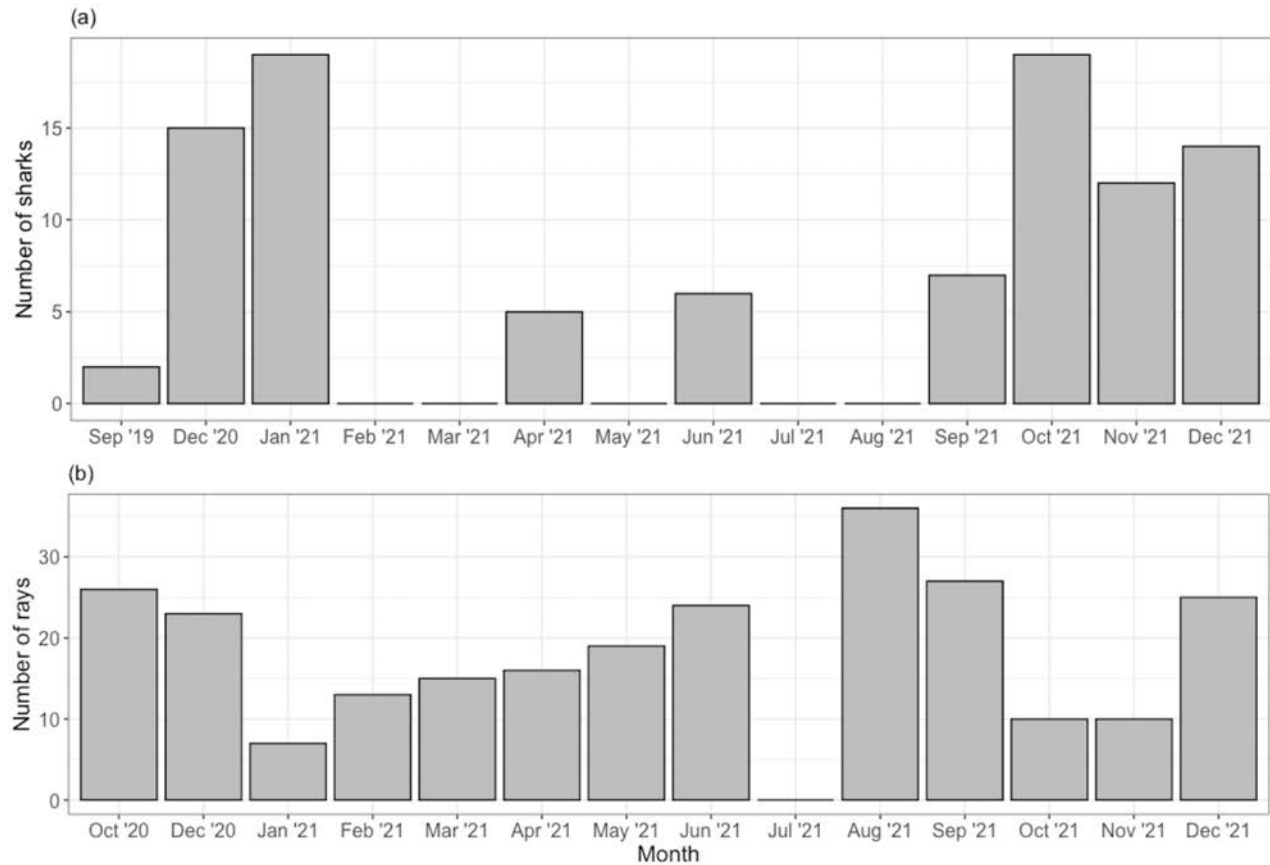


Fig 3.1. Supply by numbers of (a) Sharks (that matched the description of the blackspot shark (*C. sealei*) and Indonesian whaler shark (*C. tjutjot*), and (b) bluespotted maskrays (*Neotrygon* spp.), by month and year.

3.1.2 Dissection and collection of key body parts

The specimens were either dissected immediately upon purchase or were stored frozen until dissection. Specimens were photographed, sexed (males with claspers, females without claspers) and weighed. Stretched total length (STL - measured with dorsal portion of tail bent straight/stretched so upper lobe lies along body midline), fork length (FL) and pre-caudal length (PL) as described in Francis (2006), measured to the nearest mm for sharks and disc width (DW) measured to the nearest mm for rays (Figures 3.2, 3.3).

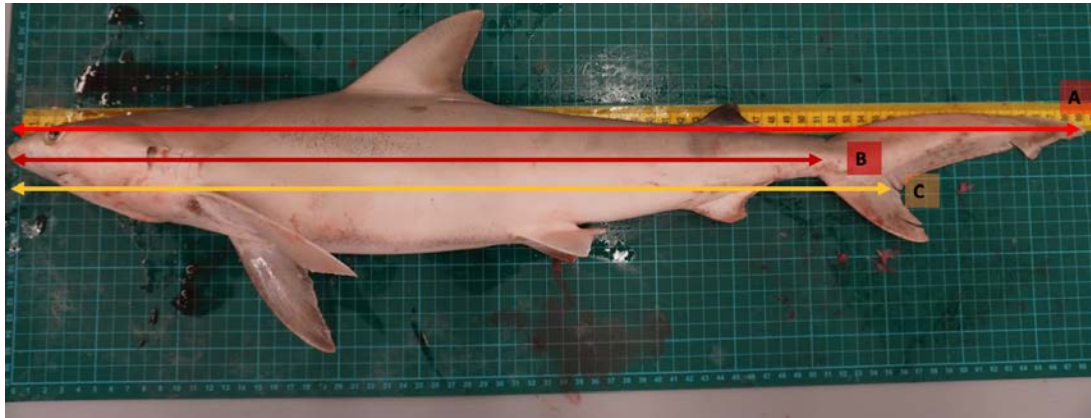


Fig 3.2. Measurements taken of shark specimens: (A) Stretched Total Length (STL), (B) Pre-caudal length (PCL), and (C) Fork Length (FL)

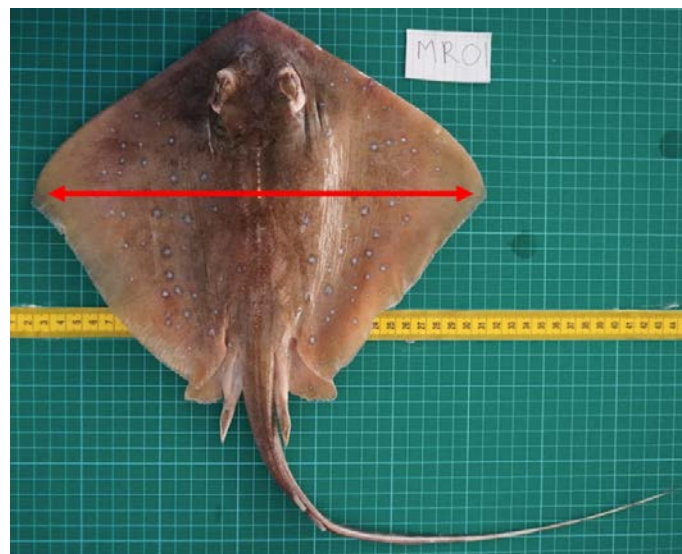


Fig 3.3. Measurements taken of ray specimens included Disc Width (DW).

The specimens were then dissected, and the liver removed and weighed. The stomach was removed for dietary analysis (as outlined in chapter 5), and the gonads of specimens were examined to assign maturity status and breeding state for each individual. Maturity was assigned following Walker, (2005) as outlined in (Table 3.1). Vertebrae were removed for age and growth analysis (as outlined in chapter 6 and 7). A fin clip from the anal fin of all specimens was taken for DNA analysis to

verify the species of each specimen (see 3.1.3) and stored in 70% ethanol for up to a week and then transferred to 90% ethanol.

Table 3.1. Reproductive indices used to determine maturity stage. Adapted from Walker (2005)

Organ	Index	Description	Binary maturity condition
Female Uterus	U = 1	Uteri uniformly thin and white tubular structure. Small ovaries and with no yolked ova	Immature
	U = 2	Uterus thin, tubular structure that is partly enlarged posteriorly. Small yolked ova developing in ovary	Immature
	U = 3	Uterus uniformly enlarged tubular structure. Yolked ova developing in ovary	Mature
	U = 4	Uterus enlarged with in utero eggs or embryos microscopically visible - pregnant	Mature
	U = 5	Uterus enlarged, flaccid and distended tubular structure - postpartum	Mature
Male clasper	C = 1	Pliable with no calcification	Immature
	C = 2	Partly calcified	Immature
	C = 3	Rigid and fully calcified	Mature

3.1.3. Confirming species composition through analysis of mitochondrial COI gene

The CTAB DNA extraction protocol (modified from Adamkeicz and Harasewych, 1996 by Carolyn Smith-Keune) was performed for all samples to examine the COI (maternal) gene. 700 ml CTAB buffer and 10 ml Proteinase K enzyme was added to an Eppendorf tube. An approximately 100 mg

piece of tissue was added to each tube and cut into fine pieces. Tubes were then placed in a vortex for up to 10 seconds, and then incubated at 65°C - 75°C for 1-2 hours until tissue was digested with minimal visible particles. 700 ml Chloroform-isoamyl was added to each tube (with digested tissue) and then centrifuged at 16,000 g for 10 minutes. After the centrifuge was complete, the supernatant was pipetted off with care not to break the layer of division, and the supernatant was added to a new set of tubes with 600 ml Chloroform isoamyl. The new tubes with 600 ml Chloroform isoamyl and the supernatant were shaken and centrifuged at 16,000 g for 10 minutes.

After centrifuging, the supernatant was again pipetted off with care not to break the layer of division and added to a new set of tubes with 600 ml of cold (-20°C) Isopropanol. The new tubes with Isopropanol and the supernatant were placed in the freezer (-20°C) for one hour, and then centrifuged at 4°C at 16,000 g for 30 minutes. After the centrifuge, each tube was inspected to check that a pellet had formed. If the pellet was not visible the tube was placed back in the centrifuge for another 10 minutes under the same conditions. All the supernatant was then poured/pipetted off being careful not to lose the pellet and 1 ml of 70% ethanol was added to each tube. The tubes were centrifuged at 4°C for another 10 minutes. The ethanol was then poured/pipetted off being careful not to lose the pellet, and the tubes were air dried for 10-20 minutes until all traces of ethanol had gone. 30 ml of 1 x TE was added, and the samples were run through gel electrophoresis for 40 minutes at 90 voltage. 1 ml of each sample was also checked through a nanodrop spectrophotometer to assess the purity of DNA.

Samples which had a poor purity reading on the nanodrop spectrophotometer were not sent for PCR and the CTAB method was performed again for these samples. DNA barcoding was performed using Fish F1 primer (only Forward reactions as Reverse reactions yielded poor results for the first few samples). F1 sequences were trimmed in Genious Prime and blasted against the GenBank COI database. The 'grade' of match to online accession numbers was used to determine the species of shark, while ray accession numbers were searched in Borsa et al, (2016) as they have not yet been updated in Genbank.

3.2. Results

3.2.1. Mitochondrial DNA analysis for blackspot sharks

Mitochondrial DNA analysis revealed 98 sharks to be the blackspot shark while results for five sharks were inconclusive. However, as they originated from the same catch location and were morphologically identical to the other 98 specimens, they were considered as blackspot sharks for further analysis. Mitochondrial DNA analysis revealed no Indonesian whaler sharks in the sample, possibly because they are not found in the area that the blackspot sharks were caught from (Figure 4.1).

3.2.2. Mitochondrial DNA analysis for bluespotted maskrays (and further investigation through nuclear DNA analysis)

Mitochondrial DNA analysis (whereby accession numbers were matched to Borsa et al, 2016) revealed 146 of the rays to be Oriental bluespotted maskrays (*Neotrygon orientalis*), 69 to be mahogany maskrays (*Neotrygon varidens*), and 36 yielded no result. While for certain individuals the mitochondrial DNA and the morphology aligned (per species descriptions in Last et al. 2016; Figure 3.4), discrepancies for other individuals were observed (Figure 3.5).

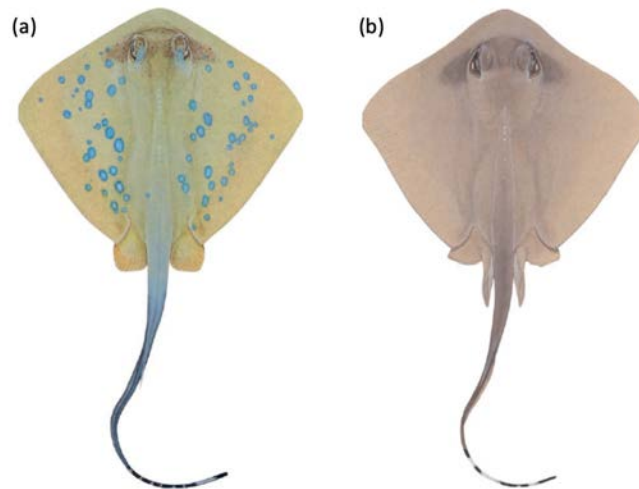


Fig 3.4. Illustrations from Last et al. (2016) 'Rays of the World' of (a) the oriental bluespotted maskray (*Neotrygon orientalis*) with morphological features including an upper surface that is yellowish brown with multiple medium-sized bluish spots and a short, broad snout; and (b) the mahogany maskray (*Neotrygon varidens*) with morphological features including an upper surface that is usually brown to reddish brown and has rarely more than three blue spots, and a short snout that is more angular.

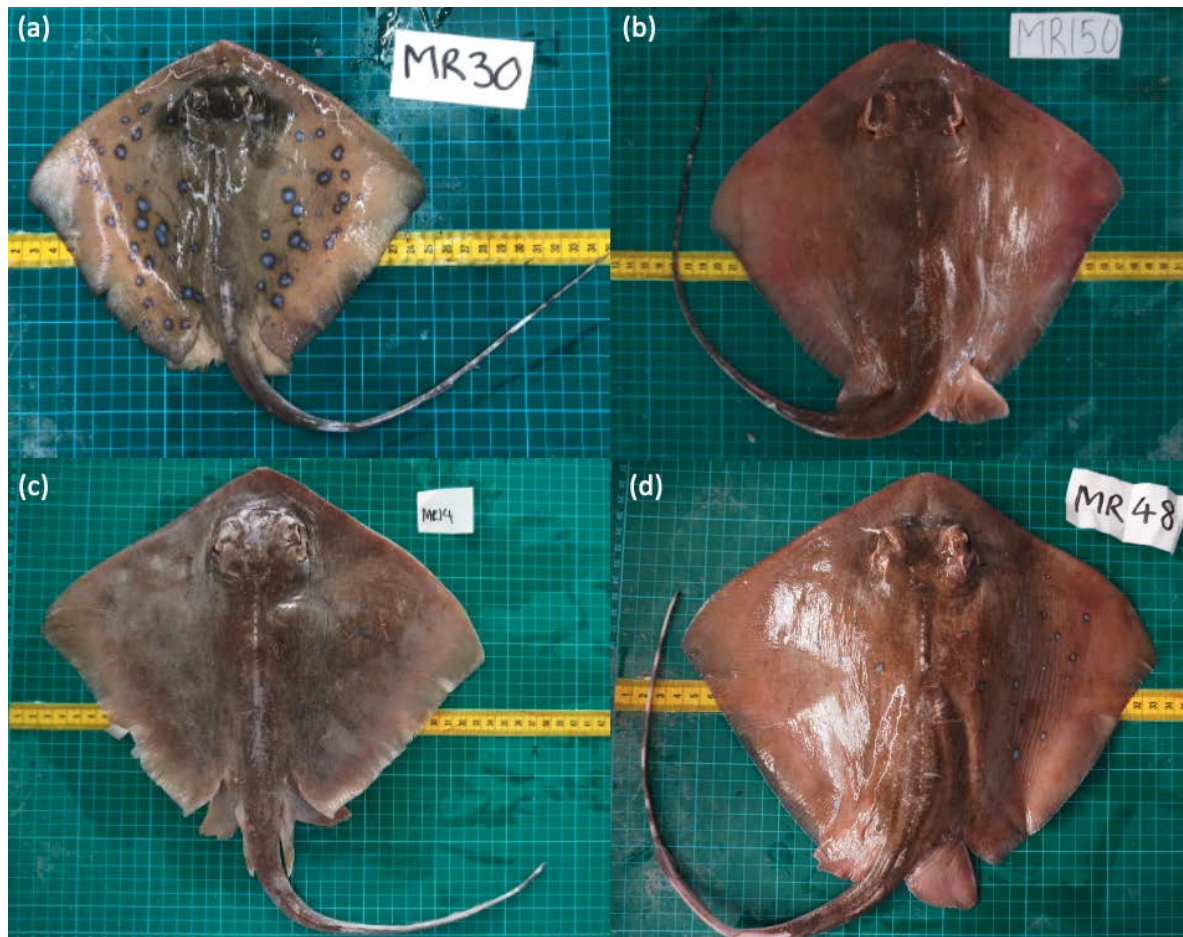


Fig 3.5. Alignment and discrepancies between mitochondrial DNA (matched to accession numbers in Borsa et al. 2016) and morphological descriptions (per Last et al. 2016) of bluespotted maskrays: (a) mitochondrial DNA and morphology align to identify the individual as the oriental bluespotted maskray (*Neotrygon orientalis*); (b) mitochondrial DNA and morphology align to identify the individual as the mahogany maskray (*Neotrygon varidens*); (c) mitochondrial DNA identifies the animal as the oriental bluespotted maskray (*Neotrygon orientalis*) but morphologically the animal shows similarities to the mahogany maskray (*Neotrygon varidens*) including a more angular snout and few blue spots; and (d) mitochondrial DNA identifies the animal as the mahogany maskray (*Neotrygon varidens*), but morphologically the animal shows similarities to the oriental bluespotted maskray (*Neotrygon orientalis*) including multiple blue spots.

Because the morphology of the bluespotted maskrays did not always align with the mitochondrial DNA results, further analysis to understand population genetics and explore the possibility of hybridization or introgression was performed. Due to cost constraints, a subset of tissue samples (92 of the total 251 bluespotted maskray samples) were sent to Diversity Arrays Technology Pty Ltd (DART; Canberra, ACT, Australia) for DNA extraction and SNP genotyping (i.e. analysis of nuclear DNA) using DArTSeq™ methodology. Of the 92 samples sent for analysis, 55 had matched to the Mahogany maskray (per the mitochondrial gene), and 37 had matched to the Oriental bluespotted maskray (per the mitochondrial gene), but for many samples the mitochondrial DNA result and the morphology did not align. The data set received from Diversity Arrays Technology Pty Ltd was then imported to RStudio (R Core Team 2022) using the dartR package (version 2.9.7; <https://cran.r-project.org/web/packages/dartR>; Gruber et al, 2018) and the following filtering was performed: (1) remove monomorphic loci; (2) retain loci with repeatability greater than 1; (3) remove loci with a read depth of <10 and >50; (4) retain one SNP per 69 bp fragment (selected with the 'best' option using `gl.filter.secondaries`); (5) remove loci with a call rate of <90%; and (6) retain loci with a minor allele frequency of >5%. This reduced the SNPs from 53,981 to 3,796. Principal component analysis (PCA) was performed in dartR to visualise the 92 samples in relation to each other (Figure 3.6).

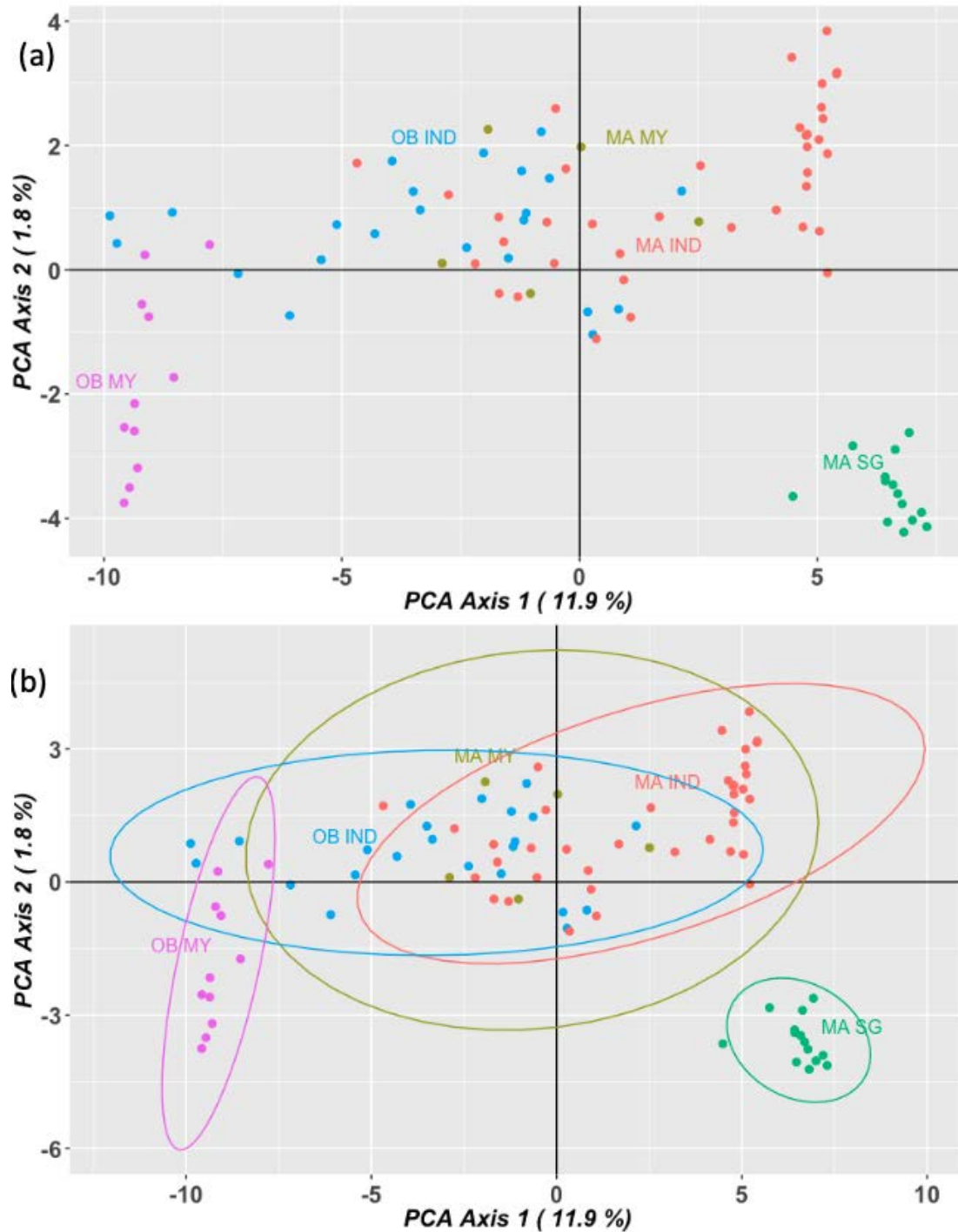


Fig 3.6. Principal Component Analysis (PCA) plot of nuclear DNA results for 92 bluespotted maskrays (a) without ellipses and (b) with ellipses. Individuals are labelled by species per the mitochondrial DNA result (OB = oriental bluespotted maskray, MA = mahogany maskray) and source population (IND = Indonesia, MY = Malaysia, SG = Singapore).

The PCA plots (Figure 3.6) show that oriental bluespotted maskrays and mahogany maskrays (per the mitochondrial DNA result) have generally separated to either side of the plots, which is expected when mapping two different species. However, while a subset of individuals (mahogany maskrays from Singapore, some mahogany maskrays from Indonesia, and oriental bluespotted maskrays from Malaysia (Figure 3.6)) form distinct clusters at either end of the plots, many individuals fall in-between these distinct clusters, which may demonstrate shared genes and thus hybridization or introgression between the two species. The low percentage on the x-axis (11.9%), suggests low genetic diversity between individuals in general.

When comparing the general morphology of animals with results from the nuclear analysis (ie. position on the PCA plot), there are noticeable differences (Figure 3.7). Oriental bluespotted maskrays from Malaysia (cluster (a); Figure 3.7) align with morphological descriptions for the species; some mahogany maskrays from Indonesia (cluster (c); Figure 3.7) align with morphological descriptions for the species; but the Oriental bluespotted maskrays and mahogany maskrays which form the middle cluster (cluster (b); Figure 3.7) often did not conform to morphological descriptions, and are the individuals suspected to represent introgression, hybridization, or undescribed animals. Additionally, the mahogany maskrays from Singapore (cluster (d); Figure 3.7) had unique thorn patterns around the upper surface of the disc (Figure 3.8), which is not included in morphological descriptions for this species (Last et al. 2016). This, combined with their isolated position on the PCA plot (Figure 3.6b) may suggest that this population is genetically isolated or represents a different species.

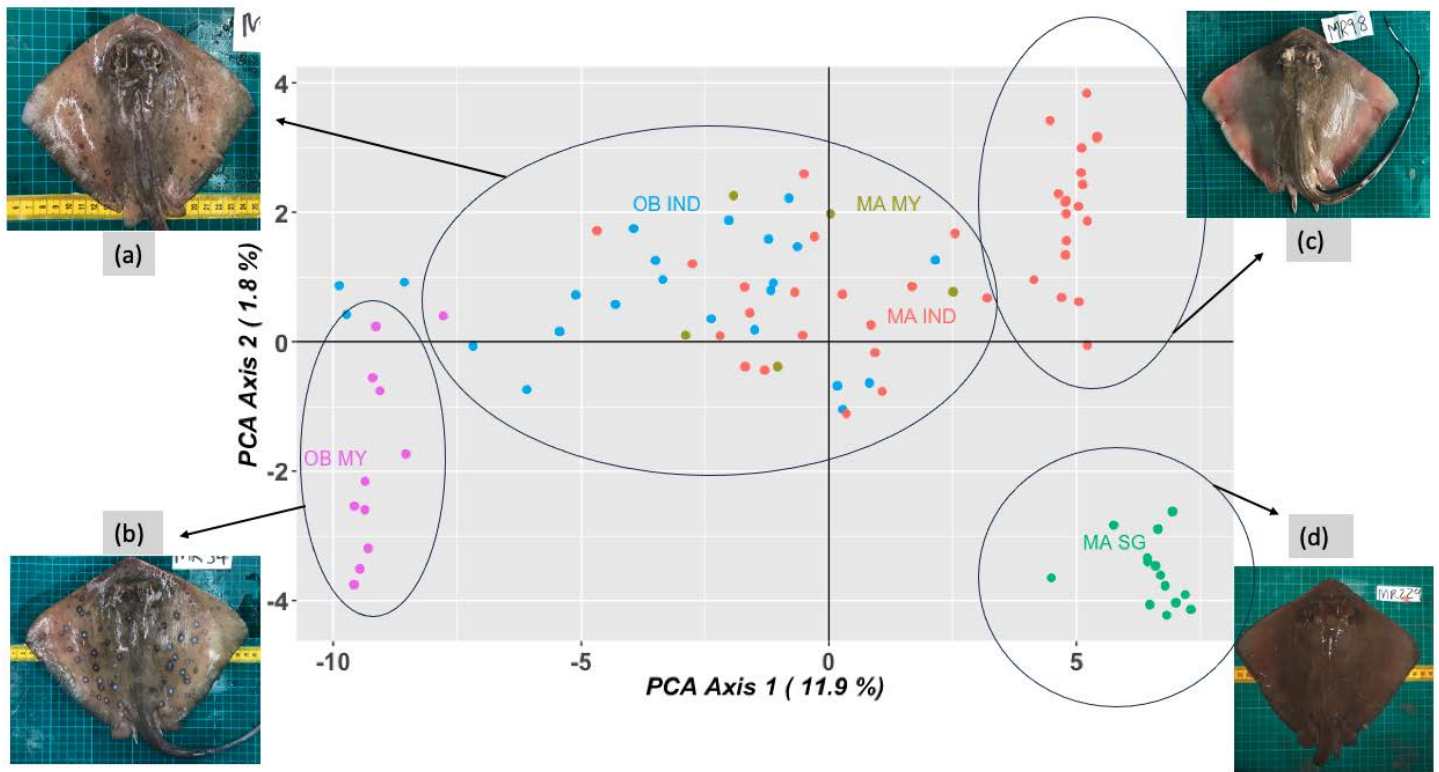
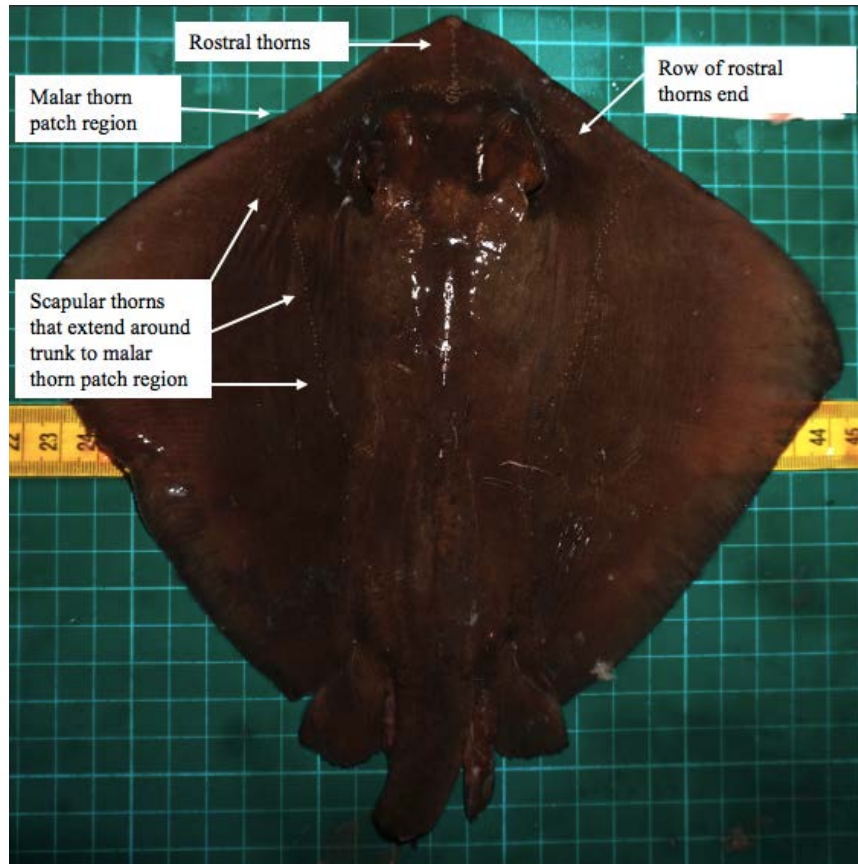


Fig 3.7. PCA plot mapping individuals by species per the mitochondrial DNA results (OB = oriental bluespotted maskray, MA = mahogany maskray) and source population (IND = Indonesia, MY = Malaysia, SG = Singapore). The PCA plot shows distinct clusters: (a) mitochondrial DNA, morphology and position of nuclear DNA on PCA plot suggests this cluster may represent hybridization, introgression or another species of bluespotted maskray; (b) mitochondrial DNA, morphology and position of nuclear DNA on PCA plot generally support the individuals being oriental bluespotted maskrays (*Neotrygon orientalis*) (c) mitochondrial DNA, morphology and position of nuclear DNA on PCA plot generally support the individuals being mahogany maskrays (*Neotrygon varidens*); (d) mitochondrial DNA, morphology and position of nuclear DNA on PCA plot support the individuals being either a genetically isolated population of mahogany maskrays (*Neotrygon varidens*) or a new species, as individuals display unique morphological features including thorns around disc (See Figure 3.8 for clearer view of thorns).



*Fig 3.8. A bluespotted maskray caught at Pulau Ubin Island in Singapore with closely spaced rostral thorns and a row of scapular thorns that extend around the trunk. Mitochondrial DNA matched to Borsa et al. 2016 analysis identified the individual as a mahogany maskray (*Neotrygon varidens*) but descriptions of the species in last et al. 2016 do not mention rostral, malar and scapular thorns as part of the species' morphology. Analysis of nuclear DNA reveals that the individual belongs to a group of bluespotted maskrays that are genetically distinct from others caught in the region (Figure 3.6; 3.7).*

3.2.3. Final sample composition - sharks

The 103 blackspot sharks originated from Indonesia (n=101) and Singapore (n=02), and consisted of 41 females and 62 males, of which 62 animals were immature, 41 were mature, and nine were gravid (Table 3.2). The sample size of sharks was skewed toward larger individuals (Figure 3.3). The supplier did not always have specimens available, so there were some months where animals were not supplied (Figure 3.10).

Table 3.2. Species composition, source country, sex, reproductive status and sizes (stretched total length, STL) of blackspot shark (*Carcharhinus sealei*) sourced from a private seafood supplier, as well as fishery ports in Singapore, between 2019 and 2021.

Source	Total	Sex		Reproductive status			Size TL (sharks) in mm	
		Male	Female	Immature	Mature	Pregnant	Range	Mean
Blackspot shark								
Indonesia	101	62	39	60	41	9	359-849	678
Singapore	2	0	2	2	0	0		
TOTAL	103							

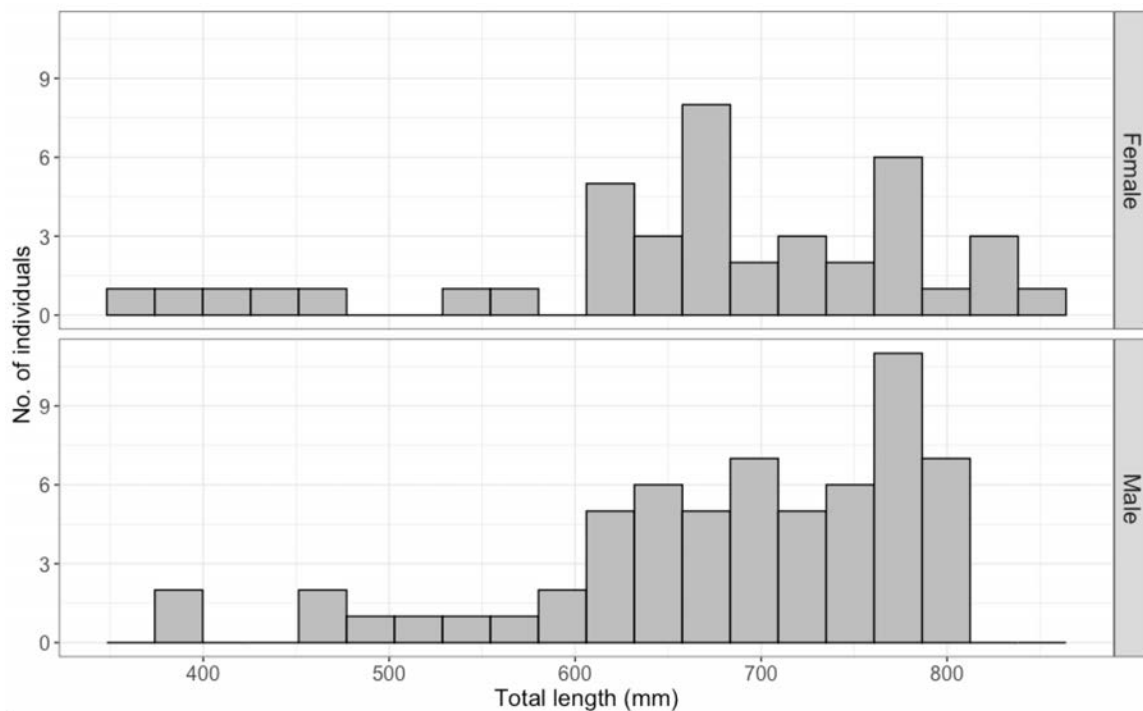


Fig 3.9. Size-frequency distribution of male (n=62) and female (n=41) blackspot sharks (*Carcharhinus sealei*) caught from fisheries in Indonesia (n=101) and Singapore (n=2) between 2019 and 2021. The sample was dominated by larger individuals (>600 mm STL) with a minimum size of 359 mm STL (from a female), a maximum size of 849 mm STL (from a female), and mean size of 678 mm STL.

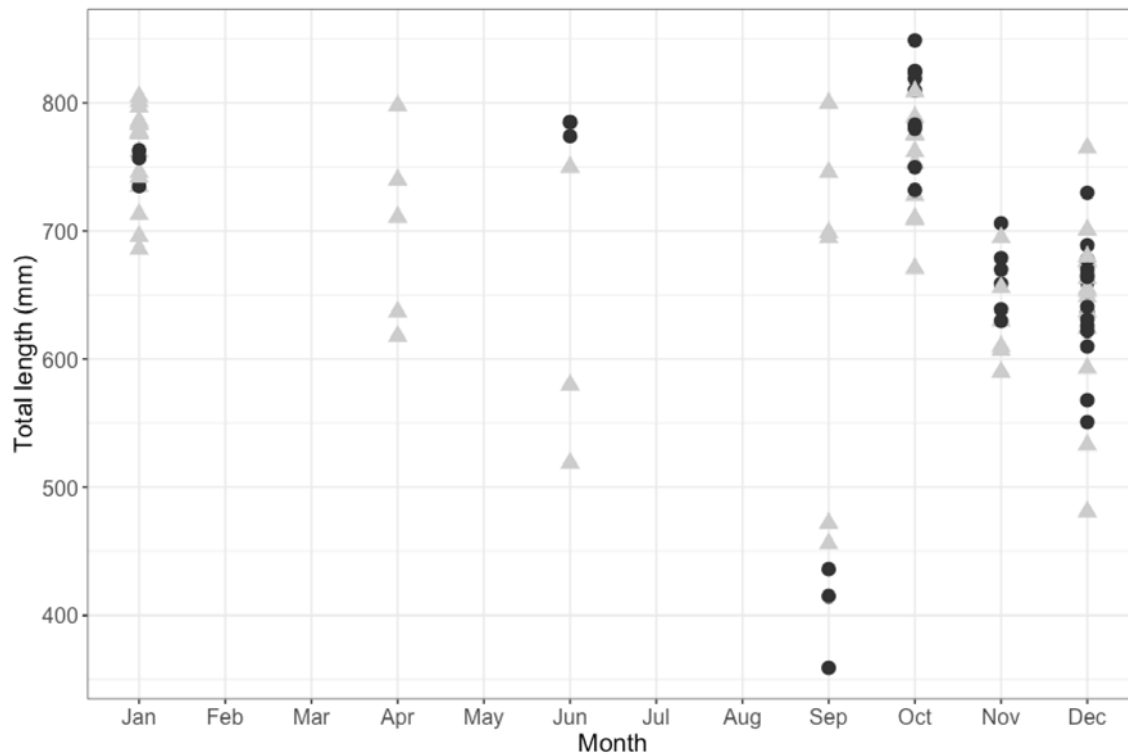


Fig 3.10. Stretched Total length of female (●, $n=41$) and male (▲, $n=62$) blackspot sharks (*Carcharhinus sealei*) supplied by month. Specimens were collected between 2019 and 2021 but were not available in the months of February, March, May, July and August due to a lack of availability from the private seafood supplier and at fishery ports. Figure excludes four animals collected in 2019 for which the month was not noted.

3.2.4. Final sample composition - bluespotted maskrays

Determining the final species composition of bluespotted maskrays presents a challenge due to the observed mismatch between the mitochondrial DNA result, morphology, and nuclear DNA analysis (Figure 3.5; Figure 3.6). For the sake of analysis, a set of criteria were used to categorise individuals as either mahogany maskrays, oriental bluespotted maskrays, or ‘undetermined’, as outlined in Table 3.3. If individuals matched a particular species in 3-4 of the four categories they were assigned that species. If an individual matched a particular species in only 1-2 of the four criteria, or particular data raised uncertainty, they remained ‘unidentified’, potentially representing introgression, hybridisation, or undescribed animals. Not all individuals had data for all four categories (e.g. not all samples were sent for nuclear DNA analysis, and some mitochondrial DNA analysis came back

inconclusive). These were assessed case by case using available data for each animal. For example, if the animal did not have mitochondrial or nuclear DNA results but the morphology (both spot pattern and snout) clearly suggested a particular species, then it was assigned that species.

Table 3.3. Criteria used to determine species composition of 251 sampled bluespotted maskrays (Neotrygon spp.) using the mitochondrial DNA, morphology, and nuclear DNA. Not all individuals had available data for all four categories (see row 3 and 4).

	Mitochondrial DNA	Morphology (spot pattern)	Morphology (snout shape)	Nuclear DNA
Description	Results from mitochondrial DNA analysis matched to accession numbers in Borsa et al. 2016.	Oriental bluespotted maskrays have multiple medium-sized blue spots, mahogany maskrays have zero or rarely more than three (Last et al. 2016)	Oriental bluespotted maskrays have short, broad snouts, mahogany maskrays have angular snouts (Last et al. 2016)	Results from nuclear DNA analysis mapped on PCA plot to understand population genetics
Sample size (out of possible 251)	215	249	250	92
Reason for sample size	36 yielded no DNA result	Two individuals' backs had sustained damage	One individual had damage to the snout	Cost constraints meant that all samples could not be analysed

Using the criteria (Table 3.3), the final species composition for analysis moving forward included 104 oriental bluespotted maskrays, 45 mahogany maskrays, and 102 individuals classified here as an 'undetermined' species of bluespotted maskray. This final species composition by country, sex, reproductive status and sizes are shown in Table 3.4.

Table 3.4. Species composition, source country, sex, reproductive status and sizes (disc width, DW) of bluespotted maskray (*Neotrygon orientalis*, *Neotrygon varidens*, and undetermined species of bluespotted maskrays) sourced from a private seafood supplier, as well as fishery ports in Singapore, between 2019 and 2021.

Source	Total	Sex		Reproductive status			Size DW (disc width) in mm	
		Male	Female	Immature	Mature	Pregnant	Range	Mean
<i>Oriental bluespotted maskray</i>								
Indonesia	91	44	47	40	51	10	181-366	268
Malaysia	13	5	8	8	4	4		
Total	104							
<i>Mahogany maskray</i>								
Indonesia	19	11	8	5	14	1	171-323	236
Malaysia	1	1	8	5	1	1		
Singapore	25	16	9	13	12	2		
Total	45							
<i>Undetermined species of bluespotted maskray</i>								
Indonesia	96	55	41	42	52	10	195-370	278
Malaysia	6	0	6	0	6	1		
TOTAL	102							

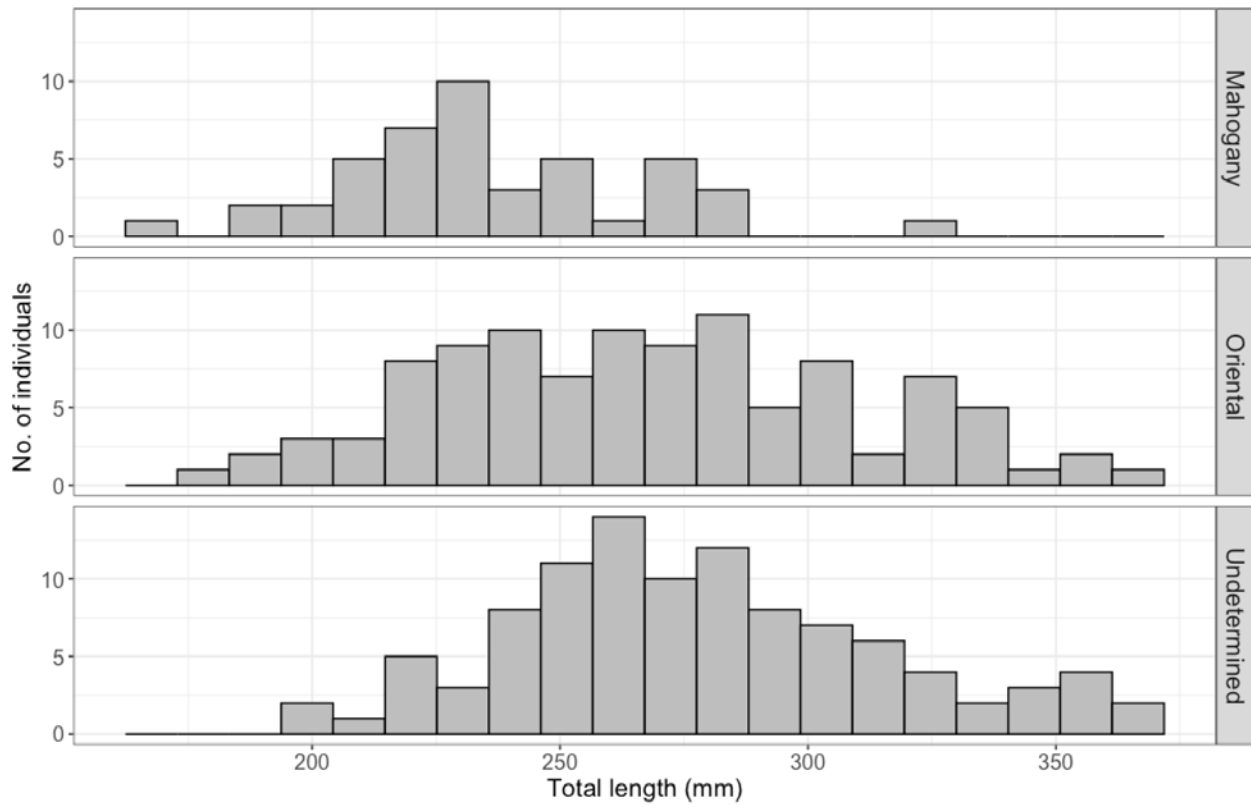


Fig 3.11. Size-frequency distribution of bluespotted maskrays. Oriental bluespotted maskray ($n=104$), mahogany maskray ($n=45$), and bluespotted maskrays where the species was ‘undetermined’ ($n=102$) caught from fisheries in Indonesia ($n=206$), Malaysia ($n=20$) and Singapore ($n=25$) between 2019 and 2021. The samples for oriental bluespotted maskrays and ‘undetermined’ species was dominated by mid-sized individuals, while the sample of mahogany maskrays was skewed toward smaller individuals, which may be because this species in general reaches a smaller size ($\sim 33\text{cm DW}$) than the oriental bluespotted maskray ($\sim 38\text{cm DW}$).

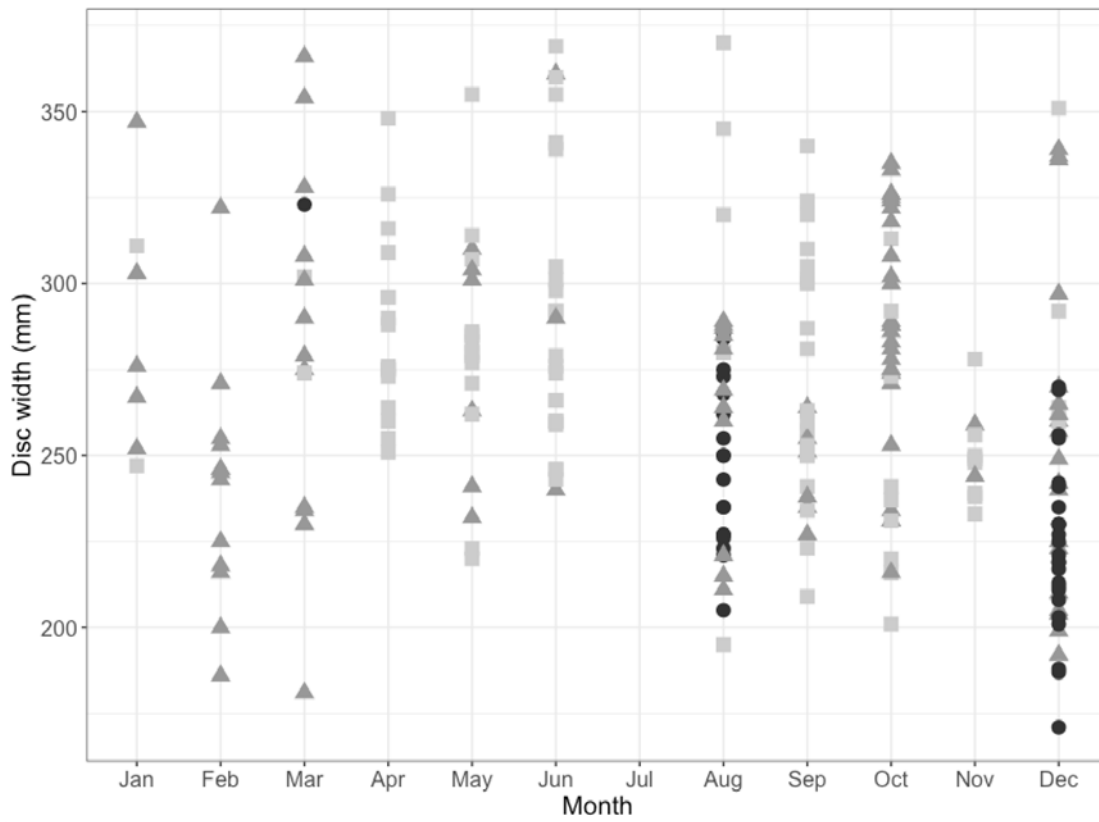


Fig 3.12. Disc width of mahogany maskray *Neotrygon varidens* (●, $n=45$), oriental bluespotted maskray *Neotrygon orientalis* (▲, $n=104$) and 'undetermined' species of maskray *Neotrygon spp.* (■, $n=102$) supplied by month, showing a good supply of bluespotted maskrays by month and size, except for mahogany maskrays which were only supplied in March, August and December. Specimens were collected between 2019 and 2021. There was a pause in sampling during July.

3.3. Concluding remarks

The analysis for the remaining thesis will be based off of the species compositions highlighted in this chapter, which include 102 blackspot sharks and 251 bluespotted maskrays.

A previous study from Brunei found that blackspot sharks and Indonesian whaler sharks occur sympatrically to each other in coastal waters, as determined through mitochondrial analysis and morphology of specimens caught by fisheries (Azri et al. 2020), but interestingly, the entire sample from the fishery location in this study (Figure 4.1), consist of only blackspot sharks. Indonesian

whaler sharks are hypothesised to have smaller, restricted ranges than blackspot sharks (Azri et al. 2020), and are reported to range to greater depths than blackspot sharks (40m vs 100m; Ebert et al., 2013; White, 2012), which may account for their absence from the fishery site in this study (Figure 4.1).

Because of the uncertainty surrounding the species composition of bluespotted maskrays, analysis throughout the thesis will examine data through three methods: (1) by bluespotted maskrays as a whole (n=251), (2) by the criteria outlined in table 3.3 (104 oriental bluespotted maskray, 45 mahogany maskray, and 102 ‘undetermined’ species of bluespotted maskray), referred to as the ‘Criteria’ method moving forward, and (3) by the mitochondrial gene (146 oriental bluespotted maskrays and 69 mahogany maskrays), referred to as ‘mitochondrial’ method moving forward, to assess if there are any differences in results. The uncertainty surrounding bluespotted maskray species, as highlighted in this chapter, raises the possibility that existing species descriptions are inaccurate (Borsa et al., 2016; Last et al., 2016), that there are undescribed species in the sample, or that there is introgression or hybridisation between species, as has been documented in other species of sharks and rays (Morgan et al. 2011; Nevatte et al., 2023; Pazmino et al., 2019). Further genetic and taxonomic investigation (outside of the scope of this thesis) is warranted to confirm which of these may be the case.

Chapter 4:

Fishery description and supplier interview

4.1. Introduction

There is growing recognition of the value of fisher knowledge to understand the biology and ecology of species, as well as the dynamics of a fishery and the options for management. The development of management measures without buy-in and support from stakeholders threatens their long-term success (Boonstra et al, 2017; Booth et al, 2019; MacKeracher, 2021). For fishers, feelings of mistrust, confusion and a lack of meaningful engagement can influence their compliance with policies and conservation efforts (Collins et al, 2020). The perspective of those involved in the fishing industry (fishers, traders, etc) is therefore vital, as ultimately they are the ones whom we expect to see behaviour change from (Booth et al, 2019; Dineshbabu et al, 2022). Such stakeholders also possess local ecological knowledge (LEK) which can provide insights into a species and their threats (Acebes et al, 2016).

The original research plan was to interview a wide group of fishers, fish traders and vendors in both Singapore as well as in Sabah, Malaysia, to record their perceptions of the fishery, trade and markets for these species, and any LEK. Unfortunately, due to COVID-19, travel into Malaysia was not possible, and Singapore's fishery ports were also closed to the public. However, we were able to engage with a private seafood supplier who was able to deliver sharks and rays to the university laboratory. This seafood supplier was interviewed, as a case study to understand more about the animals themselves (which were dissected for life-history and diet analysis; Chapter 5, 6 and 7), as well as trade and markets, and his perspective on what would work for conservation and management.

4.2. Methods

4.2.1. Interview with supplier

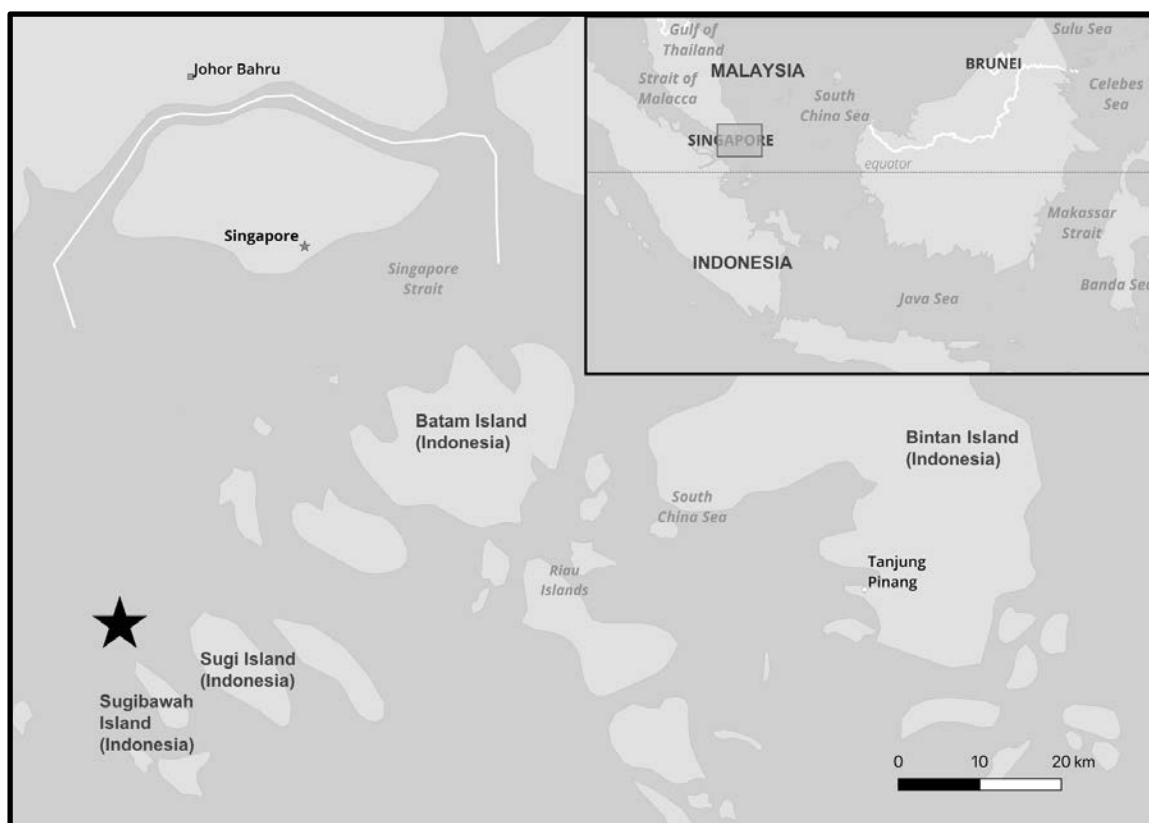
To learn more about the specimens used in this study, a private seafood supplier of blackspot sharks from Indonesia (n=92) and bluespotted maskrays from Indonesia, Singapore and Malaysia (n=225), was interviewed through a semi-structured interview consisting of 22 questions (Appendix S4 and S5). This supplier not only trades sharks and rays but other seafood in general. The interview was conducted in English, following human ethics guidelines and with signed consent, and no remuneration was given. Questions covered (1) the fishery the sharks and rays were sourced from, (2) the species themselves and trends observed, (3) the supply chain, (4) market use, and (5) solutions for their management. Some questions provided a range of answers for consideration, including the latter part of the interview ('solutions for their management'), however, responses did not have to be restricted to options provided and the supplier was encouraged to elaborate where necessary.

4.3. Results

4.3.1. The fishery and supply chain of blackspot sharks

The private seafood supplier of the 92 sharks sourced from Indonesia provided their general catch location (Figure 4.1). He reported that animals were caught by small 'sampan' fishing boats that generally stay out for no longer than 12 hours and use handlines. Fish (incl. snappers, groupers, catfish, jacks and trevallies), are the target, with blackspot sharks caught incidentally but retained. As the handlines are immediately retrieved once something is hooked, the sharks tend to still be alive when hauled in. Squid is reportedly used as bait in these handline fisheries, also confirmed by the presence of a sectioned (e.g. straight edged) piece of squid inside one of the sharks' stomachs. Generally, every species caught by these boats has commercial value, and if the fishermen can sell it they will land it ashore, unless it is not worth the ice and storage (which is reportedly rare). Within the region (Figure 4.1), blackspot sharks are also caught on longlines, and some of the sharks from this study may have come from these fisheries although the trader views that most come from handline fisheries. Longlines can stretch between 100 m

and 1 km, set at 10 to 30 m deep, and it is the bottom-set longlines (called ‘rawai’) that tend to catch blackspot sharks. As the longlines are left at sea for longer periods, the sharks are often deceased when hauled in, and the stress of pulling the longline up from depth can also cause mortality. Longline fisheries use fish of lower market value as bait such as sardines and eel flesh. Eel flesh has tough skin and stays on the hook even if smaller fish bite at it. Any blackspot sharks caught are landed on nearby Indonesian islands. The sharks are eaten locally in Indonesia for their meat but if there is a demand in Singapore they will be imported to the country via the two fishery ports (Jurong Fishery Port and Senoko Fishery Port).



*Fig 4.1. Catch location of 92 blackspot sharks (*Carcharhinus sealei*) sourced from a private seafood supplier who is based in Singapore. The sharks were caught in Indonesia (in the Riau Islands; an island chain close to Singapore) from a handline fishery predominantly targeting other species of fish, located roughly where the marker is (★)*

4.3.2. The Singaporean market for blackspot sharks over time

Within Singapore, blackspot sharks are usually sold whole in wet markets (primarily Tekka market), with Singapore's Indian population the main buyers who tend to cook it in curries at home. Historically, blackspot sharks have always been utilised for their meat, which is considered superior to other species (such as bull shark and blacktip sharks) as it is softer, but overall, the supplier reports that shark meat has never been a particularly large or prominent part of the Singaporean diet. The supplier states that supply and demand of blackspot sharks has reduced over the years and is attributed to (a) lower population numbers and fewer caught, and (b) increased availability of substitutes, including imported frozen blue shark meat which is more convenient as whole, fresh sharks (like blackspot sharks from Indonesia) have to be processed. Blackspot sharks contribute less than 5% of the supplier's business and he reports that 'not catching and selling it would not impact me', and that the market is too small to warrant it being directly 'replaced' by another species if it could not be caught or sold.

4.3.3. Perspectives on blackport shark population trends and management

The supplier has observed a decline in the availability of blackspot sharks over the years, estimating this decline at 50-70% over his 45 years in the industry. Blackspot sharks could reportedly be caught from the shore before, but now this is not the case. The species tend to be caught during monsoon seasons (around November, December, as well as in June), and he suspects this is when they aggregate for breeding. Outside of monsoon seasons the catch is more sporadic. The supplier thinks that blackspot sharks would benefit from improved management. While the release of blackspot sharks caught could be an option (as they have relatively low market value), the supplier highlights some challenges: fishermen would still pull the shark on board to remove their hook, and this rough handling on-board could result in high post-release mortality. Cutting the line while the shark is still in the water would reduce this stress, however fishermen may be unwilling to do this as they will lose the hook. If the sharks were released in such a manner, he recommends that research assess post-release survival rates. For the longline fisheries, most sharks are dead when hauled in, so release of individuals is not an option under current fishing practices. However, as long as an animal has even a small market value, fishermen would want to retain and sell them. The supplier thinks that more Marine Protected

Areas with proper enforcement would be beneficial. While requiem sharks (which includes blackspot sharks) have been added to Appendix II of CITES (as of Nov 2022), thus regulating their trade, the supplier states that enforcement is an issue, and that regulation of trade does not necessarily stop animals being caught in the first place, particularly for species primarily caught incidentally like the blackspot shark.

4.3.4 The fishery and supply chain of bluespotted maskrays

The private seafood supplier, and merchant from Jurong fishery port in Singapore, who supplied the 251 bluespotted maskrays, provided the general catch location for some of these animals (Figure 4.2). The private supplier of 225 of these bluespotted maskrays was interviewed. While two animals from Melaka in Malaysia were caught by longline (confirmed by the presence of hooks in their stomach), the rest of the bluespotted maskrays were caught in fishing traps. Such fishing traps are set in shallow waters at 5-15 metres deep and are generally just over 1 metre in width. The bluespotted maskrays are caught in traps set on mudflats while the similar-looking bluespotted lagoon ray (*Taeniura lymma*) is reportedly caught in traps set on reefs. Small fishing boats usually deploy 30-50 traps at a time: traps are often tied together (e.g. five sets of 10 tied together), and with four to five metres distance between each trap. Bigger boats can deploy 300-400 traps at a time. Traps are often left at sea for between 10 days and two weeks, with body condition of caught animals deteriorating the longer they are deployed. Over the monsoon period, traps can be left at sea for longer. Snappers and groupers are a target species for these traps, and bluespotted maskrays are incidental catches but are retained and utilised. Bluespotted maskrays are usually dead when traps are retrieved but sometimes they are still alive (they can reportedly survive for seven to 10 days without food). Longlines that catch bluespotted maskrays (e.g. the two animals caught by longline in this study) are set slightly farther offshore than the fishing traps, with snappers and groupers also the primary target species. The supplier reported that he was unaware of any practice of releasing bluespotted maskrays, across any of these fisheries.

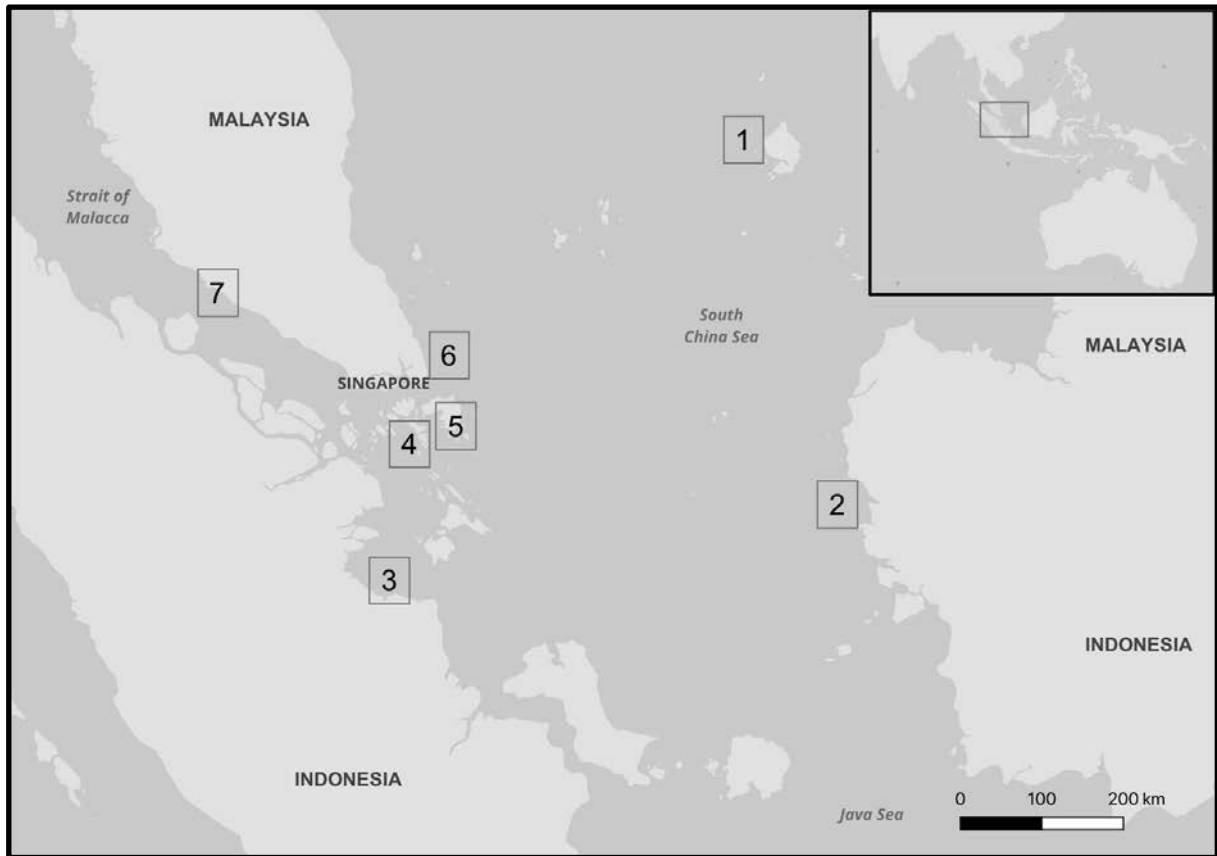


Fig 4.2. Cited catch location of bluespotted maskrays (Neotrygon spp.) sourced from a private seafood supplier who is based in Singapore. Numbers on map indicate cited origins, with (1) Natuna islands, Indonesia (n=16), (2) Kalimantan, Indonesia (n=20), (3), Jambi, Indonesia (n=10), (4) Bintan Island, Indonesia (n=26), (5) Batam Island, Indonesia (n=19), (6) Pulau Ubin, Singapore (n=25), (7) Near Melaka, Malaysia (n=4). Other cited locations which were not specific enough to include on the map include 'Peninsular Malaysia' (n=12), 'Northeastern Malaysia' (n=3), 'between Sumatra and Singapore' (n=10), 'North Indonesian waters' (n=7), 'South Central Indonesia' (n=20), 'Central Indonesia' (n=16), and 'unknown' (n=63).

4.3.5. The Singapore market for bluespotted maskrays over time

Around 30 years ago, stingrays were not a big part of the Singaporean diet and fishers would discard them at the point of catch. However, in the years since, there has been significant growth

in demand for these species. The supplier notes that the main demand for stingray in Asia comes from Singapore and Malaysia.

Bluespotted maskrays are typically an incidental catch and in Singapore, they are sold for relatively low prices from wet markets and fishery ports for their meat, with Singapore's Malay and Indian communities the main buyers who use it in curries. 'Economy rice' stalls (stalls where consumers can select a variety of vegetable and meat dishes with rice for low prices) also sell their meat. Bluespotted maskrays have never been favoured for the more expensive 'BBQ stingray' *'ikan pari bakar'* delicacy sold in Singapore. Rather, the species whitespotted whiplay (*Maculabatis gerrardi*) and sharpnose whiplay (*M. macrura*) are favoured for this dish and are targeted by fisheries. However, while demand remains high, the availability of the whitespotted whiplay and sharpnose whiplay appears to have significantly declined and their prices are now high. Stalls in Singapore that sell 'BBQ stingray' are now switching to the more affordable bluespotted maskrays and reporting that customers find it acceptable. As a result, the supplier reports that the value of bluespotted maskrays is now increasing and they are starting to be stored in cold-rooms—an indicator that they are beginning to be worth the cost of storage and there may soon be a surge in catch and imports.

4.3.6. Perspectives on bluespotted maskray population trends and management

When the supplier first started in the fishing industry, an operation in Indonesia (e.g. 3-4 medium sized boats of Indonesian standard) could catch 'one million kilograms' of stingrays per month, but now they are down to 'under 10 tonnes'. The supplier notes that in the Southeast Asia region, he believes stingrays are more threatened than sharks yet sharks still get more conservation attention.

Similar to his observations of the blackspot shark, the supplier has observed a decline in bluespotted maskray availability over the years, and estimates the supply has declined by about 50% over his 45 years in the industry. He has not noticed a particular season when the species is most abundant but he believes the species breeds year-round. The supplier thinks that bluespotted maskrays would benefit from improved management. Because bluespotted

maskrays under 1 kg in weight are preferred (as the skin gets tougher with increased size), releasing larger animals could be a solution. However, he doesn't believe fishermen would want to comply as even if they can get a little bit of money, they would want to retain them. The supplier also commented that more Marine Protected Areas (MPAs) would be beneficial, but enforcement of these areas is poor and fishermen often still fish from these areas. The supplier suggested that because the consumption of stingrays is a niche market in Singapore and Malaysia, a campaign against the consumption (e.g. targeted at consumers) would be easier than trying to improve fishery management. When asked if reduced consumption in Singapore and Malaysia would see the trade diverted elsewhere, he noted that the sambal/BBQ style of cooking stingrays is unique to Singapore and Malaysia, and while iconic in these two countries, it is not as popular elsewhere. Additionally, stingrays accumulate ammonia quickly, so freshness matters, which may prevent the long shipments of them from Southeast Asia to elsewhere. The supplier added that if there is no demand for stingray then fishermen would find something else to catch, which is not ideal as all the other species of fish in Southeast Asia are also in a 'terrible state'. He noted that cell-grown or other substitutes could help, not just for sharks and rays but all fish, and it is just about consumers getting used to (it becoming 'habit'), these alternatives.

4.4. Concluding remarks

Insights from stakeholders that work in the fishery industry are crucial for understanding species, their fisheries, and effective management (Booth et al, 2019). The interviews from this study provide valuable insights to the fisheries that the specimens themselves originate from. Additionally, they reveal perceived population trends, markets and the supply chain of the animals over the years. However, as only one subject (n=1) was interviewed in this study, moving forward a larger sample size of interview subjects is needed to provide more diverse insights regarding other fisheries, species' population trends and opinions around their management. Singapore has many stakeholders that work in the fisheries industry—with 128 fish merchants and 1,750 fish retailers, processors and buyers operating out of Jurong Fishery Port and Senoko Fishery Port alone (SFA, 2023). A wider survey which captures the LEK and perspectives of such stakeholders would have provided more holistic insights. Additionally, many of the stakeholders in Singapore are traders, processors or sellers but not fishers.

Interviews with fishers, such as from Indonesia and Malaysia, where Singapore imports sharks and rays from (Clark-Shen et al, 2021)), would provide greater insights to fishery operations, the locations/habitats of species and observed population declines in different areas.

Regardless, the interview with the supplier in this study corroborates reports that these species are threatened and in decline. According to the IUCN, the blackspot shark has experienced a suspected population reduction of 30-49% over the past 24 years (Rigby & Kyne, 2019), and the supplier interviewed in this study estimates a 50-70% over his 45 years in the industry. According to the IUCN, bluespotted maskrays have reported declines in some areas (Sherman et al, 2022a, 2022b), and the supplier interviewed in this study estimates a 50% decline over his 45 years in the industry. Overall, 72.5% of assessed shark and ray species populations in Southeast Asia are in decline, with rays more threatened than sharks (Clark-Shen et al, 2022), and the supplier interviewed in this study emphasises the general decline in populations, particularly for stingrays. Additionally, his perceptions on the supply chain, market, and trade are similar to those reported in other interviews at Singapore's fishery ports between 2017-2020 (Clark-Shen et al, 2021) which lends confidence to his answers.

Southeast Asia faces unique challenges when it comes to fisheries and management (Clark-Shen et al, 2022), and management measures which may be effective elsewhere may not prove successful in this region. For example, the supplier interviewed in this study highlighted that the CITES Appendix II listing may not be effective in reducing mortality of the blackspot shark as they are mostly caught incidentally, and indeed, regional reports highlight that most sharks and rays are caught incidentally in Southeast Asia, particularly in coastal areas (SEAFDEC, 2017). The supplier also mentioned that as long as a species even has 'little' value, fishermen will want to retain and sell them, making at-sea regulations (such as catch-and-release) unlikely to succeed on their own. Locally-appropriate economic incentives to promote management measures, such as financially rewarding fishermen for releasing animals (Booth et al, 2023) may therefore be an appropriate way forward. Future research should seek to understand other fisheries in greater depth – and the perspectives of, and impacts to, more stakeholders (fishers, traders, sellers) – to create inclusive measures that will have high compliance and ultimately, promote species recovery.

Chapter 5:

Diet and ecology of the blackspot shark (*Carcharhinus sealei*) and bluespotted maskrays (*Neotrygon orientalis* and *N. varidens*)

5.1. Introduction

Improved understanding of elasmobranch (sharks, rays and skates) ecology has highlighted their multifaceted roles in ecosystems (Barria et al, 2015; Navia et al, 2016; O'Shea et al, 2013; Roff et al, 2016; Simpfendorfer et al, 2001). Elasmobranchs occupy a range of trophic levels (Barria et al, 2015; Jacobsen & Bennet, 2013), filling the role of apex predators (Bornatowski et al, 2014; Heithaus, 2001) and mesopredators; which connect the top and lower levels of the food chain (Ajemian et al, 2012; O'Shea et al, 2013). In these roles, sharks and rays help to regulate their prey through direct consumption (Ajemian et al, 2012; Heithaus, 2001; Simpfendorfer, 2001), and by transferring energy when they are consumed by other predators such as larger sharks and marine mammals (Navarro et al, 2014; Pyle, 1999; Sherman et al, 2020b). Additionally, the foraging behaviour of stingrays contributes to bioturbation and nutrient dispersal (Crook et al, 2021).

Understanding elasmobranch ecology is crucial for the holistic conservation of species and their ecosystems (Barria et al, 2015; Costa et al, 2015; Lucifora et al, 2009). Insights to whether elasmobranchs have specialised or broad diets highlight their dependence on prey; with specialised feeders at particular risk if their main prey item is lost (Simpfendorfer et al, 2001). Dietary shifts in elasmobranchs are prevalent among ontogenetic stages (Ba et al, 2013; Bornatowski et al, 2014; O'shea et al, 2013; Saidi et al, 2009), seasons and geographic regions (Ba et al, 2013; Saidi et al, 2009), the sexes (Ba et al, 2013; Costa et al, 2015), and between urban and non-urban populations (Rangel et al, 2022), highlighting diverse resource dependencies within a species. For example, crustaceans form an important part of the diet for some younger sharks but this dependency reduces with age (Saidi et al, 2009; Simpfendorfer et al, 2001), and lobster appears a preferred prey

specifically for adult female large-eye stingray (*Dasyatis marianae*), hypothesised to provide for increased energy needs during breeding (Costa et al, 2015). Understanding diet and prey items also provides insights into feeding behaviour, revealing which ecosystems (e.g. pelagic, demersal or benthic) elasmobranchs utilise, and thus which threats they may be exposed to and which areas need conserving (Ba et al, 2013; Saidi et al, 2009; Simpfendorfer et al, 2001). Interactions between elasmobranchs that share habitats can also be identified. For example, of five species of sympatric ray on a coral reef in Western Australia, four species have similar diets and likely reduce competition through spatial partitioning, while a fifth species, consumes more crustaceans than the others, likely reducing competition through dietary partitioning (O'Shea et al, 2013). Conservation and fisheries management planning should account for these dietary needs and ecological interactions, as a loss of high quality prey items can affect survival (Chiaradia et al, 2010), and a loss of predators (such as sharks and rays) can cause trophic cascade and potential mesopredator release (Barria et al, 2015; Sherman et al, 2020b).

While the ecology of the oriental bluespotted maskray (*N. orientalis*), and mahogany maskray (*N. varidens*) has not been assessed, dietary analysis of bluespotted maskray in Australia (*N. kuhli*) revealed that annelids, and brachyurans and other prawns dominate the diets (O'Shea et al, 2013); and dietary analysis of bluespotted maskrays from Java, Indonesia, and waters off New Guinea (*N. caeruleopunctata*; *N. annotata*, and *N. leylandi*) revealed crustaceans to dominate diets (Kleinertz et al, 2002; Wagiyo et al, 2022). The diet of the blackspot shark has not been described since the species' re-description in 2012 (White, 2012). The ecology, habitat-use and behaviour of many elasmobranchs in Southeast Asia is lacking (Clark-Shen et al, 2022), and further insights could improve understanding of their ecological roles and dependencies, thus helping to develop more robust ecosystem management plans (Knip et al, 2012; Salomon-Aguilar et al, 2009).

5.2. Method

5.2.1. Excising and sorting stomach contents

Stomachs from sharks and rays collected from fishery sources between 2019 and 2021 (see Chapter 3) were excised. Stomach content was separated by taxa, and if necessary, washed by submerging

them in a beaker of tap water. Smaller items were examined under a dissecting microscope. Prey was identified to one of four taxonomic categories (species, genus, family, or above). The number of whole animals and fragments were recorded and wet weight taken for each taxonomic group. Digested tissue and fragments which could not be identified to a particular prey type were considered unidentifiable. Mucus (clear or cloudy, sticky fluid), and mucus with contents (brown, bonded clumps often filled with sand and digested matter that could not be separated), and plastic were also recorded, and their wet weight taken. All stomach content was then put in the oven at 60°C until constant weight was attained (Salini et al, 1992) and then dry weight recorded.

5.2.2. DNA testing to confirm species and gut-content

In order to collect more precise data on prey items, samples were taken from a selection of prey items which were not in highly digested states and were therefore more likely to yield results. This resulted in 19 samples, 11 from blackspot sharks, and eight from bluespotted maskrays. The CTAB DNA extraction protocol (modified from Adamkeicz and Harasewych, 1996) was performed and DNA barcoding of the COI gene performed using universal primers Fish F1 and R1 (Ward et al, 2005). Results were Sanger sequenced, trimmed in Genious Prime and blasted against the GenBank COI database.

5.2.3. Analysis of stomach contents

Percent frequency of occurrence (%FO), which is the proportion of individuals containing a prey type, and percent composition by weight (%W) including both the wet and dry weight, were calculated. For bluespotted maskrays, analysis was performed by grouping all animals together, as well as grouping animals by the ‘criteria’ method, and ‘mitochondrial’ method (Chapter 3). Plastics and contents that were suspected to be bait (e.g. straight-edged indicating preparation, attached to hooks) were excluded from %FO and %W analysis (Jabado et al, 2015), and while some studies exclude indigestible parts from such analysis (such as shells, otoliths, and cephalopod beaks) (Bornatowski et al, 2014; Dicken et al, 2017; Potier et al, 2007) as they are not considered nutritionally valuable, this study included them as in many instances they were the only identifiable parts of a prey item (Buckland et al, 2017). While many studies calculate the number of individuals

from a particular prey group (%N), this was only calculated for a subset of sharks (54.3%) in this study where whole prey items could be identified. The highly digested state of many prey (e.g. large number of fragments), and inability to separate content clumped together by mucus (Buckland et al, 2017) made it impossible to determine %N for 45.7% of sharks and 90.7% of bluespotted maskrays. The rays were particularly challenging to calculate %N for because of the frequent occurrence of adhesive mucus which bound fragments, and the inability to determine if the marine worm ‘strands’ were whole organisms or fragments (the heads of marine worms often could not be determined). For the subset of sharks with %N calculated, metrics were combined to create the index of relative importance (IRI), which is defined as: $IRI = (\%N + \%W) \times \%FO$, and expressed as a percentage.

The Bray-Curtis coefficient (20 stress runs) and ADONIS (significance $P < 0.05$) were performed using the Vegan package in R (Version 2022.12.0, R Core Team, 2022) to assess similarity and differences in diet between species, maturity, sex, breeding state and country. Non-metric multidimensional scaling analysis (NMDS) was performed with the ‘metaMDS’ function in the Vegan package of R-Studio to visualise the variation in diet between the three species using %FO, %W (wet), and %W (dry). Similarity percentages (SIMPER) were then performed using PRIMER v6 (Clarke & Gorley, 2006) to confirm where these differences occurred. As these analysis were sufficient in generating insights, other types of dietary analysis (e.g. Morisita-Horn index (Horn 1966)) were not deemed necessary.

5.3. Results

Of the total sample size of blackspot sharks, 8.7% (n=9) had empty stomachs. This left a total sample size of 94 for further analysis (Table 5.1). Diet analysis revealed that for the blackspot sharks, crustaceans, fish and cephalopods were the dominant prey items. Of the total sample size of rays, 33.9% (n=85) had empty stomachs. This left a total sample size of 166 for further analysis (Table 5.2). Diet analysis revealed that for the bluespotted maskrays, crustaceans and marine worms were the dominant prey items.

Table 5.1. Stomach content composition of prey items for 94 Blackspot shark (*Carcharhinus sealei*) caught from Indonesia ($n=92$) and Singapore ($n=2$). Results are summarised by Frequency of occurrence (%FO) and Weight (%W) (Wet (W) and dried (D)). Stomach mucus, plastic and bait are excluded from this analysis, and Number (%N) was not done due to prey items being too fragmented to determine how many individuals they derived from.

Prey category		%FO	%W (W)	%W (D)
Teleostei (total)	Fish	53.19	40.58	44.97
<i>Herklotsichthys dispilonotus</i>	Blacksaddle herring	2.13	0.91	1.29
<i>Muraenidae</i> spp.	Moray eel	1.06	1.20	1.17
<i>Plotosus</i> spp.	Eeltail catfish	1.06	0.43	0.40
<i>Synodontidae</i> spp. (incl. <i>Saurida undosquamis</i>)	Lizardfish (incl. brushtooth lizardfish)	2.13	1.28	1.01
<i>Teleostei</i> spp. (unidentified)	Fish (unidentified)	46.81	36.76	41.1
Cephalopoda (total)	Cephalopod	41.49	41.06	37.44
<i>Decapodiformes</i> spp.	Squid	12.77	16.77	11.58
<i>Octopoda</i> spp.	Octopus	5.32	4.14	2.57
<i>Sepiida</i> spp.	Cuttlefish	3.19	4.34	4.22
<i>Cephalopoda</i> spp. (unidentified)	Cephalopod (unidentified)	20.21	15.81	19.07

Table 5.1 continued...

Gastropoda (shelled)	Gastropod	2.13	0.18	0.18
Crustacea (total)	Crustacean	41.49	12.40	10.68
<i>Stomatopoda</i> spp.	Mantis shrimp	3.19	0.82	0.83
<i>Decapoda</i> spp. (excluding <i>matudidae</i> spp.)	Prawn, shrimp, lobster	12.77	2.28	2.84
<i>Matudidae</i> spp.	Matutidae crab	1.06	1.94	1.27
<i>Brachyura</i> spp.	Crab	1.06	0.04	0.02
<i>Isopoda</i> spp.	Isopod	2.13	0.02	0.02
<i>Ostracoda</i> spp.	Ostracod	1.06		
<i>Crustacea (unidentified)</i>	Crustacean	20.22	7.3	5.7
Marine Worms and worm-like invertebrates (total)	Marine worm	3.19	0.20	0.07
<i>Sipunculidae</i> spp.	Peanut worm	1.06		
<i>Marine worm (unidentified)</i>	Marine worm (unidentified)	2.13		
Protista spp.	Algae	1.06	0.01	0.02

Sand/rock		7.45	0.69	0.57
Unidentified or digested		29.79	4.76	5.42

Table 5.2. Stomach content composition of prey items for 163 bluespotted maskrays (*Neotrygon* spp.) caught from Indonesia ($n=128$), Malaysia ($n=11$) and Singapore ($n=23$). Results are summarised by Frequency of occurrence (%FO) and Weight (%W) (Wet (W) and dried (D)). Stomach mucus, plastic and bait are excluded from this analysis, and Number (%N) was not done due to prey items being too fragmented to determine how many individuals they derived from.

Prey category		%FO	%W (W)	%W (D)
Teleostei (total)	Fish	1.20	0.29	0.31
<i>Teleostei (unidentified)</i>	Fish	1.20	0.20	0.31
Cephalopoda (total)	Cephalopod	0.60	5.03	5.72
<i>Decapodiformes</i> spp.	Squid	0.60	5.03	5.72
Gastropoda (total)	Gastropod	9.04	1.56	2.98
<i>Gastropoda</i> spp. (shelled)	Shelled gastropods	8.43	1.14	2.60
<i>Gastropoda</i> (unshelled)	Sea slug	0.60	0.43	0.38
Crustacea (total)	Crustacean	56.63	15.77	15.29
<i>Decapoda</i> spp. (excluding <i>matudidae</i> spp.)	Prawn, shrimp, lobster	15.66	8.03	5.50

Table 5.2 continued...

<i>Crustacea (unidentified)</i>	Crustacean	40.97	7.74	9.79
Marine Worms and worm-like invertebrates (total)	Marine worm	57.23	52.59	45.46
<i>Eunice aphroditois</i>	Sand striker worm	0.60	51.17	42.32
<i>Marine worm (unidentified)</i>	Marine worm	56.7	1.42	3.14
Protista spp. (Total)	Algae	22.89	0.94	1.985
Bryozoa spp. (Total)	Bryozoa	1.20	0.00	0.00
Echinodermata spp. (Total)	Echinoderm	0.60	0.00	0.00
Sand/rock		18.07	0.48	1.18
Unidentified or digested		53.01	18.37	21.43

5.3.1. Plastics, bait and DNA of prey

Six bluespotted maskrays had plastics in their stomach. These plastics were all similar in structure; small (< 100 mm) blue or green strands that were sometimes coiled, and looked like they could have originated from fishing gear. Five rays had one strand in their stomach and one ray had two strands

in their stomach. Five of the rays with plastic in their stomach came from Indonesia and one came from Malaysia.

Three bluespotted maskrays and one blackspot shark had bait in their stomach. All bait was cephalopod meat. The three rays that had bait in their stomach came from Malaysia, and were the only rays in this study reportedly caught using longlines; which was also confirmed as a hook was found in each stomach.

DNA analysis was successful for only two of the 19 samples taken from prey items; revealing a Brushtooth lizardfish (*Saurida undosquamis*) and Lizardfish (*Trachinocephalus myops*), both of which came from *C. Sealei*. The remaining samples either produced no result at all; the shark or ray species they came from (e.g. *C. sealei* or *N. orientale*); or a bacteria. Additionally, one prey sample from *C. Sealei* came back as the Grey reef shark (*C. brachyurus*), however the sample was taken from a bony fish so the result was dismissed.

5.3.2. Feeding differences between species

Due to low sample sizes at the species level, ADONIS and NMDS were generated using the broad prey groupings bony fish (*Teleosti*), cephalopod (*cephalopoda*), crustacean (*crustacea*), marine worm, gastropod (*gastropoda*), echinoderm (*echinodermata*), bryozoan (*bryozoa*), and algae (*protista*). Animals which only had unidentifiable prey in their stomachs were excluded from this analysis leaving 145 bluespotted maskrays and 84 blackspot sharks for further analysis. NMDS based on %FO had lower stress values than those based on %W, and as %FO is generally considered to provide the most robust measure of diet composition (Baker et al, 2014), it was used for all further analysis. The NMDS (Figure 5.1.) and ADONIS value reveal significant differences in the diet between blackspot sharks and bluespotted maskrays ($P=0.001$, 82.69 average dissimilarity). The main driver of this difference was the higher %FO of fish and cephalopods in blackspot sharks while bluespotted maskrays had a higher %FO of marine worms, algae and crustaceans (Figure 5.2).

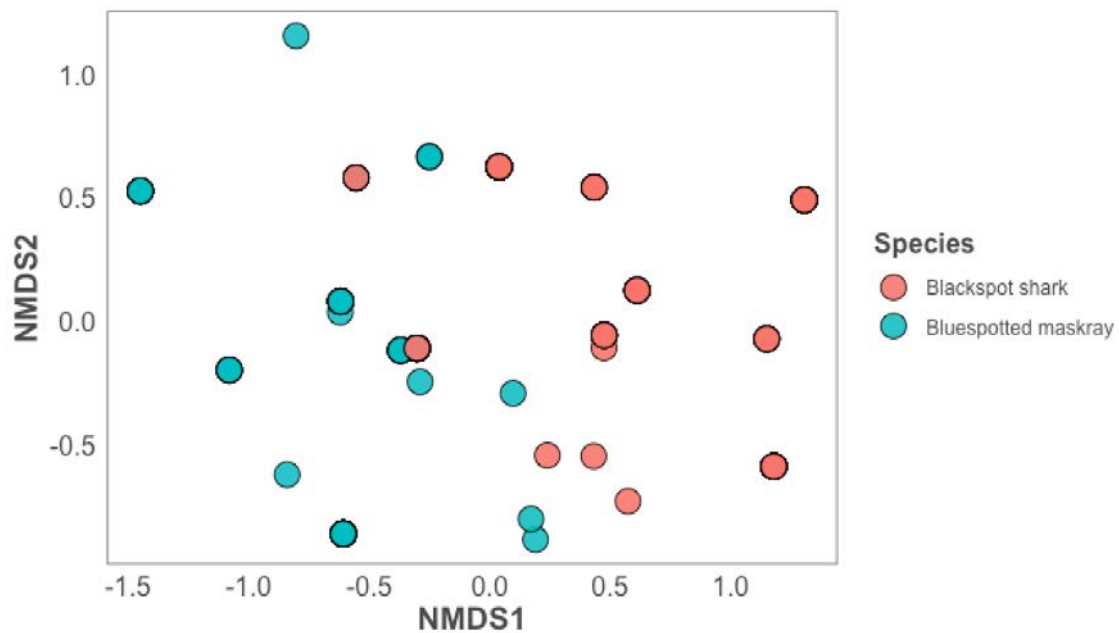


Fig 5.1. Non-metric multidimensional scaling ordination for blackspot sharks (*C. sealei*) ($n=84$) and bluespotted maskrays (*Neotrygon* spp.) ($n=145$) based on percent frequency occurrence (%FO) reveals high dissimilarity in diets (stress value=0.07799, ADONIS value=0.001***).

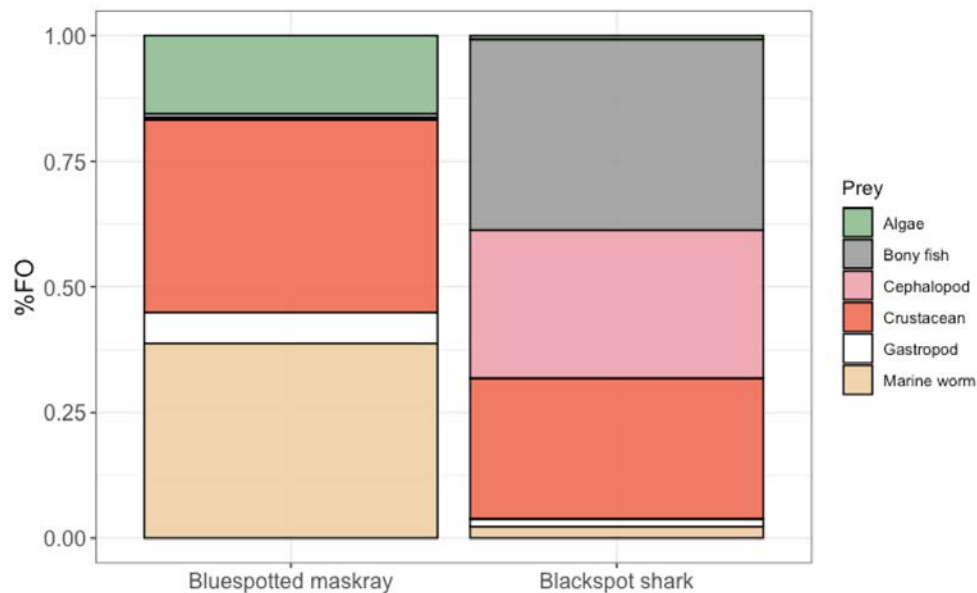


Fig 5.2. Composition of identifiable prey found in blackspot shark (*C. Sealei*) ($n=84$) and bluespotted maskray (*Neotrygon* spp.) ($n=145$) based on percent frequency occurrence (%FO). Prey items which occurred in low quantities (bryozoan ($n=2$), echinoderm ($n=1$)) and could not be properly visualised on the graph were not included.

5.3.3. Diet of the blackspot sharks

The IRI could be calculated for a subset ($n=51$) of blackspot sharks for which %FO, %W (wet) and %N could be obtained. Bony fishes were most important prey items for both immature and mature males (58.33% IRI and 56.47% IRI respectively) followed by Cephalopods (28.31% IRI and 41.44% IRI respectively). For female blackspot sharks this trend was reversed; Cephalopods were most important for both immature and mature females (36.33% IRI and 52.37% IRI respectively), followed by bony fishes (32.29% IRI and 39.60% IRI respectively). Crustaceans were far more important for immature females and immature males (31.37% IRI and 13.35% respectively), compared with mature females and males (8.02% IRI and 1.74% IRI respectively).

ADONIS and SIMPER analyses of the %FO provided further confirmation of the intra-specific differences in blackspot shark diet indicated by the IRI (section 5.3.1). In these analyses of %FO, dissimilarities occurred between males and females ($P=0.028$, 54.76 average dissimilarity). The main driver of this difference was the higher %FO of bony fishes in males, while females had higher %FO of cephalopods (for mature females only) and crustaceans (for immature females only). Another dissimilarity occurred between the age groups ($P=0.014$, 54.17 average dissimilarity), with immature sharks of both sexes having a higher %FO of crustaceans, and mature sharks having a higher %FO of cephalopods and bony fishes, with an exception among males, where immature sharks ate more cephalopods than mature sharks.

When analysing these data further, differences were observed between immature males and immature females ($P=0.025$, average dissimilarity 54.91). The main driver is that immature males have a higher %FO of bony fishes while immature females had a higher %FO of crustaceans. Another difference was observed between mature males and mature females ($P=0.025$, average dissimilarity 54.38). The main contributor comes from mature males having a higher %FO of bony fishes, and mature females having a higher %FO of cephalopods. No differences were detected between pregnant and non-pregnant specimens.

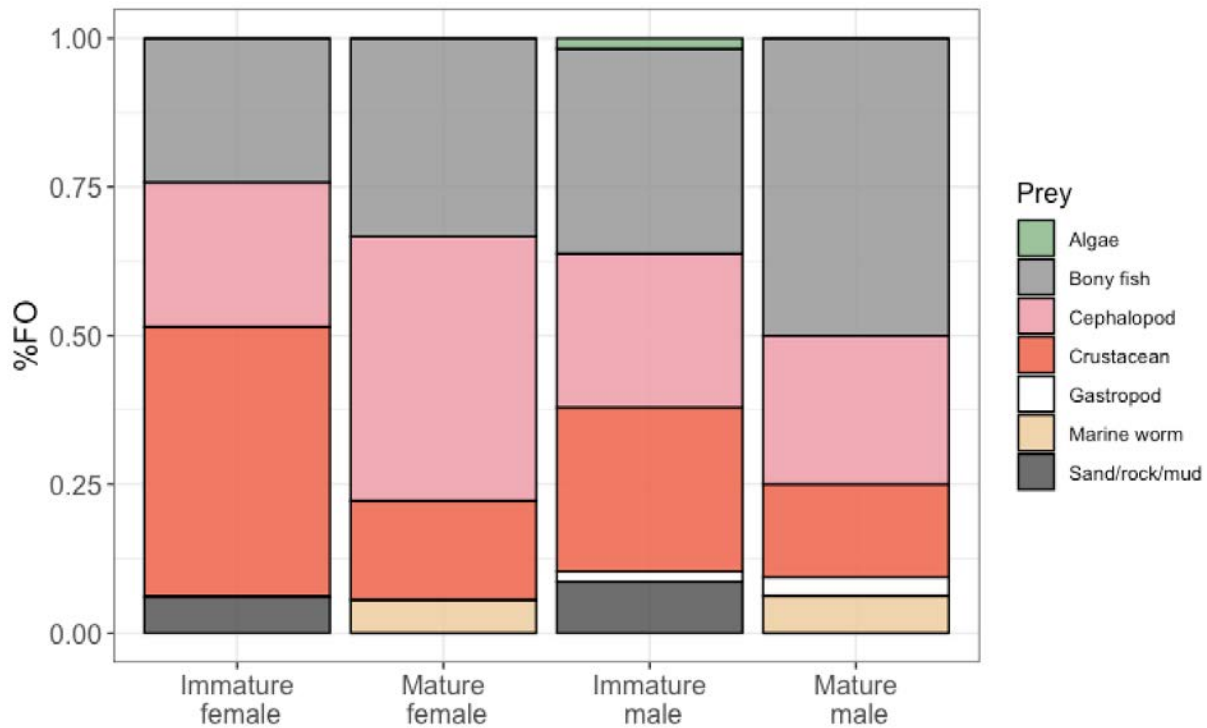


Fig 5.3. %FO of stomach content for blackspot sharks (*Carcharhinus sealei*) from Singapore ($n=2$) and Indonesia ($n=82$) with identifiable prey in their stomach ($n=84$): immature female ($n=20$), mature female ($n=12$), immature male ($n=32$), mature male ($n=20$).

5.3.3. Diet of the bluespotted maskrays

NMDS and ADONIS analysis based on %FO did not reveal any significant differences in the diet between bluespotted maskray species when categorising them based on the ‘criteria’ method ($P=0.096$) or by using the ‘mitochondrial’ method ($P=0.297$) (Figure 5.4a, b; see section 3.2.2 and 3.2.4 for descriptions of the ‘criteria’ and ‘mitochondrial’ methodology for determining species). Undetermined species of bluespotted maskray were also removed from analysis, but ADONIS still revealed no significant differences in the diet between oriental bluespotted maskray and mahogany maskray ($P=0.094$).

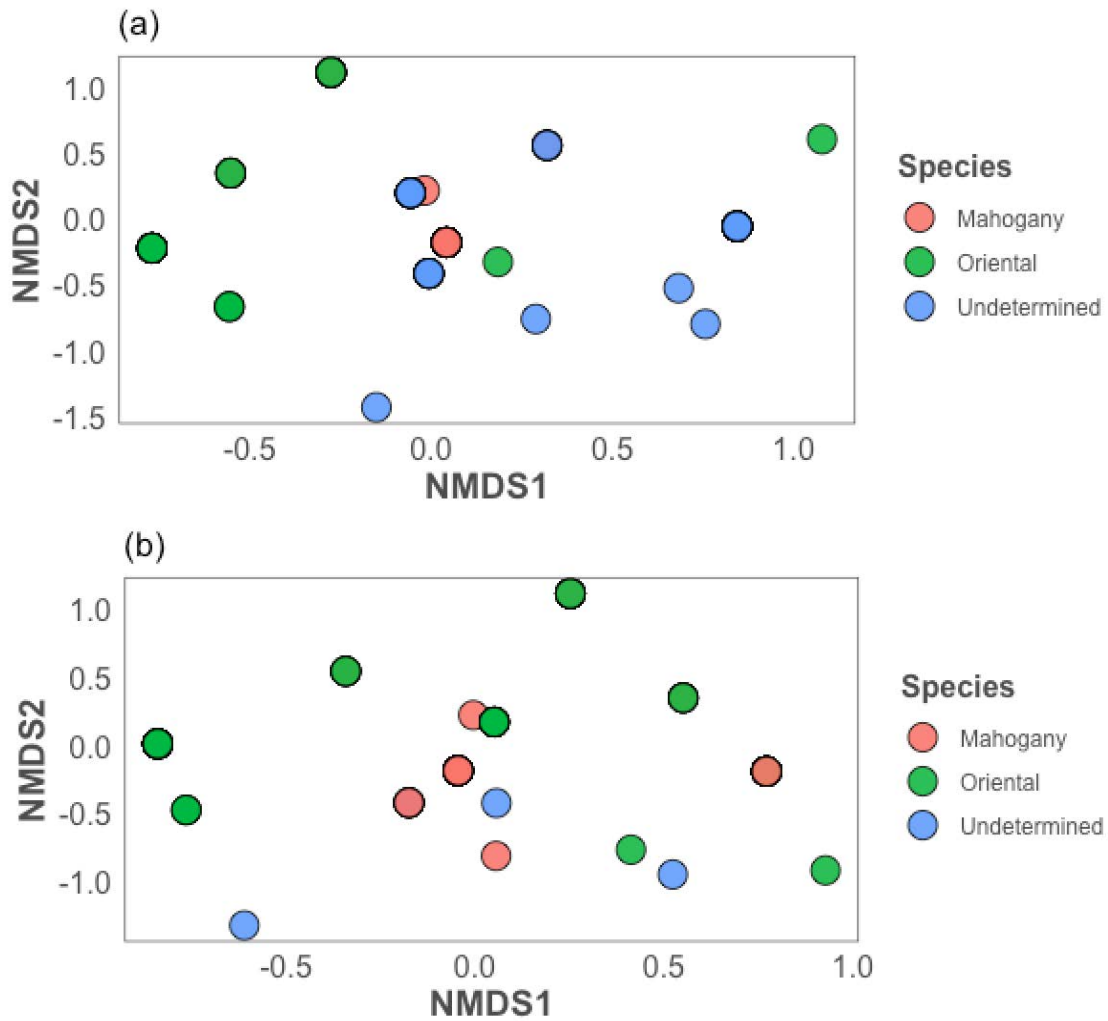


Fig 5.4. Non-metric multidimensional scaling ordination based on % Frequency Occurrence of diet analysis for (a) mahogany maskray, *Neotrygon varidens* ($n=35$), oriental bluespotted maskray *Neotrygon orientalis* ($n=47$), and undetermined species of bluespotted maskray ($n=64$) based on the 'criteria' method (as outlined in Table 3.3, section 3.2.4), (stress value=0.047, ADONIS p -value=0.096) and (b) mahogany maskray, *Neotrygon varidens* ($n=54$), oriental bluespotted maskray *Neotrygon orientalis* ($n=76$), and undetermined species of bluespotted maskray ($n=15$) using the 'mitochondrial' method (as outlined in section 3.2.2), (stress value=0.033, ADONIS p -value=0.297)

ADONIS revealed a significant difference in the diet between mature and immature oriental bluespotted maskrays (as defined by ‘criteria’ method, outlined in Table 3.3., section 3.2.4) ($P=0.05$, average dissimilarity=53.08). The main driver of this difference was the higher %FO of marine worms in mature animals, and higher %FO of crustaceans in immature animals (Figure 5.5). No significant difference was observed between the sexes of oriental bluespotted maskrays, and no significant differences were observed between the sexes and maturity in mahogany maskrays.

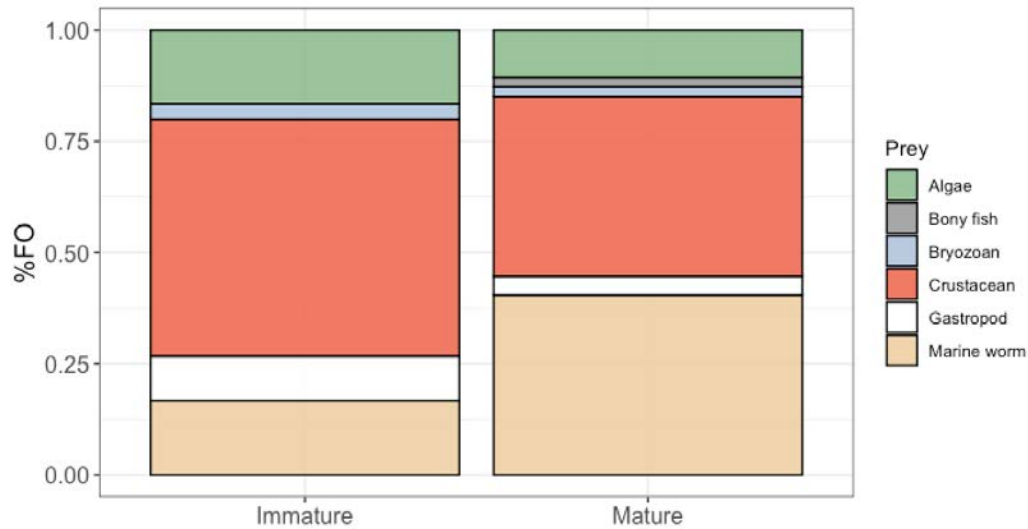


Fig 5.5. %FO of stomach content for oriental bluespotted maskrays (*Neotrygon orientalis*) ($n=47$) with species defined based on ‘criteria’ (as outlined in Table 3.3, section 3.2.4), from Malaysia ($n=3$) and Indonesia ($n=44$) with identifiable prey in their stomach: immature ($n=19$), mature ($n=28$).

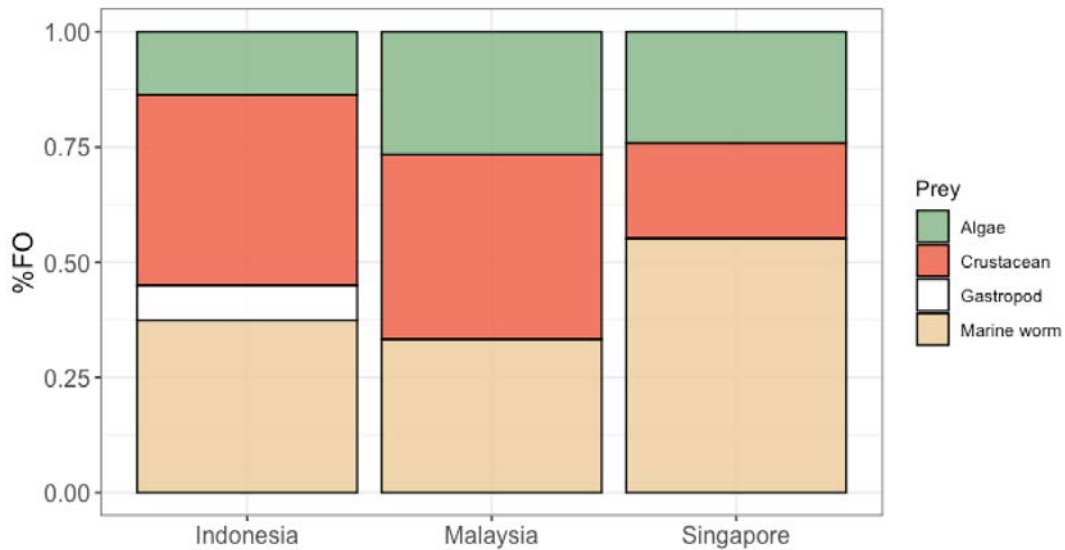


Fig 5.6. %FO of stomach content for all bluespotted maskrays (*Neotrygon orientalis*, *Neotrygon varidens* and undetermined species of bluespotted maskray) from Indonesia ($n=114$), Malaysia ($n=9$) and Singapore ($n=22$) with identifiable prey in their stomach. Fish ($n=2$), echinoderm ($n=1$), cephalopod ($n=1$), bryozoan ($n=2$) were removed from the plot due to small sample size and poor visualisation.

Aside from the difference identified in the diets of immature and mature oriental bluespotted maskrays, no further inter or intraspecific differences were observed, and so all samples were grouped together for further analysis ($n=145$). No differences were observed between sex or maturity of the combined grouping, but ADONIS analysis revealed a significant difference between country ($P=0.028$, 49.68-54.93 average dissimilarity), with the main driver of this difference animals from Indonesia and Malaysia having a higher %FO of crustaceans and animals from Singapore having a higher %FO of marine worms (Figure 5.6). All the animals from Singapore were mahogany maskrays, and when comparing the diets of mahogany maskrays from Singapore with mahogany maskrays from Indonesia, the animals from Singapore had a higher %FO of marine worms and the animals from Indonesia had a higher %FO of crustaceans (Figure 5.7). A significant difference was also observed between sites in Indonesia ($P=0.006$, 7.03-56.51 average dissimilarity), with animals from Batam having a higher %FO of crustaceans than animals from the Natuna islands, Jambi and Kalimantan (Fig 5.7). A significant difference was also observed between seasons ($P=0.028$, 47.3-57.33 average dissimilarity), with a higher %FO of crustaceans consumed

during the inter-monsoon period and southwest monsoon period compared to the Northeast monsoon period (Fig 5.8).

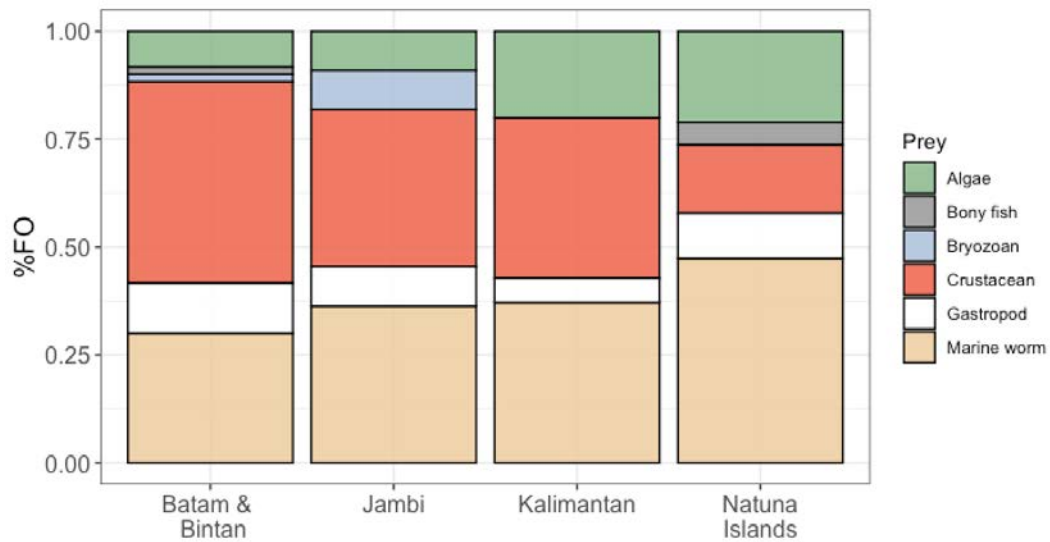


Fig 5.7. %FO stomach content for all bluespotted makrays (*Neotrygon orientalis*, *Neotrygon varidens* and undetermined species of bluespotted maskray) at five locations in Indonesia; Batam and Bintan ($n=33$), Jambi ($n=7$), Kalimantan ($n=19$), and Natuna Islands ($n=9$).

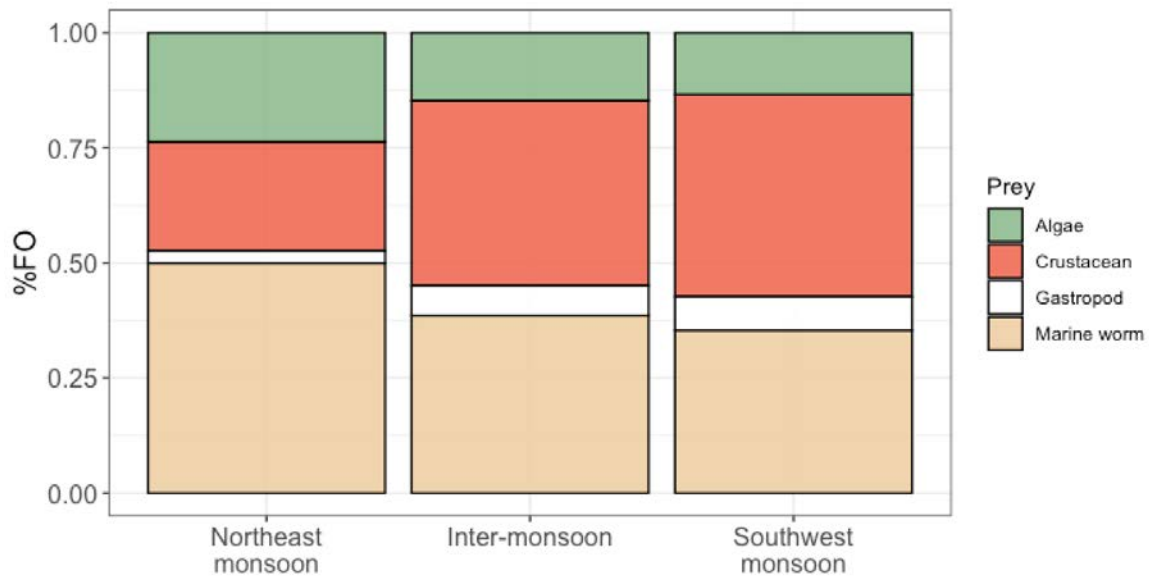


Fig 5.8. %FO stomach content for all bluespotted makrays (*Neotrygon orientalis*, *Neotrygon varidens* and undetermined species of bluespotted maskray) during three seasons between 2019 to 2021: Northeast monsoon (December-February, $n=28$), Southwest monsoon (June to September, $n=51$), and inter-monsoon period (March to May and October to November, $n=69$).

5.4. Discussion

This study provides insights to the diets of coastal elasmobranchs in Southeast Asia, a region which faces a paucity of ecological information (Clark-Shen et al. 2022). Differences were observed between the diets of blackspot sharks and bluespotted maskrays, with blackspot sharks primarily consuming bony fishes, cephalopods and crustaceans, and bluespotted maskrays primarily consuming marine worms and crustaceans. Significant differences were observed between the diets of male and female, and mature and immature blackspot sharks, while such dietary differences were less clear in bluespotted maskrays—possibly owing to the diluted sample of animals (from multiple countries and regions) and taxonomic confusions surrounding species identification.

The blackspot shark appears to be a generalist feeder, consuming a broad range of prey at the taxonomic and species level, with bony fishes (incl. herrings, catfish, lizardfish, moray eels), cephalopods (incl. squid, octopus and cuttlefish), and crustaceans (incl. shrimps, crabs, isopod and ostracod) dominating the diet. These three taxonomic groups are commonly observed as dominant prey items in sharks (Akilesh et al, 2013; Barria et al, 2015; Bornatowski et al, 2014; Saidi et al, 2009; Simpfendorfer et al, 2001). The bluespotted maskrays in this study specialise in crustaceans (primarily decapoda) and marine worms. Benthic feeding is typical for stingrays, with other studies also finding crustaceans and/or marine worms to dominate diets (Costa et al, 2015; Jacobsen & Bennet, 2013; O’Shea et al, 2013). For example, the large-eye stingray from Brazil also occupies a low niche breadth and specialises in shrimps and polychaetes (Queiroz et al, 2023), and bluespotted maskray (*Neotrygon spp.*) from waters off New Guinea had diets dominated by crustaceans (> 80%) (Wigiyo et al, 2022). The diet of the bluespotted maskray in this study differs slightly from the bluespotted maskray from Australia, which primarily eats annelids, brachyurans and few prawns (decapoda) (O’Shea et al, 2013). Admittedly, the majority of marine worms in this study were not identified to the genus level due to the high state of digestion (Table 5.2; which may be due to a prolonged period between capture and dissection) and it could be that these worms were indeed annelids. Algae also has a relatively high %FO in the diets of bluespotted maskray from this study, but this may be incidental ingestion when hunting benthic prey, as was originally speculated with algae ingestion in bonnethead sharks (Plumlee et al, 2016). However, it has subsequently been demonstrated that bonnethead sharks effectively assimilate nutrients from seagrass and are clear

omnivores (Leigh et al. 2018). Whether the algae observed in the diets of bluespotted maskrays is incidental digestion or an important component of their diet needs further exploration. No significant differences were observed between the diets of oriental bluespotted maskrays, mahogany maskrays and undetermined species of bluespotted maskrays, suggesting these animals have similar diets. However, a larger sample size of bluespotted maskray species from the same areas is needed to confirm if prey or habitat partitioning occurs, as has been found with other sympatric species of ray (O'Shea et al, 2013). This comparison was not possible in this study due to the low sample sizes of each species from each area.

Ontogenetic diet shifts were observed, with immature sharks of both sexes having a higher %FO of crustaceans and mature sharks having a higher %FO of bony fishes and cephalopods. Sand was only found in the stomachs of immature sharks, supporting this higher dependency on benthic-dwelling crustaceans. Crustaceans are also important to young smooth-hound (*M. mustelus*) and dusky (*C. obscurus*) sharks, with importance waning with increased size (Saidi et al, 2009; Simpfendorfer et al, 2001). Ontogenetic diet shifts can occur because of improved hunting abilities with age (e.g. improved gape size, speed and experience), allowing for capture of more energetically valuable prey such as fish (Dale et al, 2011; Simpfendorfer et al, 2001), expansion of home range enabling access to different prey, or habitat and prey partitioning between sizes to reduce competition (Ba et al, 2013; Costa et al, 2015; Plumlee et al, 2016). For the bluespotted maskrays, ontogenetic diet shifts were only observed in the oriental bluespotted maskray, with immature rays having a higher %FO of crustaceans and mature rays having a higher %FO of marine worms. Crustaceans are likely easy prey for smaller rays (Dale et al, 2011), and studies have found that juvenile rays often reside in shallow, sheltered habitat for protection against predators, while adults have a larger home range, which provides access to different prey (Dale et al, 2011; Costa et al, 2015; O'Shea et al, 2013; Martins et al. 2020). Tracking studies at Ningaloo reef suggests that the juveniles of five species of stingray (including bluespotted maskrays) may reside in shallow embayments that are 2-3 metres deep for up to 18 months, while adult rays are also found in these habitats but range over much greater areas, which may account for dietary differences (unpubl. Data in O'Shea et al, 2013; Martins et al. 2020). However, caution should be exercised due to the small sample size of confirmed oriental bluespotted maskrays in this study (immature=19, mature=28). When all bluespotted

maskrays were analysed together (immature=60, mature=87), no significant ontogenetic diet shifts were observed.

For the sharks, differences between the sexes were also observed: fishes were most important (in terms of IRI and %FO) for immature and mature males; cephalopods were most important for mature females (in terms of IRI and %FO), and crustaceans were most important for immature females (in terms of %FO, while IRI revealed cephalopods of most importance but this may be due to limited sample size for IRI analysis). The dominance of cephalopods (incl. squid, cuttlefish and unidentified cephalopods) in the diets of mature females may indicate that they are an energetically valuable prey for females to support reproductive activity, as has been hypothesised for adult female large-eye stingray which are known to target lobsters (Costa et al, 2015). Sex differences in diet are commonplace and can result from sex-specific food preferences and needs (e.g. breeding), as well as sexual segregation by habitat (resulting in different prey-encounter rates), and differences in stomach size (Ba et al, 2013; Costa et al, 2015; O'Shea et al, 2013; Simpfendorfer et al, 2001). More males (n=58) than females (n=34) were caught from the handline fishery which operated in a fixed area and caught 92 of the blackspot sharks used in this study, suggesting possible sexual segregation with this fishery primarily operating in a male-dominated habitat. If the species does sexually segregate, it is possible that the dietary differences observed between the sexes reflects the different geographic locations they were caught from. Some skews in sex ratio are also attributed to fishing gear, such as longlines, selecting for larger individuals (Chen et al, 2007; White & Dharmadi, 2010); however this does not appear applicable to this study, as a range of sizes of both sex were caught and the largest specimens were females (females: 359-849 mm TL, males: 384-809 mm TL).

Bluespotted maskrays from Indonesia had a significantly higher %FO of crustaceans while animals from Singapore had a significantly higher %FO of marine worms. The smaller sample size from Singapore (n=22) compared to Indonesia (n=114), may account for this difference. Additionally, all animals from Singapore were mahogany maskrays while those from Indonesia included mahogany maskrays, oriental bluespotted maskrays and undetermined species of bluespotted maskray. When comparing only mahogany maskrays from Singapore (n=22) with mahogany maskrays from Indonesia (n=12), this difference still existed: animals from Indonesia had significantly higher %FO of crustaceans while those in Singapore had significantly higher %FO of marine worms. Seasonal

factors (as Singapore animals were only caught in December, while Indonesian animals were caught in 10 out of 12 months), natural variations in habitat type between source sites (e.g. sediment type, topography), sea conditions (e.g. salinity, currents), and optimal foraging in relation to such factors (Werner, 1981), may account for these differences. Geographic variations in the diets of elasmobranchs have been observed, such as in tiger sharks, suggesting they are capable of adapting their diet based on locally abundant resources (Heithaus, 2001), which may also be the case for bluespotted maskrays.

Anthropogenic factors may also play a role: Singapore is a highly urbanised country that has contaminated waters, high shipping and aquaculture activity, and has undergone land reclamation causing extensive sedimentation—the latter of which is said to have heavily impacted coastal habitats (Sin et al, 2016). While some species of marine worm are able to live in anthropogenically impacted waters (Giangrande et al, 2005; Philips et al, 1977; Quadros et al, 2009), populations of crustaceans have been found to decrease in more urbanised environments in at least one study (Cardoso et al, 2016), which may account for this skew. Differences in dietary patterns between urban and non-urban populations of sharks has been documented (Rangel et al, 2022); in one such study, nurse sharks (*Ginglymostoma cirratum*) consumed lower-quality prey, and a lower-diversity of prey items, in urban vs non-urban habitats (Rangel et al. 2021).

Drawing conclusions about the dietary patterns observed between bluespotted maskrays in this study remain challenging. The sample size from Singapore (n=22), and Malaysia (n=9), are small compared to Indonesia (n=114); habitat-type and water parameters at each source site are unknown; sampling occurred across different seasons; and there remain taxonomic uncertainties around the animals and species composition. Nevertheless, it can at least be concluded that crustaceans and marine worms are dominant prey items for bluespotted maskrays in general.

Caution must be exercised when interpreting the results of diet analysis: identified prey are often skewed toward those that are more difficult to digest (Barria et al, 2015), and animals attracted to baited fishing gear may be in search of food and hence have empty stomachs or contain prey in the later stages of digestion (Plumlee et al, 2016). The latter does not apply for the majority of bluespotted maskray in this study which were caught in unbaited traps, but is relevant for all blackspot sharks that were caught using baited longlines. Some animals may also regurgitate prey

when caught in fishing gear, particularly gears that induce high-stress such as longlines, resulting in loss of data and a higher portion of empty stomachs (Flores-Martinez et al, 2016; Simpfendorfer et al, 2001). While a small portion of blackspot sharks in this study had empty stomachs (~8%), a large proportion of bluespotted maskrays did (~34%), which may relate to fishing traps being left at-sea for up to weeks at a time (Chapter 3), resulting in prey being digested and animals being unable to feed. For future studies, sampling from fresher specimens would help to overcome this limitation. Lastly, blackspot sharks were only sampled in seven months, and further sampling of animals from the remaining five months would have given more holistic insight to seasonal variations, and should be considered for future studies.

Chapter 6:

Life-history of the blackspot shark (*Carcharhinus sealei*)

6.1. Introduction

The vulnerability of a species—and ultimately their vulnerability to anthropogenic impacts—correlates to their life history traits: species that mature quicker, reproduce faster and have more young are better able to withstand exploitation than those that mature late, reproduce slowly and have few young (Garcia et al, 2008; Hutchings, 2002). Although life history traits vary between species (Chen et al, 2007; Chin et al, 2013a; Grant et al, 2018), elasmobranchs, in general, are known to have slow growth rates, late sexual maturity, and low reproductive potential (e.g. small litters, long inter-birth intervals), which makes them less able to withstand exploitation from fisheries (Caillet, 2015). Consequently, over a third of elasmobranchs are now threatened with extinction (Dulvy et al, 2021), and understanding species' age and growth is crucial to population assessments and fisheries management.

While age and growth analysis has not been done for the blackspot shark (*C. sealei*), their close relative, the Australian blackspot shark (*C. coatesi*), has an age-at maturity of ~5 years and maximum estimated age of 11 years (Baje et al, 2019). The age-growth relationship of closely related species, or even individuals of the same species from different regions, is variable (Gervelis & Natanson, 2013; Loefer et al, 2003), hence individual assessment of the blackspot shark is needed to inform regional specific management. By combining knowledge of the blackspot shark's life history with their ecology (diet), more holistic management and conservation plans for the species, and ecosystems, can be created.

6.2. Method

6.2.1. Vertebral processing and age and growth analysis

Samples were obtained from Jurong Fishery Port in Singapore as well as through a private seafood supplier (chapter 3). Details about the fisheries that caught these sharks were obtained through an interview with the private seafood supplier (chapter 4). The same dissection protocol as described in Chapter 3 was applied here. Sections of thoracic vertebrae were removed from individual sharks (n=103) and processed using methods described by Goldman (2005). All remaining tissue was removed from the vertebrae using a scalpel; the vertebrae were then sectioned and the five largest centra, located under the first dorsal fin, were selected as recommended by Goldman (2005) and then soaked in 5% sodium hypochlorite solution for up to 3 minutes to remove residual tissue. Centra were then rinsed thoroughly with tap water and dried in a drying oven at 45°C-60°C until dry. Two random centra per animal were selected and the posterior side of the centra (with the Hemal arch opening) were attached to a glass microscope slide using Crystalbond 509 adhesive glue and a heat pad set at 250°C. Holding the microscope slide, the centra were sanded down against fine grain (400Cw) waterproof sandpaper set in tap water, until the middle of the centra was reached. The centra were then turned over and re-set in the microscope slide. The opposite side of the centra were sanded down until only the middle section of the centra remained at ~600 µm. These sections were then examined using a dissecting microscope: translucent and opaque bands (band pairs) were counted from the birthmark (Figure 6.1), which is identified by a change in the angle of the corpus calcareum (age 0) (Caillet 2015). Each centra was photographed through a dissecting microscope (Olympus SZX7 body with a DP22 Olympus camera). To improve clarity of the band pairs, images of centra were digitally uploaded into microsoft powerpoint and Picture Editor was used to adjust contrast, colouration, and apply filters to maximise clarity of band pairs. Two independent readers then assessed the images and estimated ages for each individual by counting band pairs. Discrepancies between the counts of the first and second reader were re-analysed until a consensus was reached. The interpretability of each vertebrae was scored according to the following definitions by McAuley et al (2007): 0=unreadable; 1=bands visible but difficult to interpret; 2=bands visible but most bands difficult to interpret; 3=bands visible but a minority difficult to interpret; 4=all bands unambiguous. Average Percent Error (APE) was calculated to assess average initial disagreement

between readers with the R package ‘FSA’ (Ogle et al. 2023). Bayesian growth models using Markov Chain Monte Carlo (MCMC) enable the generation of reliable estimates with smaller sample sizes (Smart and Grammer, 2021) were used to generate age-growth curves in R (R Core Team, 2022) with the R package ‘BayesGrowth’ (Smart and Grammar, 2021) and Generalised Linear Models (GLMs) were produced to determine the age-at-maturity (L_{50} and L_{95}) using the R package MASS (Version 7.3-60) (Venables and Ripley, 2002).

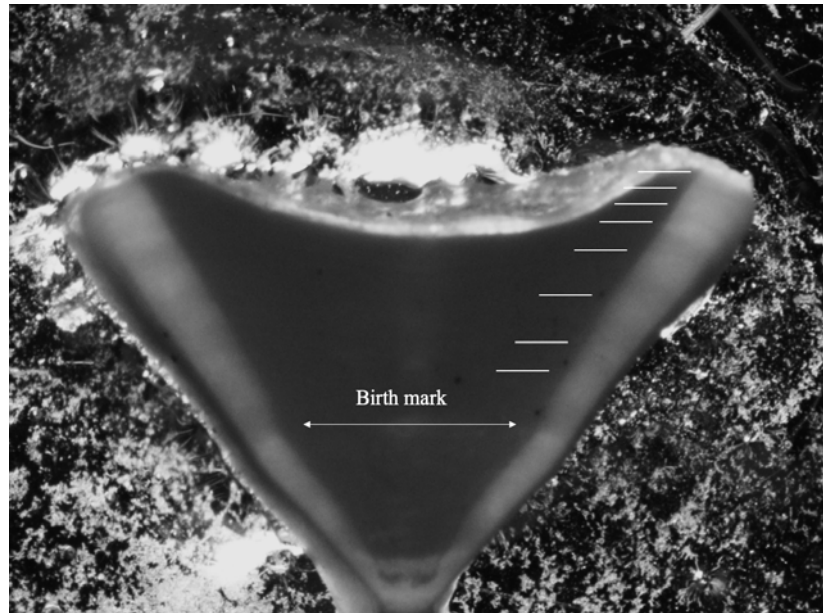


Fig 6.1. Vertebral section from an 8-year-old male blackspot shark (Carcharhinus sealei) measuring 776 mm stretched total length (STL), caught from a handline fishery in Indonesia. The birthmark and band pairs are identified. The vertebrae was given a readability score of 3.

6.2.2. Hepatosomatic Index (HSI)

The Hepatosomatic Index (HSI) is the ratio of liver weight to body weight, and is used as an indicator of energy reserves (Goede & Barton, 1990). The HSI is calculated as: $HSI = 100 * (WL/W)$ where WL = liver weight and W = body weight. A three-way ANOVA was run on R (R Core Team 2022) to test for differences in HSI values between sex, season and maturity. Low energy reserves

are typically found after events of high metabolic activity such as migrations or reproduction (Reis and Figueira, 2020).

6.3. Results

6.3.1. Age-growth analysis

The smallest mature male was 709 mm STL (with 57 mm clasper length; Figure 6.2), and the largest immature male was 786 mm STL (with 67 mm partially calcified claspers; Figure 6.2). The smallest mature female was 730 mm STL and the largest immature female was 706 mm STL. Males and females show a similar length-weight relationship, although females in the sample size attained larger sizes and heavier weights (Figure 6.3).

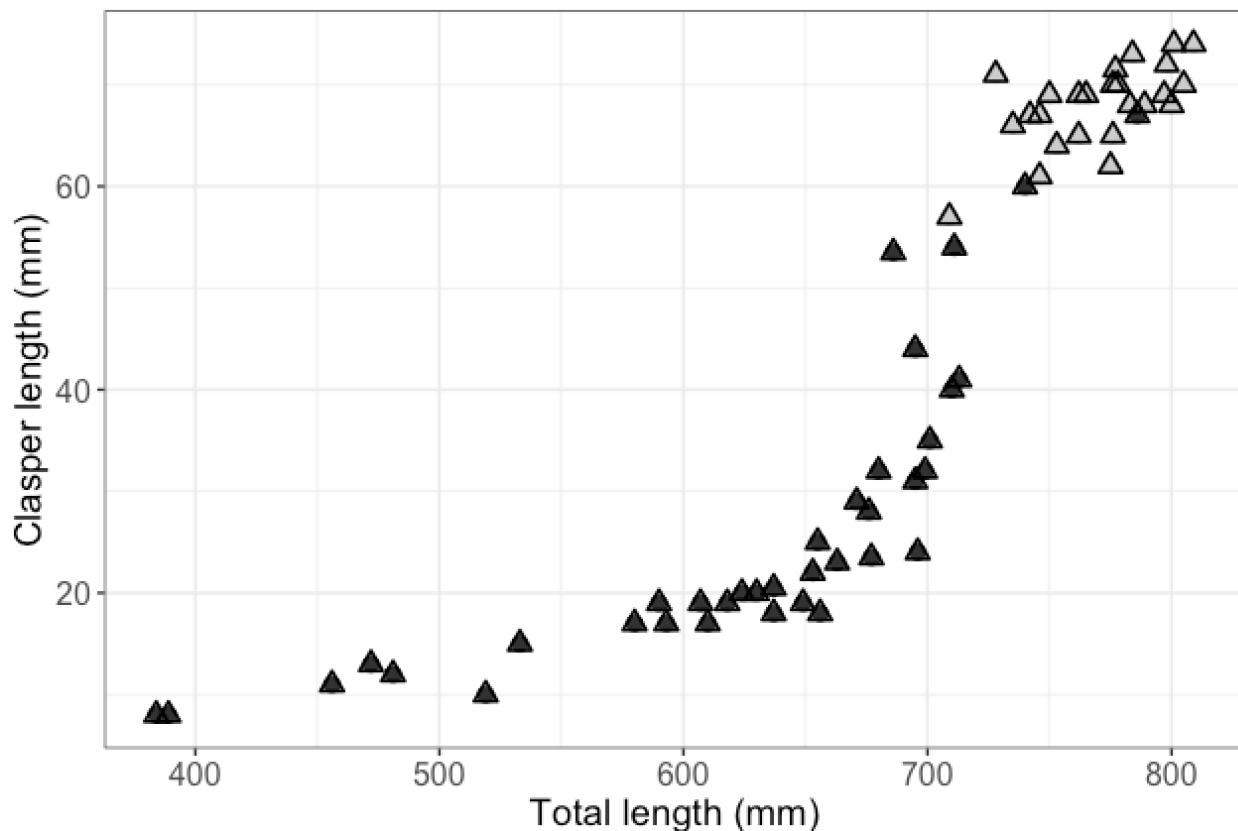


Fig 6.2. Relation of clasper length to stretched total Length (STL) and maturity (▲=uncalcified claspers, △= calcified claspers) for male (n=64) blackspot shark (*Carcharhinus sealei*), showing maturity is attained from > 709 mm STL and > 50 mm clasper length.

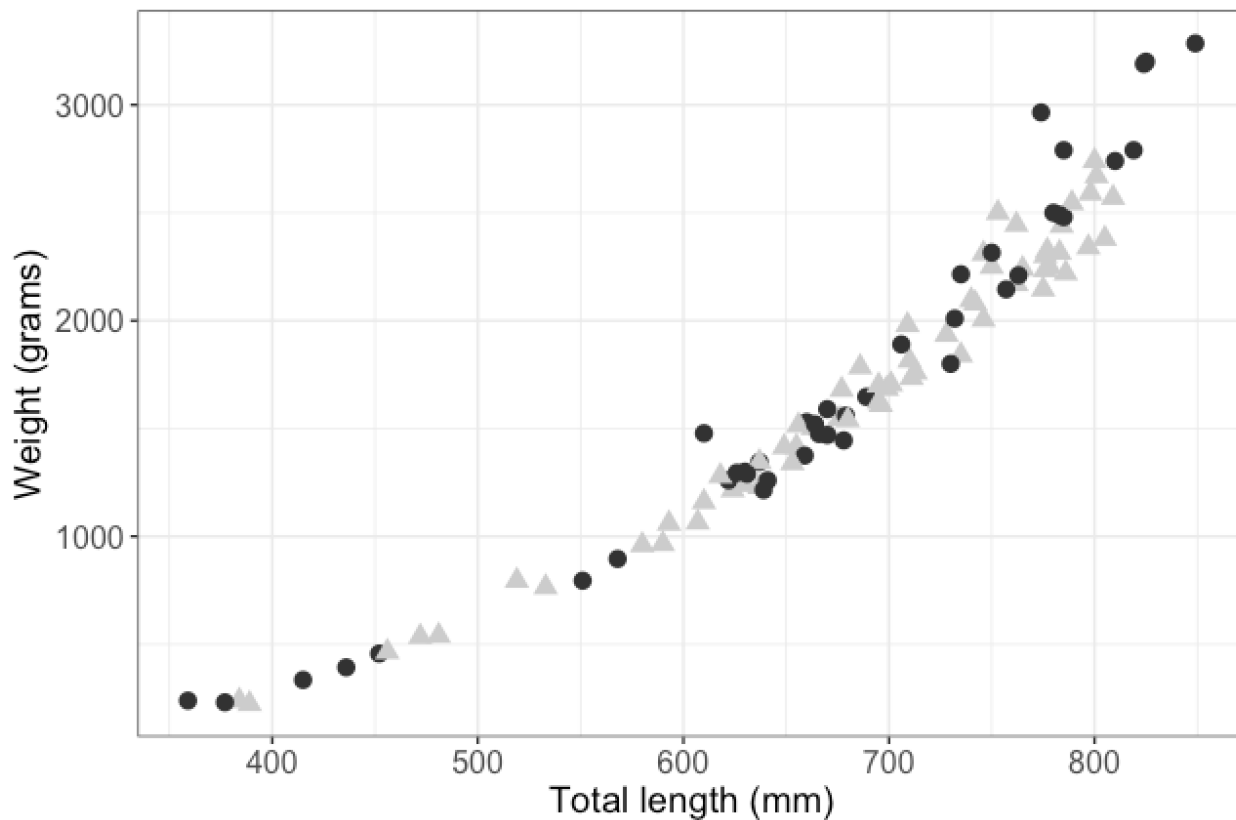


Fig 6.3. Length-weight relationship for female (●, n=41) and male (▲, n=62) blackspot shark (*Carcharhinus sealei*). Length-Weight equation (Pauly, 1983) is $W=0.0000021*L^{3.138}$.

Age-bands could be read for all 103 samples (the majority of vertebrae, >75 percent, scored two or three out of five for readability). The Average Percent Error (APE) was 14.66 percent, which is higher than the average reported APE in ageing studies (Campana 2005). The oldest agreed age (between the readers) from the study for this species was 11 years old for two females which were 757 mm STL and 825 mm STL (Figure 6.4). The oldest males in the sample were nine years old at 789 mm STL and 801 mm STL. MCMC analysis revealed that out of several potential growth models, the Logistic model was the best performing, matching the data most closely (Table 6.1). Under MCMC analysis the Log model produced a k-value of 0.37 year⁻¹ (Table 6.1). Male and female blackspot sharks matured at similar ages, with 50% of males reaching maturity at 6.15 years and 95% by 8.92 years old, and 50% of females reaching maturity at 6.12 years and 95% by 8.64 years (Figure 6.5).

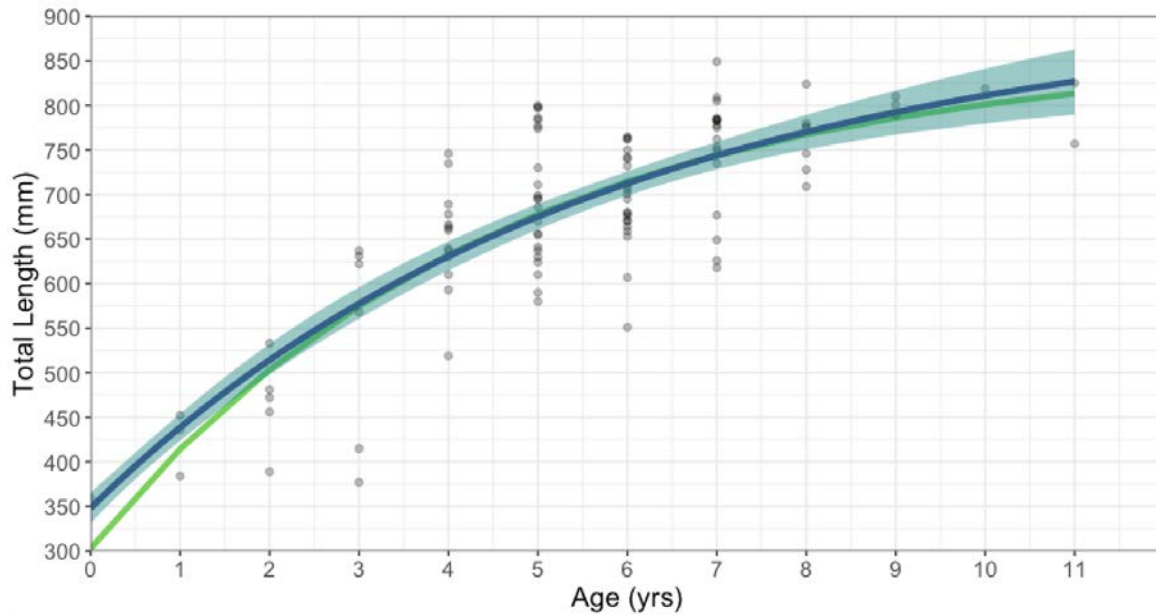


Fig 6.4. Age-growth curve for 103 blackspot sharks (*Carcharhinus sealei*) using vertebral band counts and the MCMC analysis performed using Bayesian and frequentist models. Circles (●) represent individual blackspot sharks. Lines indicate the modelled length and age values (green = Frequentist, blue - Bayesian) with light blue shading indicating the 95% confidence intervals.

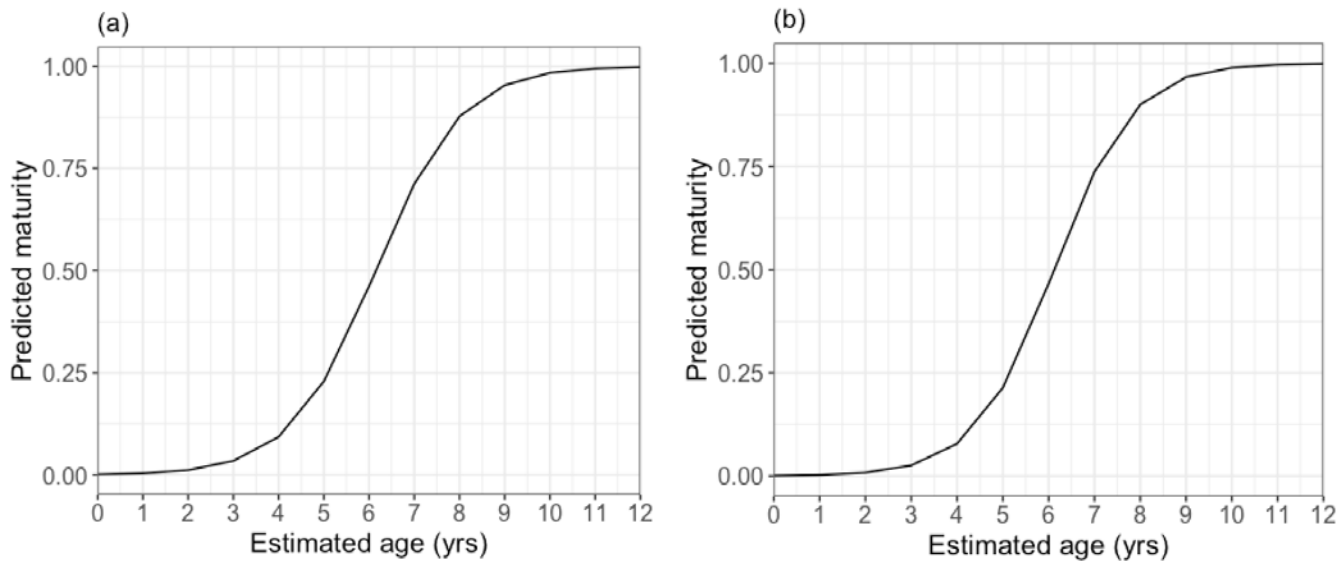


Fig 6.5. Logistic generalised linear models (GLMs) of estimated ages of (a) male and (b) female blackspot shark (*Carcharhinus sealei*) showing predictions of maturity at a given age. The model predicts a 50% age-at-maturity of 6.15, and a 95% age-at-maturity of 8.92 for males, and a 50% age-at-maturity of 6.12, and a 95% age-at-maturity of 8.64 for females.

Table 6.2. Parameter estimates and performance of models for age-growth analysis for the blackspot shark (*Carcharhinus sealei*). MCMC analysis was used to assess model performance and the best performing model was the Von Bertalanffy model which had a low LooIC. Numbers in parentheses after length at birth (L_0), asymptotic length (L_∞) and the k -value are the standard error. For the MCMC analysis, priors were set as: L_0 (350 mm, se 9.00: determined by mid-point between largest embryo and smallest specimen in the sample) and L_∞ (950 mm, se 95, after largest specimen reported by Ebert et al. 2013). The MCMC analysis generated a k -value of 0.37 year⁻¹.

Model	Model estimate			Model performance (AIC)			Model performance (LooIC) with MCMC		
	L_0 (mm)	(L_∞) (mm)	K (year-1)	AICc	AIC diff	AICc weight	LooIC	LooIC SE	LooIC Weight
Von Bertalanffy	302.9 (SE: 41.63)	861.6 (SE: 49.64)	0.222 (SE: 0.0508)	1156	1.41	0.21	1155	16.3	0.21
Logistic	327.1 (SE: 31.48)	804.5 (SE: 27.2)	0.4175 (SE: 0.0658)	1155	0	0.43	1153	15.95	0.47
Gompertz	315.1 (SE: 35.51)	825.1 (SE: 34.51)	0.3206 (SE: 0.05798)	1155	0.4	0.35	1154	16.1	0.33

6.3.2. Reproductive analysis

Of the 16 mature female blackspot sharks, nine were gravid. Two were early stage pregnancies, with two large yolky eggs inside the uterus but no embryos attached. The remaining seven gravid females had litters of two pups, however in one individual it appeared that one of the two pups had failed to develop properly. The largest embryos observed (Figure 6.6a: 341 mm and 333 mm STL from the same mother) were fully developed, and the smallest shark provided from the fishery measured 359 mm STL, suggesting length at birth falls between 341 mm and 359 mm STL. The largest embryo (341 mm STL) was 43% the size of the mother (785 mm STL) implying that, while blackspot sharks have small litters, this reproductive limitation is balanced by the relatively large size-at-birth which increases survival (Gutteridge et al, 2013). Of the nine gravid females, six had embryos that could be sexed, of which five were males and five were females (total embryos=10), meaning the sex ratio did not significantly differ from 1:1 (chi-square: 0, df=1, P=1.00). The youngest gravid female was five years old, and the oldest was ten years old.

Females showed various stages of pregnancy during the course of the year (Figure 6.6a). Non-gravid mature females showed various ova diameter throughout the year (Figure 6.6b), suggesting reproduction is asynchronous and year-round. Noticeably smaller ova were observed, however, during December, suggesting a potential 'pause' during this month.

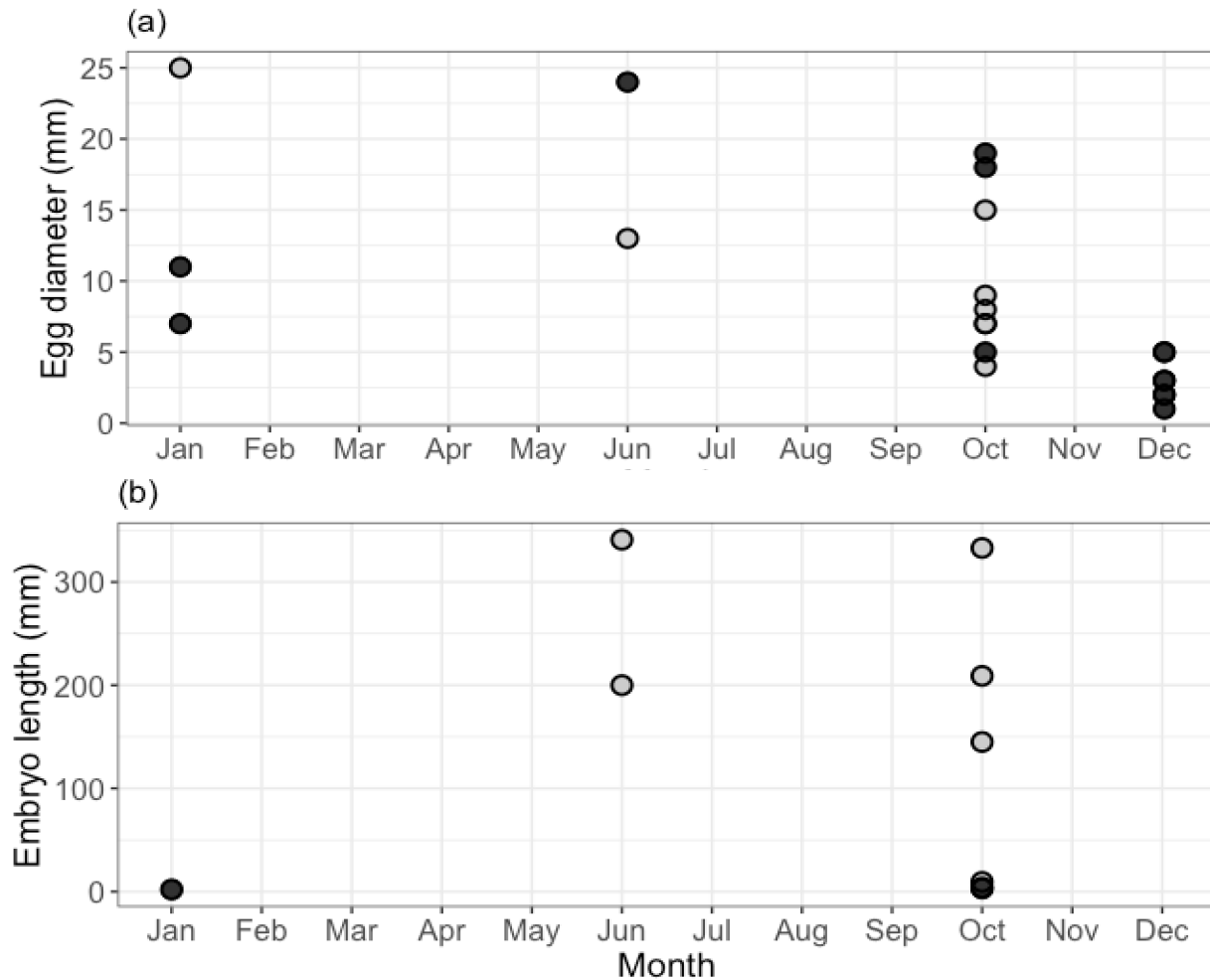


Fig 6.6. Reproductive data for female blackspot sharks (*Carcharhinus sealei*) showing (a) embryo (O) and implanted egg (●) length by month for nine gravid individuals (where females carried two embryos, only the largest was selected for visualisation), and (b) Largest ovarian egg diameter by month for all mature females ($n=16$), showing both gravid (O) and non-gravid individuals (●). Of gravid females ($n=9$) only eight are visualised as the ova in one gravid female was damaged and not measurable.

6.3.3. Hepatosomatic Index (HSI)

Results of the three-way ANOVA revealed a statistically significant effect of sex on HSI (females overall had significantly higher HSI than males ($P=0.0212-0.0261$)) but not of month, maturity or in the interaction of sex and month, or of sex and maturity (Table 6.2, Figure 6.7). The highest HSI

value (5.9375) was observed in a mature female with an early stage pregnancy (presence of two large yolky eggs in uterus). The second highest HSI value (5.2910) was observed in an immature female in November. The lowest HSI values (<2.00) were observed in a mature female (Caught in June), a mature male (caught in April) and an immature male (caught in December).

*Table 6.3. Results of two-way analysis (ANOVA) for the Hepatosomatic index (HSI) for male (n=41) and female (n=30) Blackspot shark (Carcharhinus sealei) for which liver weight was recorded, with significant results marked with *. Interaction of three variables could not be calculated due insufficient data.*

	Sum of sqrs	d.f	Mean square	F	P
Sex	3.36	1	3.356	5.609	0.0212*
Month	4.31	6	0.718	1.201	0.3188
Sex X Month	4.50	5	0.900	1.504	0.2023
Residuals	35.31	59	0.598		
Sex	3.36	1	3.356	5.174	0.0261*
Maturity	0.00	1	0.000	0.000	0.9866
Sex x Maturity	0.01	1	0.007	0.010	0.9200
Residuals	44.11	68	0.649		
Maturity	0.03	1	0.0255	0.040	0.843
Month	5.80	6	0.9664	1.499	0.194
Maturity X Month	3.61	5	0.7220	1.120	0.360
Residuals	38.04	59	0.6448		

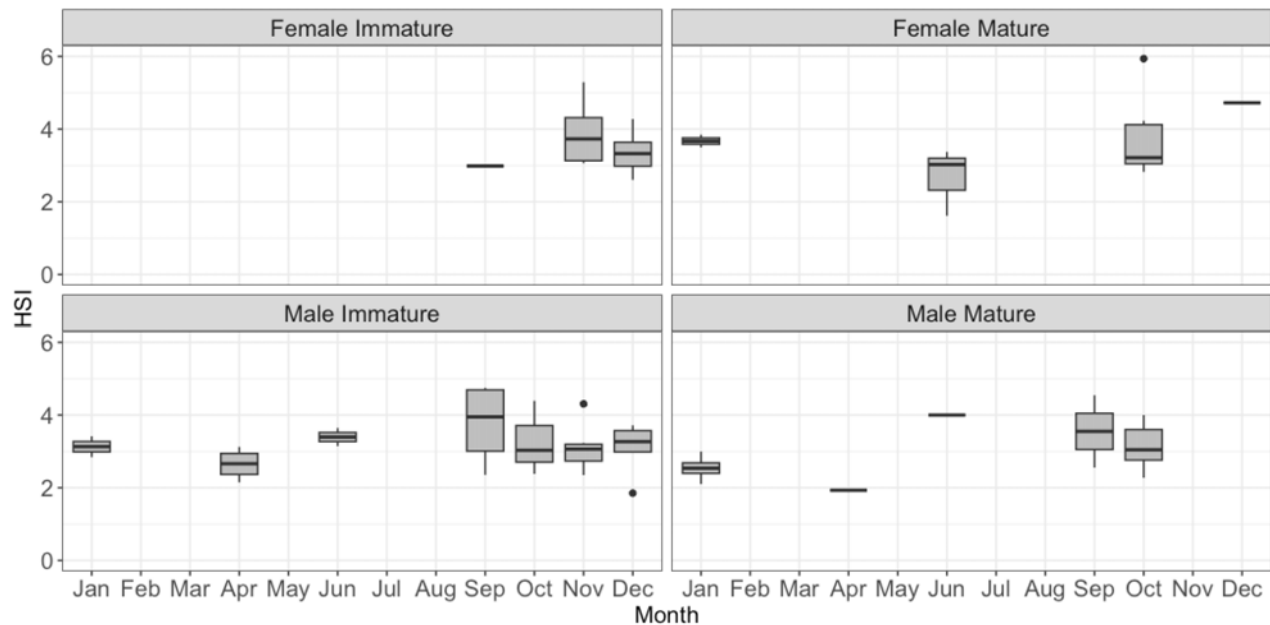


Fig 6.7. Hepatosomatic index (HSI) for male ($n=41$) and female ($n=30$) blackspot shark (*Carcharhinus sealei*) for which liver weight was recorded, between sexes and across months. Dark bars within each box represent the median value, the upper and lower boundaries of each box represent the interquartile range, the whiskers represent the total range, and the points outside the box represent outliers.

6.4. Discussion

This study provides a comprehensive account of the biology of the blackspot shark, a species that is widely taken in fisheries across Southeast Asia (SEAFDEC, 2017a). This study of blackspot sharks is based on samples mainly taken from Indonesia with significantly more males ($n=58$) than females ($n=34$) evident in the sample used in this study. This may suggest the occurrence of sexual segregation, with this fishery primarily operating in a male-dominated habitat. Sexual segregation is often seen in animals for reasons relating to social aspects or habitat (Wearmouth & Sims, 2008). The phenomena is commonly observed in sharks, including spottail sharks (*C. sorrah*) in Australia, where females use shallower habitats than males (Knip et al, 2012), blacktip reef sharks (*C. melanopterus*) in French Polynesia, where females frequent lagoons while males frequent fore-reefs

(Mourier et al, 2013) and blacktip reef sharks in Australia, where adult males are transient and largely absent from areas used by adult females (Chin et al. 2016). However, in this study, females were supplied in five of the seven months that males were, suggesting at least some cohabitation, or that the fishery also ranges into female habitat. The sample size of blackspot sharks from this study was also skewed toward larger individuals for both males and females, which may be attributed to size-related habitat segregation, feeding habits, or fishing gear selectivity, as some gear such as longlines may select for larger individuals (Chen et al, 2007; White & Dharmadi, 2010).

This study suggests that male and female blackspot sharks reach maturity at about the same age with 50 percent maturity at 6.1 years, and 95 percent maturity by 8.6-8.9 years. However, some individuals mature as early as four years old, and the youngest gravid female was five years old and 750 mm STL. A maximum age of 11 years was recorded during the study for this species, which is typical for small, in-shore Carcharhinid species (Gutteridge et al, 2013). It was determined that the species has a k -value of 0.37 year⁻¹ which is considered a moderately rapid growth rate (Branstetter, 1990; Chen et al, 2007). While this is considerably faster than some larger species such as the dusky shark (*C. obscurus*), which matures at 17 to 23 years old with a k -value of 0.043 (Simpfendorfer et al, 2002), it is slower than some other small bodied species such as the Australian sharpnose shark (*R. taylori*), which matures at one year of age and has a k -value of 1.33 for males (Simpfendorfer, 1993)). The blackspot shark matures later than its close relative the Australian blackspot shark (*C. coatesi*) from Papua New Guinea, which attains 50 percent age-at-maturity by 5.1-5.3 years, 95 percent age-at-maturity by 6.4-7.4 years, and has a maximum age of 11 years as well (Baje et al, 2019)).

While the blackspot shark has a moderately fast growth rate, this study found that the species has a small litter size of only two pups which is considered among the lowest for a carcharhinid (Gutteridge et al, 2013; Last & Stevens, 2009). The large size-at-birth limits the number of pups a female can bring to full gestation, for instance the largest embryo in this study was 43 percent the size of the mother. While this reproductive strategy reduces fecundity, a large size at birth increases neonate survival helps to compensate for this low fecundity (Branstetter, 1990). Reproduction appears to be asynchronous (year-round) likely due to the absence of temperature fluctuations in the tropics. Outside of the tropics, many elasmobranchs are known to breed synchronously in-line with

optimal seasons (Harry et al, 2013). Overall, species like the blackspot shark may take longer to recover from exploitation, as regeneration takes longer due to low reproductive output (Smith et al, 1998). This, coupled with the blackspot sharks relatively late age-at-maturity (6.1 years), suggests the species is less productive and therefore more vulnerable to fishing pressure, as is also suspected for the Australian blackspot shark (Baje et al, 2019). Additionally, small-bodied Carcharhinids have high natural mortality as they can experience predation across all age classes (Branstetter, 1990). The blackspot shark is reported to have experienced a suspected population reduction of 30-49% over the last 24 years (Dulvy et al, 2020), which corresponds with the more detailed and local account (see Chapter 4) where the seafood supplier estimated a 50-70% decline in availability over the last 45 years. Aside from intrinsic sensitivity, any species exposed to exploitation faces potential risk (Shermann et al, 2022), and focusing conservation efforts on large species, as often happens, leaves smaller species without the protection they need (Garcia et al, 2008).

This study is the first to assess the biology of the blackspot shark in Southeast Asia. While the sample size was considerable (n=103), collection of specimens across all months, and a larger sample size of females, would have given more confidence to the interpretation of reproductive data.

Chapter 7:

Life-history of the bluespotted maskray (*Neotrygon orientalis* and *N. varidens*)

7.1 Introduction

Stingrays are mesopredators (mid-ranking predators) and have important roles in ecosystems; they provide food for a variety of predators, have symbiotic relationships with other taxa, and are ‘bioturbators’ (disturbing sediments), which is important to nutrient dynamics (Crook et al, 2021; Flowers et al, 2020; Shermann et al, 2020b). In addition, they provide economic value in terms of tourism (Haas et al, 2017), and commercial usage (e.g. for food and leather) (Dulvy et al, 2021; Sahubawa & Pertiwinigrum, 2020; D’Alberto et al. 2022). Despite their value to ecosystems and people, stingrays receive relatively little conservation attention and are highly exploited and threatened (Dulvy et al, 2021). While stingrays are targeted in some fisheries (Clark-Shen et al, 2021), their benthic-dwelling nature also leaves them highly vulnerable to being taken as bycatch (Reis & Figueira, 2020). They are often caught in benthic fishing gear including trawlers (SEAFDEC, 2017), fishing traps, and ‘Brother Hooks’, (which is a translation from mandarin; the gear may also go by the name ‘rawai tiada umpan’; Burdon & Parry, 1954), which are a set of hooks dragged along the floor specifically to target benthic animals like stingrays (Personal comms. Seafood supplier).

Knowledge of a species’ ‘vulnerability’ is useful for devising conservation and management plans (Simpfendorfer et al, 2011). There are two key elements that determine how vulnerable elasmobranch species are to exploitation: (1) their productivity, which is dependent on the species’ life-history characteristics (i.e. their growth rate, sexual maturity, litter size, interbirth interval), and (2) the degree to which exploitation (e.g. fisheries) negatively impacts them (Patrick et al, 2009; in press: Shermann et al, 2022). Elasmobranchs, in general, are considered highly vulnerable because of their ‘slow’ life histories (e.g. late maturity, low fecundity, long gestation, compared to bony fishes) (Cailliet, 2015), and high capture rates (both targeted and as bycatch) in mostly unmanaged

fisheries (Dulvy et al, 2021; Oliver et al, 2014; Simpfendorfer & Dulvy, 2017). However, the number of life-history studies on stingrays is low compared to sharks (Dale and Holland, 2022; Torres-Palacios et al, 2019), and there is limited reporting of their catch rates and landings at the species level (FishStatj, 2016). More, in depth, studies on the life history of stingrays is urgently needed to inform conservation and management strategies of this group (Garcia et al, 2007).

The bluespotted maskray complex (*Neotrygon* spp.) is a group of small, benthic dwelling stingrays characterised by blue spots. They occur throughout the Indian ocean and Indo-pacific region, but confusion persists regarding their taxonomy, and the group has only recently been separated into multiple species that inhabit restricted ranges (Borsa et al. 2016; Last et al, 2016). Consequently, the species in this complex are in need of research and conservation attention. Stingray catch is high in Southeast Asia, and there are significant data deficiencies (Clark-Shen et al. 2023). Bluespotted maskrays are traded in large volumes for their meat, and may be increasingly targeted (Chapter 4). This chapter looks at the life-history of bluespotted maskrays (age-growth, reproduction), to further understand vulnerability to exploitation, and potential management options.

7.2. Method

7.2.1 Vertebral processing and age and growth analysis

Stingray ageing followed standard protocols as described in Chapters 3 and 5. Samples were obtained from Jurong Fishery Port in Singapore as well as through a private seafood supplier (chapter 3). Details about the fisheries that caught these rays were obtained through an interview with the private seafood supplier (chapter 4). The same dissection and processing protocol used for sharks (Chapter 5) was applied here. Sections of thoracic vertebrae from the bluespotted maskray (n=251) were processed using methods described in Goldman (2005). Tissue from the vertebrae was removed using a scalpel; the vertebrae was then sectioned and the five largest centra were chosen (Goldman, 2005). The centra were soaked in 5% sodium hypochlorite solution for up to 2 minutes to remove residual tissue, and then rinsed thoroughly with tap water and dried in a drying oven at 45-60°C until dry. Two centra per animal were randomly selected and the posterior side of the centra (with the hemal arch opening) attached to a microscope slide using crystalbond 509 adhesive glue

and a heat pad set at 250°C. The centra were sanded down using fine grain waterproof sandpaper (400Cw) set in fresh water, until the middle of the centra was reached. The centra were then flipped over and re-set in the microscope slide. The opposite side of the centra were then sanded down until only the middle of the centra remained at a >600µm section. These sections were then examined under a dissecting microscope: translucent and opaque bands (band pairs) were counted from the birthmark (Figure 7.1), identified by a change in the angle of the corpus calcareum (age 0) (Caillet, 2015). Microsoft powerpoint was used to enhance the colouration and contrast of images, and band pairs were read independently by two readers. The interpretability of each vertebrae was scored according to the following definitions by McAuley et al. (2006): 0=unreadable; 1=bands visible but difficult to interpret; 2=bands visible but most bands difficult to interpret; 3=bands visible but a minority difficult to interpret; 4=all bands unambiguous. Average Percent Error (APE) was calculated to assess average initial disagreement between readers with the R package 'FSA' (Ogle et al. 2023). Differences between the counts of the first and second reader were re-analysed with a third reader until agreed upon. Vertebrae with un-interpretable band counts were excluded from further analysis. Bayesian growth models (the von Bertalanffy growth function (VBGF); von Bertalanffy, 1938), the logistic function (Ricker, 1979), and the Gompertz function (Ricker, 1975)) were generated using Markov Chain Monte Carlo (MCMC) analysis (Smart & Grammer, 2021) in R (R Core Team, 2022) using the R package 'BayesGrowth' (Smart & Grammar, 2021). Generalised Linear Models (GLMs) were produced to determine the age-at-maturity (L_{50} and L_{95}) using the R package MASS (Version 7.3-60; Venables & Ripley, 2002).

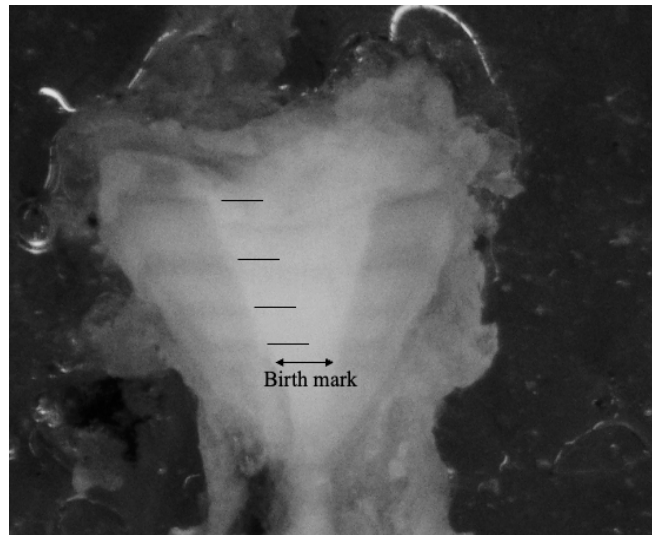


Fig 7.1. Vertebral section from a 4-year-old male Oriental bluespotted maskray (N. orientalis) measuring 276 mm Disc Width (DW), caught from a trap in Indonesia. The birthmark and band pairs are identified.

7.2.2. Hepatosomatic index (HSI)

The Hepatosomatic Index (HSI) is used as an indicator of energy reserves (the ratio of liver weight to body weight) (Goede & Barton, 1990). The HSI is calculated as: $HSI = 100(WL/W)$ where WL = liver weight and W = body weight. The HSI was calculated for sex, month and maturity to identify periods of differing energy reserves. Two-way and three-way ANOVA was run on R-Studio to test for variations in HSI relating to sex, month and maturity. A Tukey's Honestly Significant Difference test (Tukey's HSD) was run on R-Studio to identify where significant differences occurred.

7.3. Results

The Average Percent Error (APE) was 13.2 percent, which is higher than the average reported APE in ageing studies (Campana 2005). Of the 251 samples of bluespotted maskray, 15 vertebrae were unreadable and excluded from further analysis, leaving a total sample size of 236 samples for life-history analysis. Due to uncertainties around the population genetics and species composition of the bluespotted maskrays (as outlined in Chapter 3), three approaches to assign the sampled stingrays to species were considered: (1) the 'criteria' method (outlined in Table 3.3, section 3.2.4) (2) the 'mitochondrial' method (section 3.2.2) and (3) by grouping all bluespotted maskrays together

regardless of species. For the purposes of age and growth analysis and upon advice from a taxonomic and genetic expert (Gavin Naylor pers comm), the ‘criteria’ method, was predominantly used for further analysis. This assignment process resulted in 45 samples of mahogany maskray *N. varidens* (17 female, 28 male), 104 samples of oriental bluespotted maskray *N. orientalis* (55 female, 49 male), and 102 samples of undetermined species (47 female, 55 male).

7.3.1. Age-growth analysis

Based on the method outlined in Table 3.3 (section 3.2.4) whereby species composition was determined using the ‘criteria’ method, mahogany maskrays matured at a smaller size (smallest mature male 208 mm DW, smallest mature female 187 mm DW) compared to oriental bluespotted maskrays (smallest mature male 221 mm DW, smallest mature female 199 mm DW) and the undetermined species of bluespotted maskrays (smallest mature male 243 mm DW, smallest mature female 238 mm DW). Males from all three groups showed similar clasper length at maturity (Figure 7.2).

Mahogany maskrays also attained a smaller size in general (323 mm maximum recorded DW) compared to oriental bluespotted maskrays (366 mm maximum recorded DW) and undetermined species of bluespotted maskrays (370 mm maximum recorded DW) (Figure 7.3). Females from all three groups reached larger sizes and heavier weights than males (Figure 7.3).

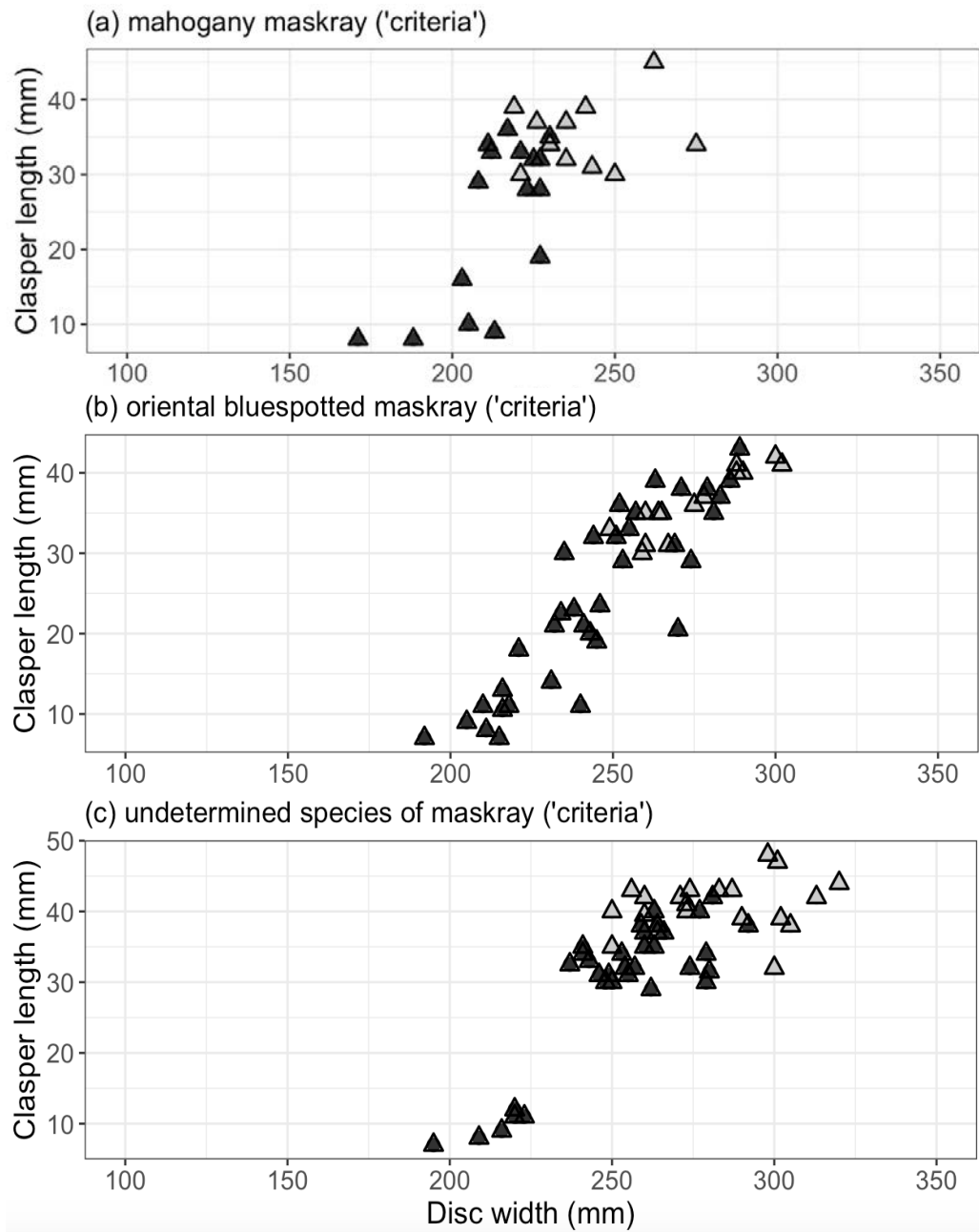


Fig 7.2. Relation of clasper length to disc width (DW) and maturity (black $\blacktriangle$ =uncalcified claspers, grey $\triangle$ =calcified claspers) for male maskrays: (a) 28 mahogany maskray (*Neotrygon varidens*), (b) 49 oriental bluespotted maskray (*Neotrygon orientalis*), (c) 53 undetermined species of maskray (*Neotrygon* spp.), as determined by the 'criteria' method (outlined in Table 3.3, section 3.2.4), showing maturity is attained from 219 mm DW and 30 mm clasper length for mahogany maskrays, 249 mm DW and 30 mm DW for oriental bluespotted maskrays, and 250 mm DW and 32 mm clasper length for undetermined species of bluespotted maskrays.

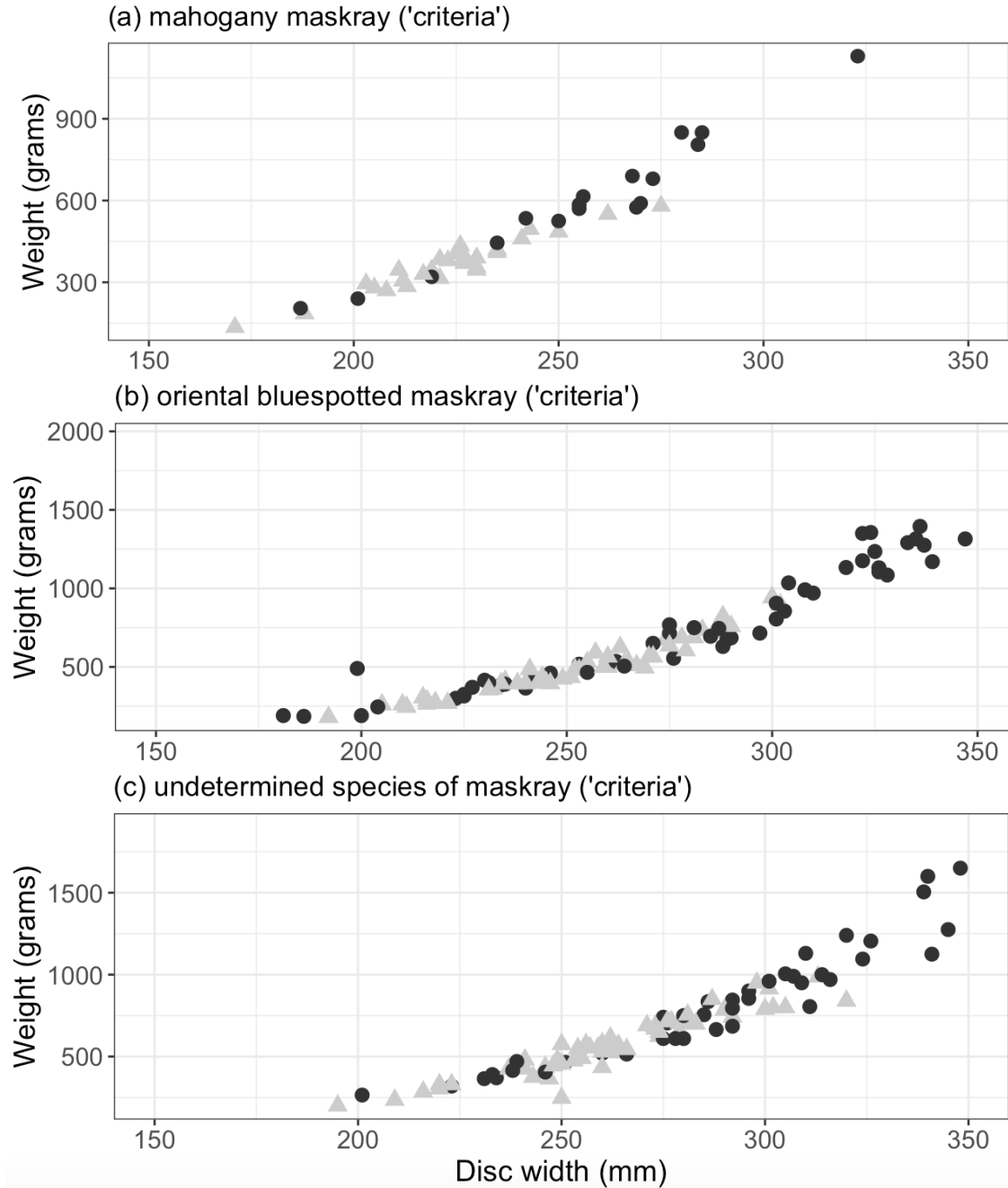


Fig 7.3. Length-weight relationship for female (●, $n=119$) and male (▲, $n=132$) maskrays: (a) 45 mahogany maskray (*Neotrygon varidens*), (b) 104 oriental bluespotted maskray (*Neotrygon orientalis*), and (c) 102 undetermined maskrays (*Neotrygon* spp), as determined by the 'criteria' method (outlined in Table 3.3, section 3.2.4). Length-weight equation (Pauly, 1983) is $W=0.3358821*L^{1.3611}$ for all females and $W=0.5277392*L^{1.232}$ for all males.

Using the ‘criteria’ method for determining species (as outlined in Table 3.3, section 3.2.4), the oldest agreed age (between the readers) from the study was 11 years old for two females (both undetermined species of maskray) which were 309 mm DW and 326 mm DW respectively (Figure 7.4). The oldest male in the sample was an undetermined species of bluespotted maskray which was eight years old at 273 mm DW.

MCMC analysis revealed that out of several potential growth models, the Von Bertalanffy (VB) model was the best performing, matching the data most closely (Appendix S6). The VB model produced a k -value of 0.2 year⁻¹ when analysing all maskrays together regardless of species and sex, and a k -value that ranged between 0.05-0.23 year⁻¹ when examining species groupings separately (Table 7.1). Female maskrays reached maturity earlier (female L_{50} maturity = 3.10-3.75 years across species groupings where female sample size was sufficient) compared to males (male L_{50} maturity = 3.75-4.61 years across species groups) (Figure 7.5, Table 7.1).

Comparison of age-growth results by the various approaches to categorising species reveal some differences (Figure 7.4, Table 7.1). Oriental bluespotted maskrays (by ‘criteria’ method, outlined in Table 3.3. Section 3.2.4) had a k -value of 0.21 year⁻¹ and L_{50} maturity of 3.59 years (females) and k -value of 0.58 year⁻¹ and L_{50} maturity 4.52 years (males), while oriental bluespotted maskrays (by ‘mitochondrial’ method, outlined in section 3.2.2) had a k -value of 0.17 year⁻¹ and L_{50} maturity of 3.66 years (females) and k -value of 0.49 year⁻¹ and L_{50} maturity of 4.61 years (males) (Figure 7.4, Figure 7.5, Table 7.1). Either way, males grew faster but reached maturity at later ages.

Mahogany maskrays (by ‘criteria’ method, outlined in Table 3.3. Section 3.2.4) had a k -value of 0.33 year⁻¹ and L_{50} maturity of 2.08 years (females), while males had a k -value of 0.44 year⁻¹ and L_{50} maturity of 3.75 years (males). Mahogany maskrays (by ‘mitochondrial’ method, outlined in section 3.2.2) had a k -value of 0.52 year⁻¹ and L_{50} maturity of 1.11 years (females) while males had a k -value of 0.26 year⁻¹ and L_{50} maturity of 3.79 years (males) (Figure 7.4, Figure 7.5, Table 7.1). The low sample size of immature female mahogany maskrays ($n=2$, using ‘criteria’ method and $n=3$ using the ‘mitochondrial’ method; Table 7.1) may account for the low L_{50} maturity reported for females of this species.

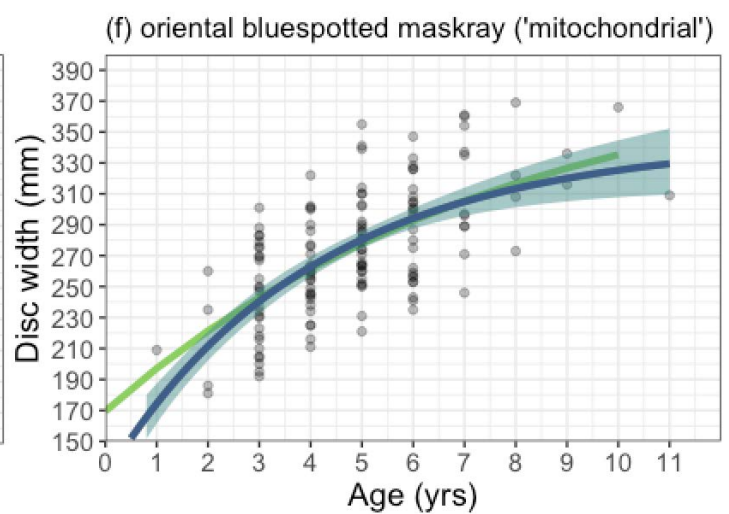
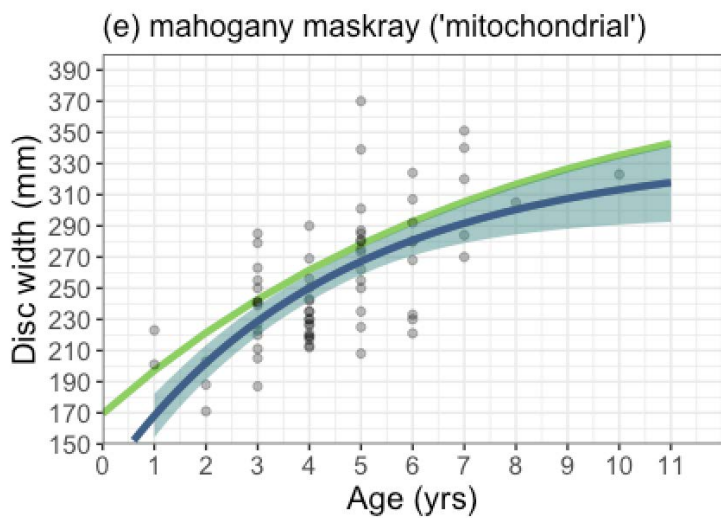
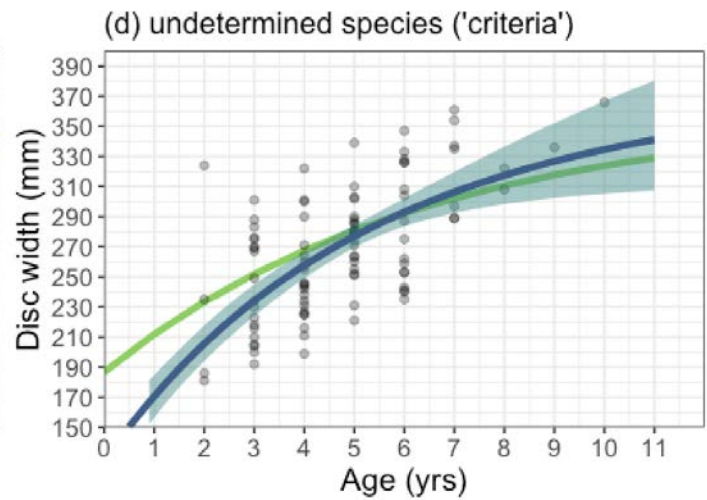
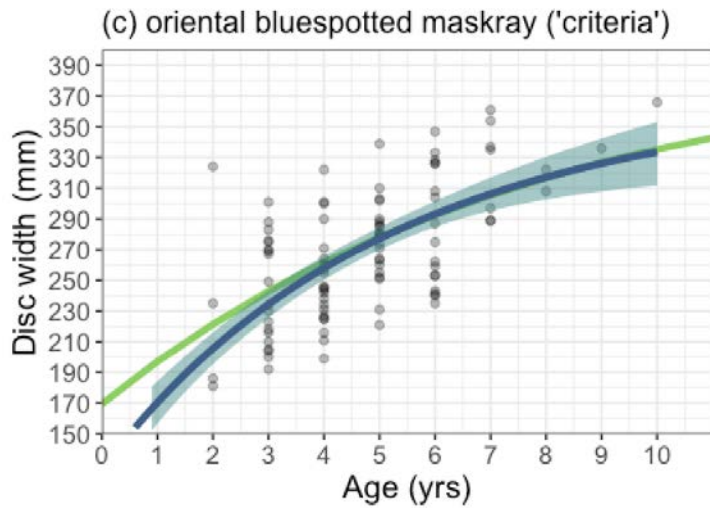
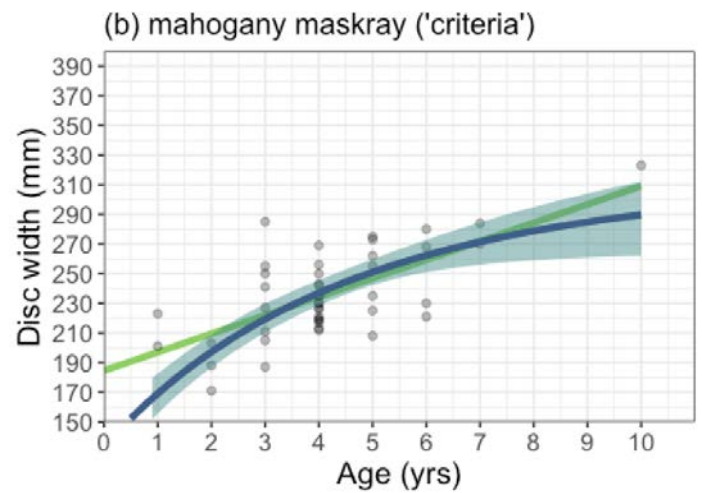
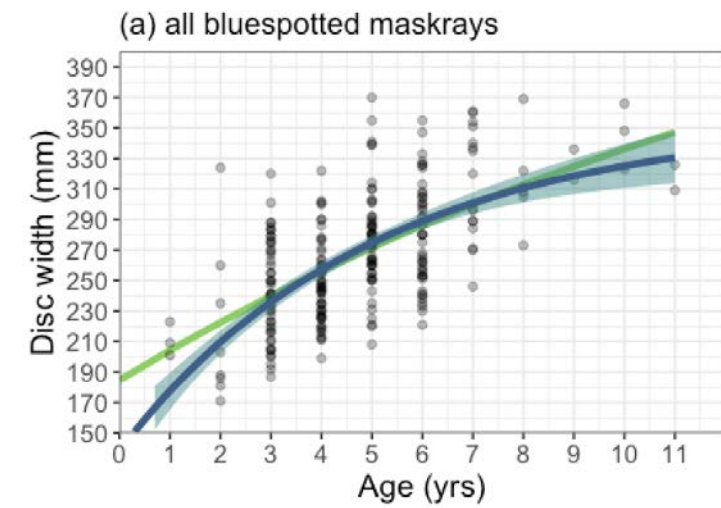
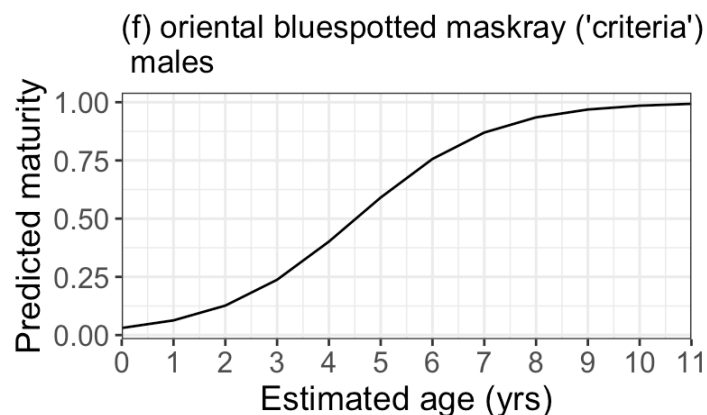
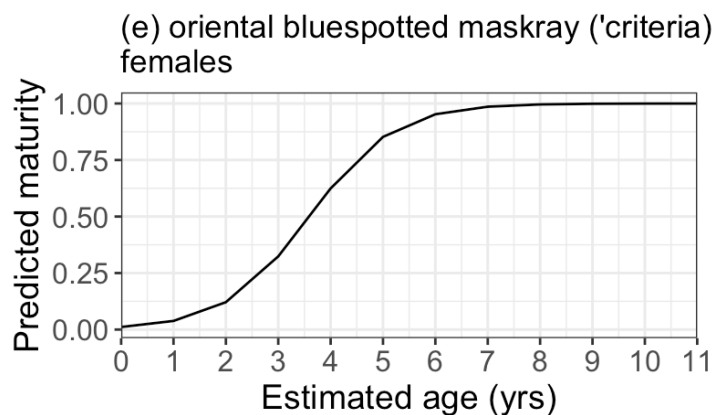
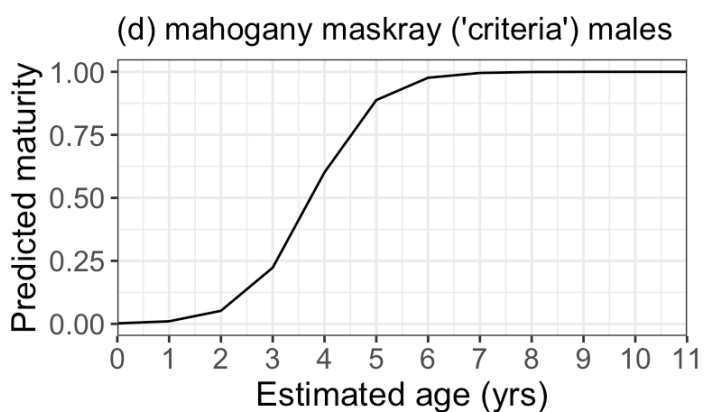
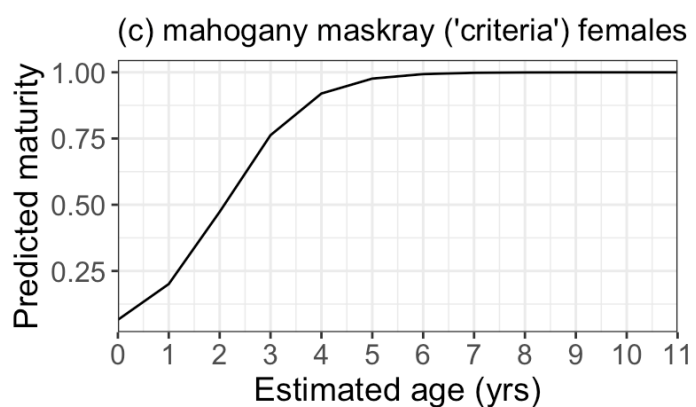
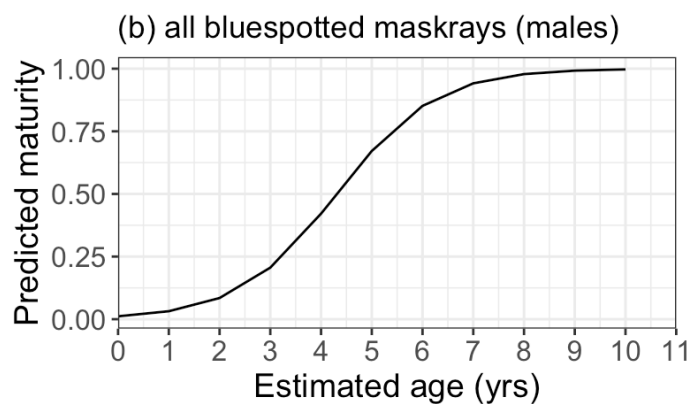
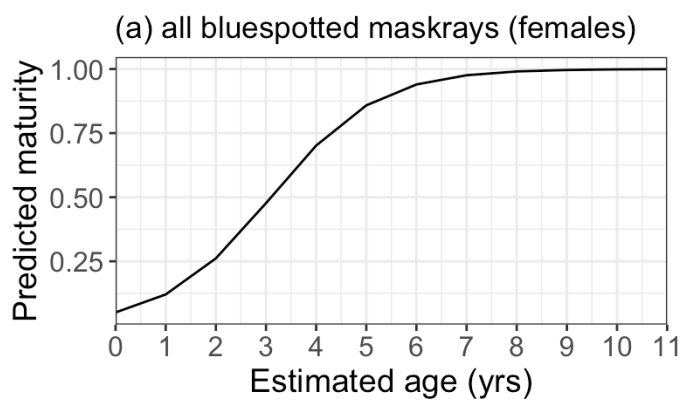


Fig 7.4. Age-growth curve for 236 bluespotted maskray (Neotrygon spp.) using vertebral band counts and the MCMC analysis performed using Bayesian and frequentist models. Circles (●) represent individual rays. Lines indicate the modelled length and age values (green = Frequentist, blue - Bayesian) with light blue shading indicating the 95% confidence intervals: (a) all bluespotted maskrays (n=236) regardless of species, (b) mahogany maskrays (n=45) categorised using 'criteria' method (outlined in Table 3.3, section 3.2.4), (c) oriental bluespotted maskrays (n=104) categorised using 'criteria' method (outlined in Table 3.3, section 3.2.4), (d) undetermined species of bluespotted maskray categorised using 'criteria' method (outlined in Table 3.3, section 3.2.4), (e) mahogany maskray (n=69) categorised using 'mitochondrial' method (as outlined in section 3.2.2) and (f) oriental bluespotted maskrays (n=135) categorised using 'mitochondrial' method (as outlined in section 3.2.2).



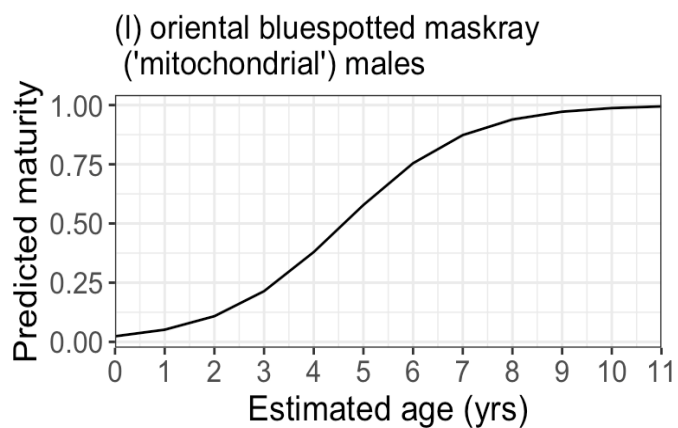
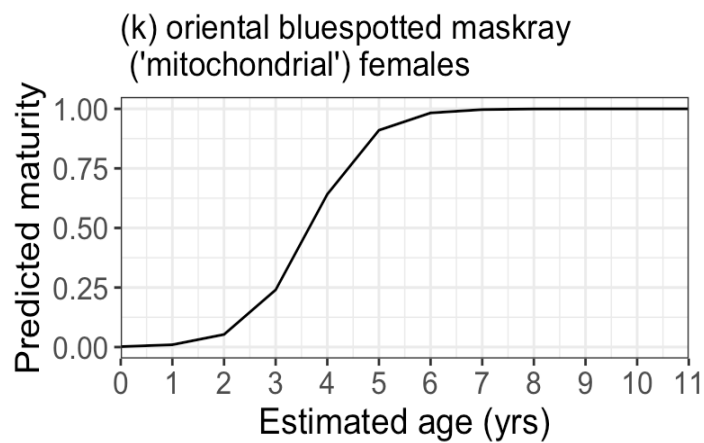
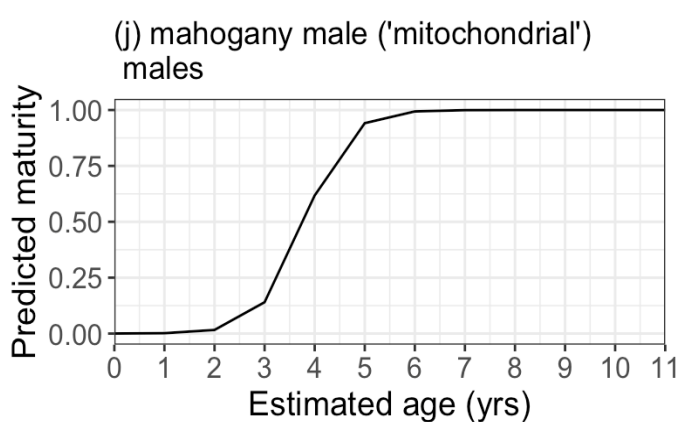
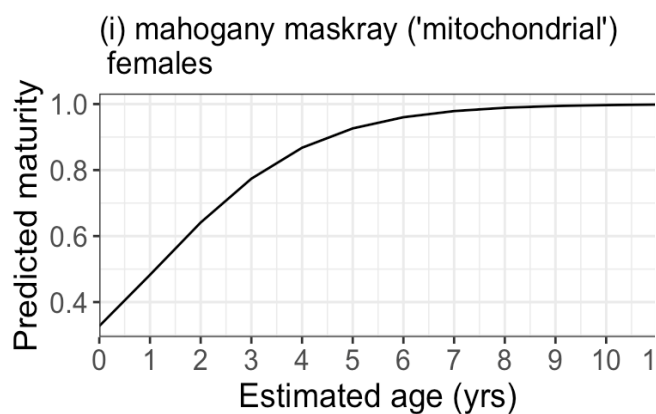
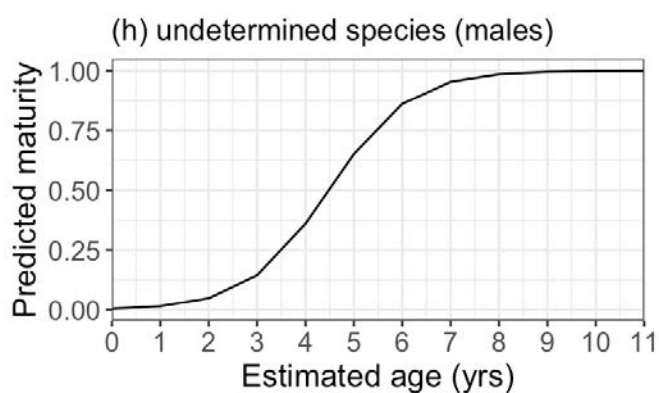
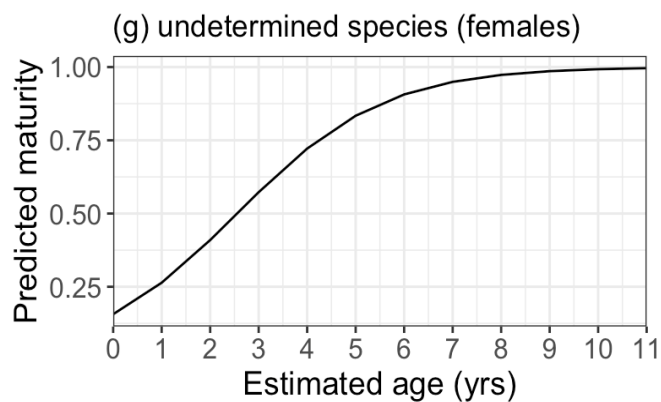


Fig 7.5. Logistic generalised linear models (GLMs) of bluespotted maskray showing predictions of maturity at a given age. (a) female and (b) male bluespotted maskrays (n=236) regardless of species, (c) female and (d) male mahogany maskrays categorised using 'criteria' method (outlined in Table 3.3, section 3.2.4), (e) female and (f) male oriental bluespotted maskray categorised using 'criteria' method (outlined in Table 3.3, section 3.2.4), (g) female and (h) male undetermined species of bluespotted maskray, categorised using 'criteria' method (outlined in Table 3.3, section 3.2.4), (i) female and (j) male mahogany categorised using 'mitochondrial' method (as outlined in section 3.2.2). (k) female and (l) male oriental bluespotted categorised using 'mitochondrial' method (as outlined in section 3.2.2).

Table 7.1. Results of the General Linear model (GLM) showing 50% length-at-maturity (L50) and 95% length-at-maturity (L95), as well as k-value using MCMC analysis (parameter estimates and performance of age-growth models in Appendix S6) for: (a) female and (b) male bluespotted maskrays (n=252) regardless of species, (c) female and (d) male oriental bluespotted maskray categorised using 'criteria' method (outlined in Table 3.3, section 3.2.4), (e) female and (f) male mahogany maskrays categorised using 'criteria' method (outlined in Table 3.3, section 3.2.4), (g) female and (h) male undetermined species of bluespotted maskray categorised using 'criteria' method (outlined in Table 3.3, section 3.2.4), (i) female and (j) male oriental bluespotted maskray categorised using 'mitochondrial' method (as outlined in section 3.2.2) and (k) female and (l) male mahogany categorised using 'mitochondrial' method (as outlined in section 3.2.2). An * indicates model outputs that are not reliable (e.g. potentially from too small a sample size).

Group	Sex	Sex (n)	Maturity	Maturity (n)	L50 (years)	L95 (years)	k-value	k-value
	Bluespotted maskray (all)							
a	Female	111	Immature Mature	22 89	3.10	6.21	0.23 (sd: 0.05)	0.2 (sd: 0.04)
b	Male	123	Immature Mature	64 59	4.31	7.16	0.52 (sd: 0.12)	
	Oriental bluespotted maskray (criteria)							
c	Female	52	Immature Mature	14 38	3.59	5.96	0.21 (sd: 0.05)	0.2 (sd: 0.04)
d	Male	43	Immature Mature	23 20	4.52	8.36	0.58 (sd: 0.17)	
	Mahogany maskray (criteria)							

Table 7.1 continued...

e	Female	17	Immature Mature	2 15	2.08	4.39	0.33 (sd: 0.14)	0.23 (sd: 0.08)
f	Male	28	Immature Mature	14 14	3.75	5.53	0.44 (sd: 0.22)	
Undetermined species of bluespotted maskray (criteria)								
g	Female	42	Immature Mature	6 36	2.56	7.02	0.44 (sd: 0.22)	0.2 (sd: 0.04)
h	Male	53	Immature Mature	28 25	4.48	6.94	0.75 (sd: 0.27)	
Oriental bluespotted maskray (mitochondrial)								
i	Female	63	Immature Mature	14 49	3.66	5.36	0.17 (sd: 0.06)	0.25 (sd: 0.05)
j	Male	71	Immature Mature	38 33	4.61	8.26	0.49 (sd: 0.13)	
Mahogany maskray (mitochondrial)								
k	Female	31	Immature Mature	3 28	1.11	5.64	0.52 (sd: 0.12)	0.23 (sd: 0.05)
l	Male	37	Immature Mature	18 19	3.79	5.07	0.26 (sd: 0.05)	

7.3.2. Reproductive analysis

Of the 95 mature females in this study, 28 were gravid. Of these, six females had litters of two pups, 18 had litters of just one pup, and four were at an early stage where the number of embryos could not be determined. Litters of two pups were only seen in females of a larger size (Figure 7.6). The youngest gravid females were three years old ($n=4$, ranging from 260-301 mm DW) and the oldest gravid female was 11 years old (326 mm DW). The youngest female showing signs of having given birth recently (postpartum; Figure 7.7a) were four years old ($n=3$, ranging from 199-322 mm DW), and the oldest female showing signs of having just given birth was 11 years old (309 mm DW). The largest embryo observed (Figure 7.7b: 160 mm DW) was 44.3% the size of the mother (361 mm DW). Females across all species groupings showed various ova diameter (Figure 7.7a) as well as stages of pregnancy and signs of postpartum (Figure 7.6b) during the course of the year, suggesting reproduction is asynchronous and year-round. Of the 19 embryos that could be sexed, 10 were female and 9 were males, meaning the sex ration did not significantly differ from 1:1 (chi-square: 0.05, $df=1$, $p=1.00$).

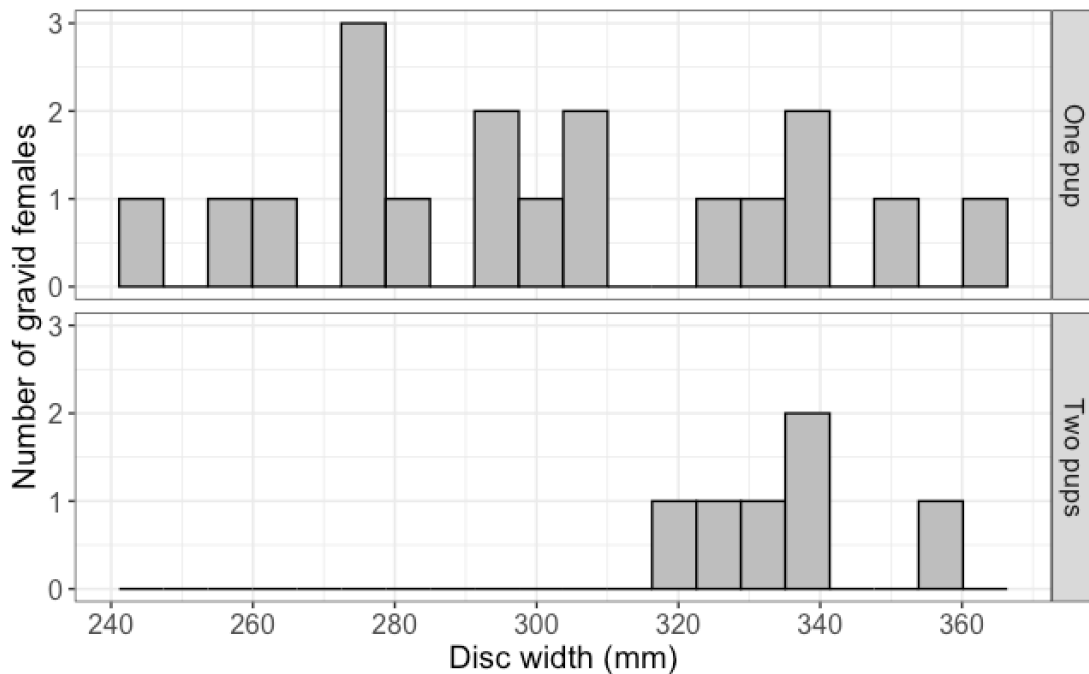


Fig 7.6 Disc width (DW) of gravid female bluespotted maskrays ($n=29$) and their litter size, showing litters of two pups were only seen in larger individuals. The six females carrying litters of two pups were aged 5-11 years old.

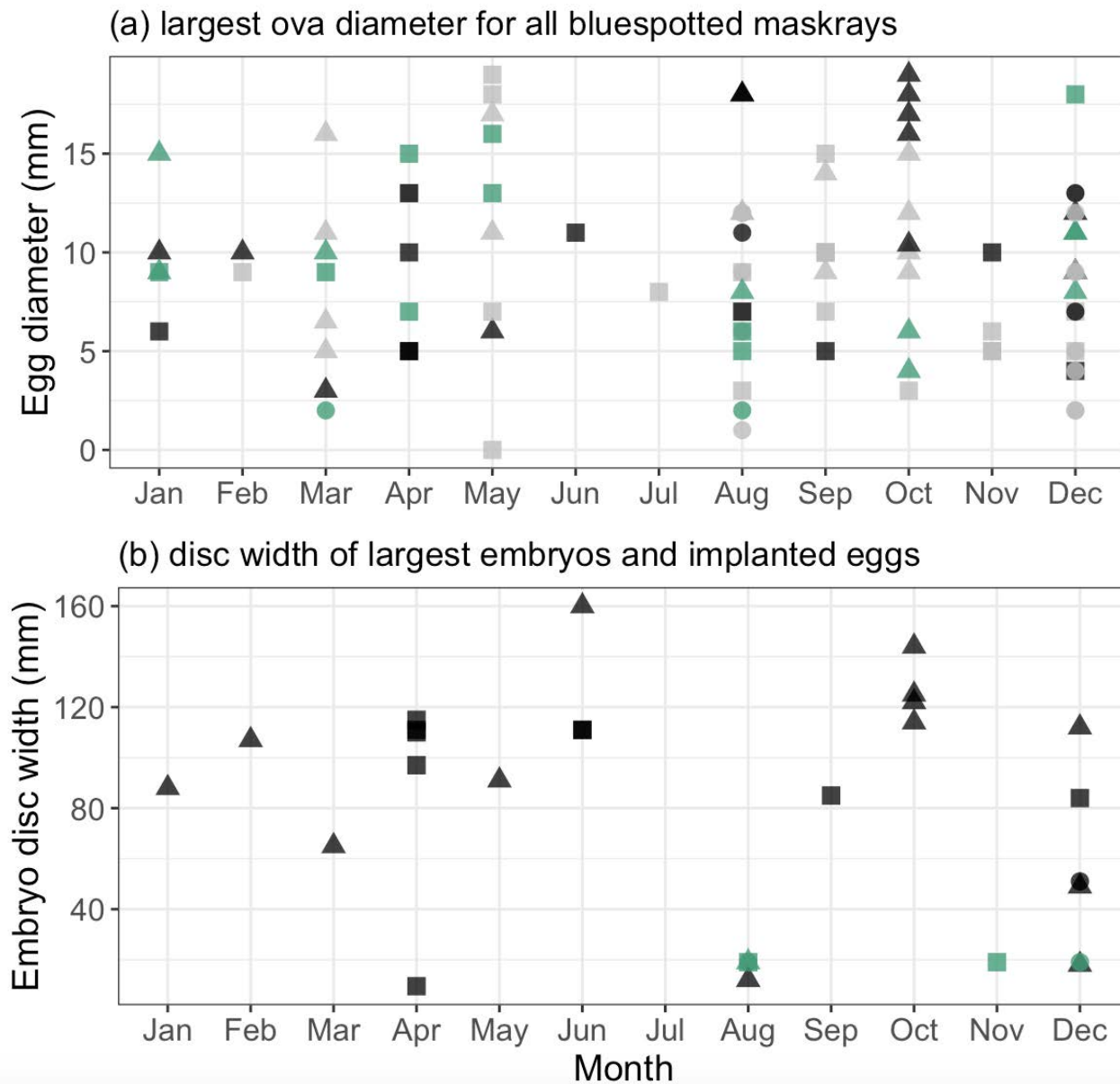


Fig 7.7. Reproductive data for female bluespotted maskrays ($n=94$) ($\blacktriangle$) oriental bluespotted, maskray, *Neotrygon orientalis* ($\bullet$) mahogany maskray, *Neotrygon varidens* and ($\blacksquare$) undetermined species of bluespotted maskray, *Neotrygon* spp. categorised using 'criteria' method (outlined in Table 3.3, section 3.2.4), showing a lack of seasonal patterns in egg or embryo size, and thus asynchronous reproduction: (a) largest ovarian egg diameter by month for gravid (black), postpartum (green) and non-gravid / non-postpartum individuals (grey), and (b) disc width of embryos (black) and length of implanted eggs (green).

7.4.2. Hepatosomatic index (HSI)

Results of the three-way ANOVA revealed a statistically significant effect of sex, month, and maturity on HSI as well as the interaction of sex and month, sex and maturity, and maturity and month (Table 7.3, Figure 7.7). Mature females had significantly higher HSI values than immature females ($P=0.007$), mature males ($P<0.0001$), and immature males ($P<0.0001$). Among mature females, HSI values in December were significantly higher than most other months ($P<0.0001$ - 0.031), and significantly higher than HSI values of immature females in December. There were no significant differences among males pertaining to maturity or month. The highest HSI values (> 6.0) were observed in two mature females, one of which had an early stage pregnancy. The lowest HSI values (>1.25), were observed in two mature males and one mature female (not gravid or post-partum), and one immature female and three immature males. Although post-partum females had a lower mean HSI value (2.84) compared to gravid (3.09) and mature but not gravid or post-partum (3.24) females, this was not significant.

Table 7.3. Results of three-way analysis (ANOVA) for the Hepatosomatic index (HSI) for male (n=109) and female (n=91) bluespotted maskray (*Neotrygon spp.*) for which liver weight was recorded, with significant results marked with *, significance value of $P=0.05$

Source	SS	d.f	Mean square	F	P
Sex	12.40	1	12.401	19.119	2.20e-05***
Month	27.74	10	2.774	4.276	2.73e-05***
Maturity	9.45	1	9.448	14.566	0.0002***
Sex X Month	16.59	10	1.659	2.557	0.0068**
Sex X Maturity	4.59	1	4.589	7.074	0.0086**
Maturity X Month	5.15	9	0.572	0.882	0.5423
Maturity X Month X Sex	8.28	7	1.182	1.823	0.0862
Residuals	103.78	160	0.649		

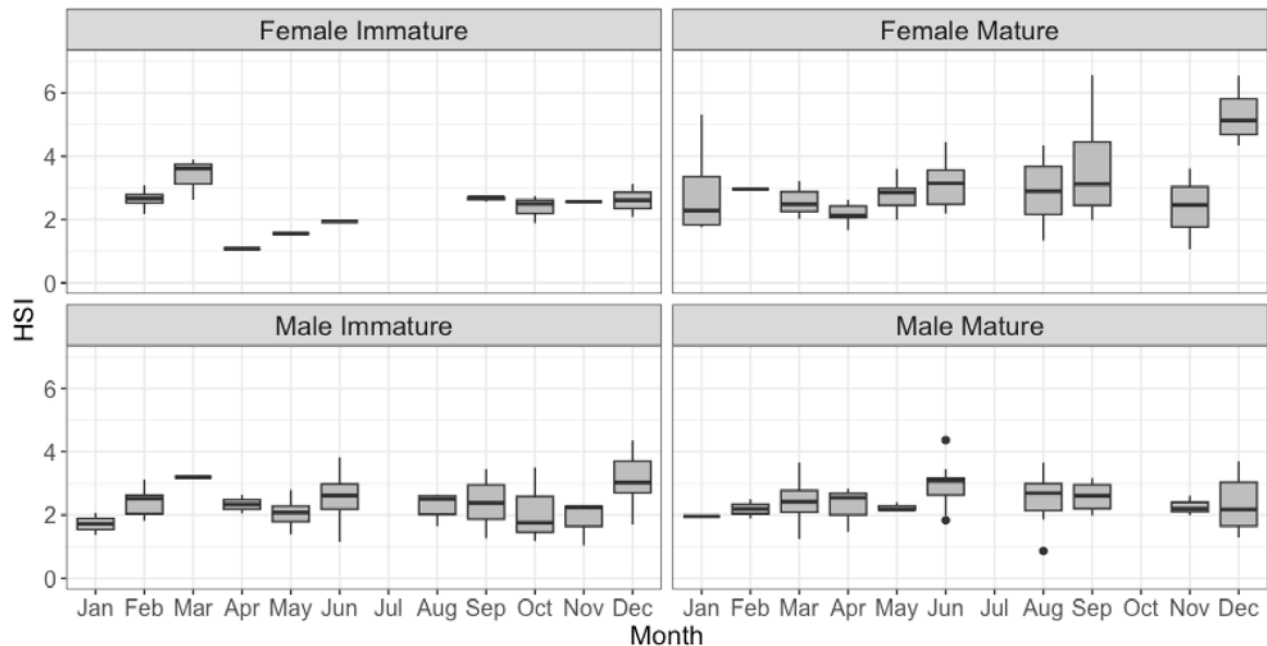


Fig 7.8. Hepatosomatic index (HSI) for male ($n=109$) and female ($n=91$) bluespotted maskray (*Neotrygon* spp.) for which liver weight was recorded, between sexes and maturity and across months. Dark bars within each box represent the median value, the upper and lower boundaries of each box represent the interquartile range, the whiskers represent the total range, and the points outside the box represent outliers.

7.5. Discussion

When grouping all bluespotted maskrays together, as well as examining them by species groups, this study suggests that females mature earlier than males. For all bluespotted maskrays grouped together, females attain 50 percent maturity at 3.10 years and 95 percent maturity at 6.21 years, compared to males which attain 50 percent maturity at 4.31 years and 95 percent maturity at 7.16 years. This contradicts other studies that generally report male stingrays (Dale & Holland, 2012; Ebert & Cowley, 2009) and sharks (Chin et al, 2013a; Grant et al, 2018) as maturing earlier than females. This study also founds that females attain larger sizes and weights than males: the largest female in the sample was 14% larger in disc width, and 50% heavier in weight, than the largest male in the sample. Females also reach older ages: a maximum age of 11 years was recorded for two

females, while the oldest recorded age for a male was eight years. This finding is similar to other studies that report female stingrays (Reis & Figueira, 2020; Schieber et al, 2023; Torres-Palacios et al, 2019) and female sharks (Gervelis & Natanson, 2013; Natanson et al, 2006) attaining larger sizes and older ages than males, including in maskrays (*Neotrygon* sp.) from Australia (Jacobsen & Bennett, 2010). One hypothesis for why females attain larger sizes is that larger uteri can carry more embryos and thus increase fecundity (Klimley, 2013). Indeed, this study found that only larger females carried two pups while smaller females carried one pup, supporting this theory.

A reproductive strategy of just 1-2 pups per litter means the bluespotted maskrays in this study have a low fecundity (Gutteridge et al, 2013; Last & Stevens, 2009). However, similarly to the blackspot shark, the largest embryo in this study was 44.3% the size of the mother; and this large size-at-birth increases neonate survival and compensates for this low fecundity (Branstetter, 1990). Bluespotted maskrays from this study appear to be asynchronous breeders, with gravid females, carrying embryos at various stages, observed in 11 out of 12 months, and post-partum females (which may be from natural births or catch-induced abortion which is reported in some dasyatid rays (Smith et al, 2007) observed in seven out of 12 months. For some species of stingray, such as the blue stingray (*D. chrysonota*) from Southern African waters, breeding is synchronous and during summer months as this may be energetically more optimal (Ebert & Cowley, 2009). However, due to temperature stability in the tropics, this likely has less importance to species in Southeast Asia hence breeding is asynchronous (Harry et al, 2013). Although gestation period could not be established in this study, bluespotted maskrays in Australia reportedly have a gestation period of four months, with one litter produced each year (synchronous breeding during the summer months) (Pierce et al, 2009). If a similar assumption is applied to bluespotted maskrays in Southeast Asia, then a litter of 1-2 pups per year from age three (youngest gravid female observed in this study) to age 11 (oldest gravid female in this study), then one female may only produce 8-16 pups over their lifetime, which is a very low reproductive output, as is reportedly common among Dasyatid rays (Ebert & Cowley, 2009). This limitation may also explain why female bluespotted stingrays mature earlier than males; they need to begin reproducing as early as possible to ensure that they produce enough offspring to maintain population viability. However, bluespotted maskrays in Southeast Asia may have more than one litter per year due to asynchronous breeding, which needs further investigation.

Using the ‘criteria’ method for categorising species, oriental bluespotted maskrays reached larger sizes than mahogany maskrays (9-12% larger by disc width for males and females respectively, and 39-43% larger by weight for males and females respectively) which aligns with existing descriptions of the species whereby mahogany maskrays have a smaller maximum size than oriental bluespotted maskrays (Last et al, 2016). Oriental bluespotted maskrays also appear to mature later (between 3.69 and 7.16 years) than mahogany maskrays (between 2.08 and 5.53 years). These pattern remains when grouping animals using the ‘mitochondrial’ method. Differences in age-at-maturity between species of maskray (*Neotrygon* sp.) were also reported from Australia: age at first maturity for the speckled maskray and plain maskray was 2-3 years compared to 5-7 years for Kuhl’s maskray (Jacobsen & Bennett, 2010). However, caution must be exercised in this study due to the low sample size of immature female mahogany maskrays (n=2 when categorising animals using the ‘criteria’ method, and n=3 when grouping animals using the ‘mitochondrial’ method). A larger sample size of immature female mahogany maskrays would help to confirm these findings. The observed ages-at-maturity from this study are younger than some species of ray, such as the brown stingray which matures from 15 years for females and 8.3 years for males (Dale & Holland, 2012), but slower than others, such as the yellow stingray where males and females reach maturity before one year of age (Schieber et al, 2023). Again, the relatively young age at maturity evident in the present study may reflect the species’ requirement to begin reproducing as soon as possible given their limited fecundity over a relatively short lifespan.

This study found no difference between the lifespans of the two species: a maximum age of 10 years was recorded for both a female oriental bluespotted maskray and a female mahogany maskray, and a maximum age of seven years was recorded for a male oriental bluespotted maskray and six years for a male mahogany maskray (using the ‘criteria’ method for categorising species). Two females of undetermined maskray species had a maximum age of 11 years. A study on speckled maskrays from Australia found a maximum age of 11 for males and 18 for females (Jacobsen & Bennett, 2010). Bluespotted maskrays in Southeast Asia may have shorter lifespans, or older individuals may be rarer to come by, which could apply if larger individuals are disproportionately caught by fishing gear (Marquez-Farias et al, 2022). There may also be size-related habitat segregation, as larger-bodied animals are safer from predators and can venture further offshore (O’Shea et al, 2013). If bluespotted maskrays are segregating by size, larger and older individuals may not have been

encountered in these particular near-shore trap fisheries. The largest individuals encountered in this study were five females ranging in size from 366 mm to 371 mm, and three of these individuals came from longline fisheries which, according to the seafood supplier interviewed in this study, operate farther offshore than the fishing traps (Chapter 4), suggesting that indeed, larger individuals may occur farther offshore. The largest reported size for the species is 380 mm disc width (Last et al, 2016) suggesting the species may live beyond the 11 years estimated in this study.

Considering this study found sex-related differences for maximum sizes and age-at-maturity, age-growth analysis was performed separately for males and females. The animals show a fairly fast growth rate – including when grouping all bluespotted maskray species together (k-value of 0.23 year⁻¹ for females vs k-value of 0.52 year⁻¹ for males) – and when categorising species using the ‘criteria’ method: oriental bluespotted maskrays (k-value of 0.21 year⁻¹ for females vs 0.58 year⁻¹ for males); mahogany maskrays (k-value 0.33 year⁻¹ for females vs 0.44 year⁻¹ for males). Therefore an overall k-value of 0.21-0.33 year⁻¹ for females, 0.44-0.58 year⁻¹ for males (and 0.21-0.58 when grouping both sexes of these species together) can be applied generally to Southeast Asia’s oriental and mahogany bluespotted maskrays. When analysing males and females together, all species groupings (all together, ‘criteria’ method, and ‘mitochondrial’ method) generated a k-value of 0.2 year⁻¹. This is similar although not identical to maskrays (*Neotrygon* sp.) in Australia (the speckled maskray, plain maskray, and kuhl’s maskray) which have reported k-values of between 0.08-0.31 for females and 0.08-0.20 for males (Jacobsen & Bennett, 2010).

While the results of this study reveal differences between the size, maturity and weight of mahogany maskrays and oriental bluespotted maskrays, the analysis finds similarities between oriental bluespotted maskrays and undetermined species of bluespotted maskrays. This may suggest that the rays for which species could not be determined (because of potential hybridisation, introgression, or simply inability to correctly ID; as outlined in Chapter 3), may be more similar to oriental bluespotted maskrays than mahogany maskrays in terms of biology. There is much confusion within the bluespotted maskray complex, with species being continually re-described and new species classified (Borsa et al, 2016; Borsa et al, 2018; Last et al, 2016). Within this study, there was confusion regarding how to classify species, with mis-matches between a species’ morphology and their DNA (as described in Chapter 3). This created difficulties analysing life-history data as a large

portion of samples were declared ‘undetermined species’. Therefore, establishing broad life-history findings, and general management measures that can represent multiple species in this complex, may be a more practical way forward. Overall, this study suggests that bluespotted maskrays in Southeast Asia experience a fairly rapid growth (with a broad k -value = 0.17-0.75 when analysing species via different species-groups), a moderate age-at-maturity (from 2-3 years old), a relatively short life-span (max age eight for males and 11 for females, but possibly longer), and a low fecundity (1-2 pups per litter). The low fecundity of bluespotted maskrays in particular, as highlighted in this study, as well as in other regions (Pierce et al, 2009), makes them vulnerable to exploitation and population depletions as populations are less able to rebound quickly. Although bluespotted maskrays in Asia are currently listed as ‘Least Concern’ by the IUCN (Sherman et al, 2022a, 2022b), the seafood supplier interviewed in this study estimates a population decline of 50% over his time in the industry, and there may be a potential exploitation shift to this species as the *Maculabatis* sp. populations decline (as outlined in Chapter 4). Given the life-history data from this study, and potential changes in the market which will potentially exacerbate exploitation, further monitoring of catches and landings, along with further age and growth assessment (to clarify age and growth parameters in this study) should be executed. Together, this information can be used for an updated risk assessment for these species.

Chapter 8:

Concluding remarks and discussion

8.1. Introduction

Understanding the conservation-needs of a species requires a multi-disciplinary approach (Booth et al. 2019; Cheddadi et al. 2020; Garcia et al. 2008). Biological data (e.g. age-growth relationships, reproduction) provides insight to a species' intrinsic ability to proliferate and the biological limits to exploitation (Garcia et al, 2008); ecological studies (e.g. examining diet) give insight to how animals spatially arrange themselves, as well as trophic interactions which are the foundations of ecosystems (Van der Putten et al. 2004); genetic and taxonomic work sheds light on evolutionary processes and population interactions (Frankham 2003); and exploring human dimensions such as LEK (Booth et al, 2019), can shed light on key animal habitats and behaviour (Berkstrom et al. 2019), as well as reveal how, why and where animals experience mortality. Through inclusion of biological, ecological, genetic and social science, this thesis has provided holistic insights to the life and death of the blackspot shark (*C. sealei*) and bluespotted maskray (*Neotrygon* spp.), which combined, provides a better understanding of how to manage and conserve them.

8.1. Summary of findings

Genetics analysis revealed all sharks in the sample to be blackspot sharks. Despite having a moderately fast growth rate ($k=0.37$), blackspot sharks have a late age-at-maturity (~6 years old), especially considering their short life-span (maximum recorded age of 11 years old for females and nine years old for males in this study), and a low fecundity (2 pups per litter), which makes it harder for populations to rebound from exploitation. Indeed, it is suspected that the species has experienced a population reduction of 30-49% over the last 24 years (Dulvy et al, 2020), and the private supplier interviewed in this study estimated a decline of 50-70% over the last 45 years (Chapter 4). While the declines reported by the IUCN are inferences of global population trends, and the reports by the supplier speculative, these trends may suggest that the species is at high risk of over-exploitation

and may be experiencing declines. The bias toward males in the sample, and observed dietary differences between the sexes, suggests resource or spatial partitioning; with mature males consuming more bony fishes, mature females consuming more cephalopods, and immature animals consuming more crustaceans than mature animals.

The bluespotted maskrays were more challenging to study due to conflicting results in relation to species identification from genetic and morphological techniques (Chapter 3) which raised questions about existing species descriptions (Borsa et al. 2016). Genetic and morphological analysis suggested a mixed sample of oriental bluespotted maskrays and mahogany maskrays, but also indicates potential hybridisation, introgression and even a possible distinct species in Singapore (Chapter 3). Considering these uncertainties, establishing findings which can be applied to the species-complex as a whole, may be a more practical approach. Overall, while inter and intra-species difference were revealed (Chapter 7), this study suggests that when grouped together, species in the bluespotted maskrays complex in Southeast Asia experience a fairly rapid growth (k -value = 0.17-0.75), a moderate age-at-maturity (from 2-3 years old), a relatively short life-span (max age eight yrs for males and 11 yrs for females, but possibly longer), and a low fecundity (1-2 pups per litter). Although bluespotted maskrays are listed as ‘Least Concern’ by the IUCN (Sherman et al. 2022a), their low fecundity may make them vulnerable to exploitation. This risk may be exacerbated if fisheries begin to target these species which may be beginning to occur. The private supplier interviewed, reported a change in species availability due to the reduced availability of larger dasyatid species that may be declining (Sherman et al. 2020a), and estimates a decline in availability of 50% during his time in the industry. Further investigation should examine if this could be explained by changes in sourcing and trade (Chapter 4), and if mesopredator release may be occurring in areas where other top predators have been removed, as is the case for the blue-spotted lagoon ray (Sherman et al, 2020b). The diet of the bluespotted maskrays reveal a specialised diet of crustaceans and marine worms, and this specialised diet also puts them at risk if their main prey item is lost (Simpfendorfer et al, 2001).

8.2. Management and conservation implications

8.2.1. Blackspot sharks

The life-history results reveal that blackspot sharks mature late and have low fecundity, which suggests that unregulated exploitation could be detrimental to their populations and improved management should be considered. All requiem sharks are now listed on CITES Appendix II, meaning international trade cannot occur without a permit declaring that it is not detrimental to the survival of the species in the wild (CITES, 2021). The enactment of this listing for *Carcharhinidae* spp. was enforced from November 2023, and includes the blackspot shark (CITES, 2022). As mentioned by the seafood supplier interviewed during this study (Chapter 4), the blackspot shark is often caught incidentally, so any trade restrictions resulting from a CITES Appendix II listing may not reduce mortality, and different fishing gears will require different interventions. Sharks caught by hand lines are reportedly retrieved immediately, when the animal is still alive (see Chapter 4), so training fishers to safely handle and release animals (Poisson et al, 2014) could be a management option. Willingness of fishers to do this should, however, be explored and post-release survival assessed (Booth et al. 2023; Ellis et al. 2017). Sharks caught by longlines are reportedly often deceased by the time the gear is retrieved, so efforts to limit initial capture and mortality are needed. Interventions to reduce shark bycatch recommended for other longline fisheries include reduced number of hooks, attaching lights (although only certain colours may reduce bycatch), reducing soak time, avoiding wire leaders and changing hook and bait type (Afonso et al, 2021; Swimmer et al, 2021; Yulianto et al, 2018). The blackspot shark is morphologically similar to the Indonesian whaler shark (*C. tjotjot*), and somewhat similar in appearance to other coastal Carcharhinid species found in Southeast Asia including blacktip reef sharks and juvenile spottail sharks (they all have black spots on fins although in different places and quantities), and these species are also taken in regional fisheries (Clark-Shen et al, 2022; SEAFDEC, 2017). Difficulties in identification may therefore lead to ineffectual strategies if using a single species approach. Hence, similar management measures could be applied to multiple species (given that all requiem sharks will be listed on CITES Appendix II), or identification training may need to be carried out to help fishers to distinguish between lookalike species (Macbeth et al. 2018). However, additional measures should be considered to manage domestic catch, consumption and use as CITES only regulates

international trade. Hence, country specific management actions including conducting risk assessments, identifying relevant fisheries limits and regulations, considering spatial and/or temporal closures, and creating management strategies to enforce these arrangements, need to be completed.

8.2.2. Bluespotted maskrays

Although the bluespotted maskrays sampled in this study were reportedly caught incidentally in traps and longlines, they still have value and a market once caught, and the seafood supplier interviewed in this study suggests that they may be increasingly targeted as other more valued species of stingrays (such as the whitespotted whipray and sharpnose whipray (*Maculabatis gerrardi* and *M. macrura*), which are targeted for Singapore's 'BBQ stingray' delicacy (Clark-Shen et al, 2021)) experience further declines. For the ease of regulation, efforts that can be broadly applied to all species within the species-complex (e.g. release all bluespotted maskrays over a certain size; protect all bluespotted maskrays regardless of species), may be simpler and more effective than trying to distinguish between similar species whose populations and home ranges may be continually evolving and expanding (Kirkpatrick & Barton, 1997). However, this study did show potentially different life-histories between oriental bluespotted maskrays and mahogany maskrays (e.g. different ages at maturity and maximum sizes), so where geographic, site and species-appropriate conservation measures can be developed and implemented effectively, they should (Lucifora et al, 2011). For example, the 'mahogany maskrays' found at an Island in Singapore (Pulau Ubin) are genetically and morphologically distinct from others in the sample (Figure 3.7). These animals could be particularly vulnerable to localised threats and extinction (Borsa et al, 2016), and so national policies (e.g. adding them to the National Protected Species list (Wild Animals and Bird Act, 2020)) and site-specific efforts (e.g. a designated MPA around Pulau Ubin, or encouraging local fishers to retrieve traps sooner and release bluespotted maskrays while still alive) that protect these animals should be strongly considered.

This study found that only larger female bluespotted maskrays (over ~ 310 mm disc width in this study) carry more than one pup, and so protecting older, larger females may be most crucial. Prioritising the protection of breeding adults is a biological foundation for elasmobranch fishery

management (Prince 2005; McAuley et al. 2006). The supplier interviewed in this study also highlighted that bluespotted maskrays under 1 kg in weight (under ~ 300 mm disc width) are the preferred size for the meat trade (in Singapore at least), and so asking fishermen to avoid, or release, larger individuals (> 300 mm disc width), could be a management option that works for both the species' biology and fishers' preferences. However, fishing gears in Southeast Asia are indiscriminate, at-sea regulations often difficult to enforce (Chapter 2; Clark-Shen et al, 2022) and bluespotted maskrays often deceased when hauled in from fishing traps (as described in Chapter 4). Additionally, the seafood supplier highlighted that even if something has little value, fishermen will want to retain it (Chapter 4), so while in theory this management approach could be beneficial, in reality it may be difficult to fully enforce. However, opinions from a larger sample size of stakeholders regarding the feasibility of management options is warranted before any are dismissed. The seafood supplier holds the opinion that reducing the consumer-demand for stingrays in general may be an easier approach than trying to tackle species-specific fisheries management (as described in Chapter 4). Considering the local delicacy 'BBQ stingray' is particularly unique to Singapore and Malaysian consumers (as described in Chapter 4), focused messaging to this audience could be effective. However, although this delicacy is unique to these two countries and consumer awareness is certainly warranted, stingray meat is also consumed elsewhere (SEAFDEC, 2017a; Chapter 2), and in some areas demand is growing: for example in the Andaman sea, consumption of ray meat has increased as teleost fish decline, and targeted fisheries for rays have developed to meet the demand (Tyabji et al, 2022). There is also a growing trade of products other than meat, including stingray skins (Chapter 2; D'Alberto et al, 2019) and their tails (Armstrong et al, 2023). Considering the capture and trade of stingrays in Southeast Asia is already at high and unsustainable volumes (Chapter 2; Clark-Shen et al, 2021; SEAFDEC, 2017a), and there is growth in other regions and markets (Tyabji et al, 2022; D'Alberto et al, 2019), species of stingray (most notably those in the Dasyatid family, which are particularly impacted by trade (Clark-Shen et al, 2021; Dale & Holland, 2012; SEAFDEC, 2017a)) could be explored as a candidate for listing on CITES Appendix II to limit their trade. Currently, no species of stingray are listed on Appendix I, II or III (CITES, 2023).

8.2.3. Ecosystem-based management

In addition to species-specific management measures, the ecosystem as a whole must be considered: species need healthy prey populations and habitats to survive (Chiaradia et al. 2010). Southeast Asia faces immense overcapacity in the fisheries sector (Pomeroy et al. 2016), with impacts not only on sharks and rays (SEAFDEC 2017), but all marine creatures (with coastal fish stocks just 5-30% of unexploited levels (Silvestre et al. 2003)). Some prey items identified as being important to blackspot sharks, such as squid and fish, are commonly caught, consumed in, and traded from Southeast Asia (FishStatJ). Additionally, six countries in Southeast Asia contribute about 47% of global shrimp landings, and the region reports declining Catch Per Unit Effort (CPUE) for shrimp fisheries (Suuronen et al, 2020). This may impact bluespotted maskrays and juvenile blackspot sharks, which both show high consumption of crustaceans including shrimp. Intense shrimp and lobster fishing have been highlighted as an issue for crustacean-dependent rays in Brazil (Costa et al, 2015). Shrimp fishing is increasingly transitioning to aquaculture farming in Southeast Asia (Suuronen et al, 2020) which may help the situation, but poses a different set of environmental problems (Cao et al, 2007), such as the over-reliance on biomass fishing for the production of aquaculture feed (Huntington & Husan 2009). The diet of bluespotted maskrays (of marine worms and crustaceans), and juvenile blackspot sharks (of crustaceans), highlight their benthic-feeding behaviour, and so bottom-trawling, a particularly destructive fishing practice which affects benthic communities, could be drastically reformed (McConnaughey et al. 2019), or banned regionally. When trawling was banned in Hong Kong, an increase in general species richness (Wang et al. 2021), as well as increased abundance and biomass of shrimp (Tao et al. 2021), was observed. Trawling also accounts for high rates of shark and ray bycatch (SEAFDEC 2017a), and so a ban or reform on this fishing gear would likely help to reduce shark and ray mortality. Indonesia bans the use of certain trawls in their waters (Chong et al, 1987), which in the Java sea resulted in observed increases of ray biomass (Tirtadanu & Suprpto, 2017), and Malaysia prohibits trawling within 0-5 nautical miles of the coast (Wahida et al. 2022), showing that there is existing recognition of how unsustainable these fishing gears are, making it a potential ‘low-hanging fruit’ in fisheries reform.

After the disappearance of primary prey, predators may shift to lower energy prey (Costa et al, 2015), exhibit lower reproductive success and eventually a reduction in population size (Chiaradia et al, 2010). Ecological studies like this one highlight the need for broader, ecosystem-driven

management, rather than solely elasmobranch-focused management, that may increase the survival of sharks and rays while not accounting for the depletion of resources they need to survive.

8.2.4. Other comments

If devising management measures relating to the life-history stage of the animals (e.g. release or avoiding capture and sale of adults or juveniles), practitioners should consider communicating such measures both in terms of size (e.g. release animals over 700 mm STL) and weight (e.g. release animals over 1kg); as while size may be practical for fishers, traders and sellers often operate in terms of weight (Pers. Obs.).

8.3. Future research

The readability of vertebrae in this study was fairly low, with above-average APE scores between readers (Campana 2005). Therefore, further studies to help validate reported findings would be beneficial. A Population Viability Assessment (PVA) utilising life-history data generated from this study, would help to reveal which segments of blackspot shark and bluespotted maskray populations are most important to protect (Carlson & Simpfendorfer, 2015). PVAs are a modelling tool that use life-history data to predict the likelihood of a population persisting into the future under different exploitation and management scenarios—for example, for smalltooth sawfishes, with an age-at-maturity of seven years old, and in the absence of fishing mortality or catastrophic climate effects, the population grew relatively rapidly and recovered in 40-50 years (Carlson & Simpfendorfer, 2015). A PVA could help to inform size and sex-related catch and release schemes, or other measures to make fisheries more sustainable (Oktaviyani et al. 2021). Additionally, collecting catch and effort data should be included in future work as density index (CPUE) is a key index to reflect the stock status, and is recommended protocol for collecting data on sharks and rays within Southeast Asia (SEAFDEC 2016).

Future research should also examine the habitat-use and movement of blackspot sharks and bluespotted maskrays, such as through tagging and tracking studies, which can be used alongside life-history information and other criteria to design appropriate spatial protection (Chin et al. 2022).

Future research could work with fishers in Indonesia to identify catch locations of blackspot sharks to establish critical habitats; determine whether sex and size segregation occurs (as is suspected from the dietary analysis and sample bias from the fishery); and examine survivability of animals when hauled in by different gear. Working with fishermen that haul in blackspot sharks that are alive (as described in Chapter 4 for the hook and line fishery that caught the animals in this study), to attach telemetry tags and then release animals, would help to determine home range size and whether segregation occurs (Chin et al. 2016), and understand post-release survivability for this species (Musyl & Gilman, 2018), which will be crucial for devising fisheries management measures that reduce mortality. Unlike some large-bodied species, small-bodied sharks are known to consistently use nearshore regions as both juveniles and adults (Chin et al. 2013b; Heupal et al. 2006; Knip et al. 2012), highlighting how critical it is to protect these habitats for overall survival. However, a population-genetics assessment of blackspot sharks in Brunei suggests that blackspot sharks may be migratory and have wider ranges than Indonesian whaler sharks (Azri et al. 2020), which if true, will need to be accounted for in MPA design or other fisheries management arrangements.

There is still limited information on stingray home-ranges and movements (Elston et al, 2022), however, a study on blue stingrays (*Dasyatis chrysonota*) in South Africa found that even though the species is small-bodied, they travel large distances (up to 200 km), use movement corridors linking different habitats, and spend time inshore and offshore, highlighting that existing MPA zonation may not be sufficient to protect them (Elston et al, 2022). And in New Zealand, there is molecular evidence to show male-biased dispersal in short-tail stingray populations (*Bathytoshia brevicaudata*), suggesting that males may range between populations more than females (Roycroft et al. 2019). Bluespotted maskrays are a widespread species-complex, across parts of Asia, Southeast Asia, and Australia (Last et al, 2016) and so similar research on their movements could have far-reaching applications in designing appropriate MPAs across these coastal areas. Additionally, bluespotted maskrays are valuable to tourism as they are commonly encountered by scuba divers, giving further incentive as to why countries should invest in their conservation.

While further research could seek to clarify species within the bluespotted maskray complex, genetics work is costly, and the importance of such results to devising conservation and management plans should be considered, especially as it is possible that the species-complex will continue to

evolve and diverge (Kirkpatrick & Barton, 1997). However, while the creation of policies to protect the species-complex as a whole may offer a more practical approach, the identification of new or distinct species may help to bolster species-protection in its own way; for example, based on morphological and genetic analysis (Chapter 3) this thesis suggests that the maskrays found in Singapore may be distinct and unique to the country. This discovery may better incentivise Singapore and its citizens to celebrate and conserve them.

8.3. Concluding remarks

Research and conservation efforts are generally more biased towards large species of elasmobranchs which are considered more vulnerable (Garcia et al. 2008), but this study of small-bodied species reveals life history, prey dependencies, and fisheries threats which put them at risk – particularly considering the large volumes in which they and their prey are captured and traded around the region (SEAFDEC 2017a; Clark-Shen et al. 2021). Conservation typically adopts a ‘reactive’, rather than a ‘proactive’, approach, with measures often only established once a population is in a critical state (Drechsler et al. 2011), and usually favouring the more charismatic species (Bellon 2019; Colleony et al. 2017). There remains a lack of urgency to proactively protect small-bodied sharks and rays; but the recently announced Extinction of the Java stingaree, a small-bodied ray found in Indonesia (Constance et al. 2023), serves as a stark warning of what else is to come if we remain placid in our approach to conservation and management.

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Appendix

Appendix S1

A comparison of elasmobranch species caught as bycatch and from target fisheries. ‘(A) Predominantly bycatch in select SEA fisheries’ are the top 10 most commonly caught elasmobranchs by number (and in order of highest to lowest) from fisheries landing their catch in Cambodia, Indonesia, Malaysia, Myanmar, Thailand and Vietnam from 2015 to 2016, with sharks and rays reported to be predominantly bycatch (SEAFDEC 2017a). The ‘Fishing gear’ column reports on studies that explicitly state which gear these species were by-caught with in respective countries (SEAFDEC 2017a; Clark-Shen et al. 2021). The ‘(B) Targeted in an Indonesian fishery’ are the top 10 most commonly caught species by number (and in order of highest to lowest) in one targeted shark fishery in Lombok Indonesia (Yulianto et al. 2018). The ‘Fishing gear’ column reports on other studies that explicitly state which gear these species were caught with in targeted shark fisheries in respective countries (Blaber et al. 2009; DoF Philippines 2009; Sulaiman et al. 2018; Clark-Shen et al. 2021).

(A) Predominantly bycatch in Select SEA fisheries					(B) Targeted in one Indonesian fishery				
Species	Environment	Size	Status	Fishing gear (supplementary)	Species	Environment	Size	Status	Fishing gear (supplementary)
Pale-edge sharpnose ray <i>Dasyatis zugei</i> (<i>Telatrygon zugei</i>)	Demersal, continental	Small	VU	Trawl (CA, MY, TH, VN)	Silky shark <i>Carcharhinus falciformis</i>	Pelagic, oceanic	Large	VU CITES II	Longline (IND, PH) Hook and line (PH)
Scaly (dwarf) whipray <i>Himantura walga</i> (<i>Brevitrygon walga</i>)	Demersal, coastal	Small	NT	Trawl (CA, MY, MYA, TH, VN, IND); gillnet (MYA); trammel (IND)	Black tip shark <i>Carcharhinus limbatus</i>	Pelagic, inshore/offshore	Large	VU	Longline (IND, PH) Hook and line (PH); Gillnet (PH)
Brownbanded bamboo shark <i>Chiloscyllium punctatum</i>	Demersal, coastal	Small	NT	Trawl (CA, MY, TH, VN, IND, SG); gillnet (VN)	Scalloped hammerhead <i>Sphyrna lewini</i>	Pelagic, coastal/semi-oceanic	Large	CR CITES II	Longline (IND, PH) Hook and line (PH)

Blue-spotted masray <i>Neotrygon spp.</i> (Consists of multiple cryptic species)	Demersal, coastal/continental	Small	NT	Longline (IND); Trawl (MY, MYA, TH, IND); gillnet (VN, IND); fish trap (IND)	Tiger shark <i>Galeocerdo cuvier</i>	Pelagic, coastal	Large	NT	Longline (IND, PH); gillnet (IND); hook and line (PH); handline (PH)
Whitespotted whipray <i>Himantura gerrardi</i> (<i>Maculabatis gerrardi</i>)	Demersal, coastal	Small	EN	Trawl (MY, TH, IND); gillnet (MYA, IND)	Blue shark <i>Prionace glauca</i>	Pelagic, oceanic	Large	NT	Longline (IND); hook and line (PH)
Hasselt's bamboo shark <i>Chiloscyllium hasselti</i>	Demersal, coastal	Small	EN	Trawl (MY, MYA, TH)	Shortfin mako <i>Isurus oxyrinchus</i>	Pelagic, oceanic	Large	EN	Longline (IND, PH); hook and line (PH)
Coral catshark <i>Atelomycterus marmoratus</i>	Demersal, coastal	Small	NT	Trawl (CA, MY, TH, VN)	Dusky whaler <i>Carcharhinus obscurus</i>	Pelagic, coastal	Large	EN	Longline (IND)
Bigeye thresher shark <i>Alopias superciliosus</i>	Pelagic, coastal / continental / oceanic	Large	VU CITE S II	Gillnet (IND); trawl (VN); longline (IND)	Spot-tail shark <i>Carharhinus sorrah</i>	Pelagic, coastal	Small	NT	Longline (IND, PH) Hook and line (PH); gillnet (PH); handline (PH)
Pelagic thresher shark <i>Alopias pelagicus</i>	Pelagic, oceanic	Large	EN CITE S II	Gillnet (IND); longline (IND); Trawl (MY, VN)	Pelagic thresher <i>Aliopias pelagicus</i>	Pelagic, oceanic	Large	EN CITES II	Longline (IND, PH) Hook and line (PH); gillnet (PH); single hook (PH)

Spottail shark <i>Carcharhinus sorrah</i>	Pelagic, coastal	Small	NT	Trawl (CA, MY, TH, VN, SG); gillnet (MYA, VN, IND)	Houndsharks <i>Hemitriakis sp.</i>	Demersal, continental	Small		Longline (IND)
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Appendix S2

Production, use and trade of elasmobranchs in Southeast Asia. Information is a snapshot of elasmobranch trade and domestic use and does not account for all aspects of trade. Any reference to 'export' and 'import' may include re-export and re-import and are not distinguished. For production rank, exporter rank and importer rank, Okes & Sant (2019) is used for latest information, with Dent & Clark (2015) defaulted to if information is not available in Okes & Sant 2019.

Country	Global production rank	Reported fisheries type	Domestic market	Global exporter rank	Export destinations	Global importer rank	Origins of imports
Brunei		Bycatch; targeted shark fishing banned (SEAFDEC 2006)				Fins: 18 (Dent and Clarke 2015)	Fins: Malaysia (Dent and Clarke 2015)
Cambodia		Bycatch (SEAFDEC 2006, 2017)	Meat consumed locally; shark fins used in local restaurant for important ceremonies; small sharks used for aquaculture feed (SEAFDEC 2006, 2017)		Whole sharks and rays: Vietnam and Thailand (Krajangdara 2011; SEAFDEC 2017)		Fins: China, Vietnam, Taiwan; shark meat: Vietnam, China, Thailand; whole rays and meat: Thailand (SEAFDEC 2006; Krajangdara 2011)
Indonesia	1 (Okes and Sant 2019)	Target and bycatch (SEAFDEC 2006, 2017)	Meat (e.g. meat balls), fins, cartilage and liver oil consumed locally; shark teeth and jaws sold locally as souvenirs; skin used for leather and processed as snacks; head and stomach used in aquaculture and	Fins: 3 Meat: 18 (Dent and Clarke 2015)	Fins: China, Hong Kong, Vietnam, Malaysia, Japan, Singapore, Taiwan; whole sharks and rays: China, Hong Kong, Bangladesh, Sri Lanka, Singapore; powdered cartilage:	Fins: 7 (Dent and Clarke 2015)	Fins: Malaysia (SEAFDEC 2006)

			animal feed (Dent and Clarke 2015; SEAFDEC 2006)		Singapore (Dent and Clarke 2015; SEAFDEC 2006; SEAFDEC 2017; Clark-Shen et al. 2020)		
Malaysia	8 (Okes and Sant 2019)	Bycatch (SEAFDEC 2006, 2017)	Meat (e.g. fish ball, fish cake, salted meat, used in Indian curry, and grilled, cooked or smoked for rays), and fins consumed locally; cartilage consumed in soup; shark teeth and jaws sold as souvenirs; leftover parts such as organs used for fishmeal, bait, and fertilizers (SEAFDEC 2006, 2017; Aswani et al. 2018)	Fins: 10 (Dent and Clarke 2015)	Fins: Singapore , Brunei, Indonesia' , Philippines, Hong Kong; ray meat: China; whole sharks and rays: Singapore; ray skin: Thailand; heads and cartilage: Hong Kong (SEAFDEC 2006, 2017; Worm et al. 2013; Dent and Clarke 2015; Howard et al. 2015; Aswani et al. 2018; Okes & Sant 2019; Clark-Shen et al. 2020)	Fins: 2 (Okes & Sant 2019)	Fins: Thailand, Vietnam, Hong Kong, Indonesia, Myanmar (SEAFDEC 2006)
Myanmar		Target and bycatch (SEAFDEC 2006, 2017)	Meat consumed fresh and dried and often used during traditional water festival and special events; shark skin boiled and made with salad, chilly and lemon juice; intestine and liver consumed		Fins: Taiwan, China, Hong Kong, Thailand, Singapore; most of shark products are exported to China; ray skin: Thailand; jaws: Thailand (SEAFDEC 2006,	Fins: 6 (Dent and Clarke 2015)	

			locally; heads and other parts used as bait (SEAFDEC 2006, 2017; DoF Myanmar 2015); small-bodied carcharhinid sharks butterflied, salted and dried and made into salted fishes; fins from small sharks dried (Personal obs. Shin Arunrugstichai)		2017; DoF Myanmar 2015)		
Philippines	31 (Dent and Clarke 2015)	Target and bycatch (DoF Philippines 2009)	Meat (e.g. fish balls, coconut-infused dish called Kinunot mainly with thresher sharks or rays, dried manta ray meat and skin a delicacy in some areas), small fins consumed locally; jaws sold in souvenir shops; tails of some rays kept to ward off supernatural forces and skin used for high-end furniture in Cebu city (SEAFDEC 2006; DoF Philippines 2009; Acebes et al. 2016b; Pomeroy et al. 2016; Personal communication,		Fins, liver oil, gill plates: large fins, liver oil and gill plates exported; shark meat: USA as fish balls. (SEAFDEC 2006; Acebes & Tull 2016b; LAMAVE 2017)		Ray skins: Indonesia (Seimbiring et al. 2015)

			Alessandro Ponzo)				
Singapore		Bycatch (Clark-Shen et al. 2020)	Meat (e.g. shark nuggets, dried shark meat, and BBQ stingray) and fins consumed locally; wedgefish head used in cartilage soup (Clark-Shen et al. 2020)	Fins: 6 Meat: 13 (Dent and Clarke 2015)	Fins: Hong Kong, China, Taiwan ++; shark meat: South Korea, Brazil, Italy++; ray meat: Malaysia, Indonesia++; bones and teeth: Australia (Boon 2017)	Fins: 4 Meat: 9 (Dent and Clarke 2015; Okes & Sant 2019)	Fins: Spain, Uruguay, Namibia ++; shark meat: Taiwan, South Korea, Mauritius, Indonesia, Malaysia++; ray meat: Malaysia, Indonesia++; bones and teeth: Australia; whole sharks and rays: Indonesia, Malaysia (Clark-Shen et al. 2020; Boon 2017)
Timor-Leste						Fins: 10 (Dent and Clarke 2015)	
Thailand	13 (Dent and Clarke 2015)	Bycatch (SEAFDEC 2006, 2017)	Meat (e.g. fish balls, salted meat) and fins consumed locally; shark jaws and teeth sold as souvenir; head skin, livers, cartilage, stomach used as fish bait and aquaculture feed. (SEAFDEC 2006, 2017)	Fins: 2 (Dent and Clarke 2015)	Fins: Japan, Australia, Russia, Singapore, Hong Kong, China, Korea, USA, Myanmar, Taiwan Mexico, Sri Lanka, Malaysia ++; shark meat: Singapore, China, Hong Kong, Taiwan, Myanmar; whole shark body: Taiwan, Myanmar;	Fins: 9 (Dent and Clarke 2015)	Fins: Hong Kong, China, Indonesia, Malaysia, Singapore++; whole sharks and meat: Malaysia, Cambodia, Taiwan, Indonesia, China, Vietnam, South Korea, Japan ++; whole rays and meat: Myanmar, Malaysia, Cambodia, Japan, Pakistan,

					whole ray body and meat: Malaysia, Singapore, Cambodia, Vietnam, China, South Korea ++; shark cartilage: Taiwan ++; shark skin: Taiwan, Hong Kong; ray skin: China, Hong Kong, Singapore ++ (SEAFDEC 2006; Dent and Clarke 2015; Krajangdara 2019)		India, Singapore, Papua New Guinea ++; cartilage: Indonesia ++; ray skins and tails: Malaysia, Indonesia, India, Pakistan, Bangladesh, Japan ++ (SEAFDEC 2006; Krajangdara 2019)
Vietnam		Target and Bycatch (SEAFDEC 2006, 2017)	Meat, stomach of shark, and shark fin consumed locally; shark teeth sold as souvenirs. (SEAFDEC 2006, 2017)	Fins: 7 (Dent and Clarke 2015)	Whole sharks, rays and skates: China; skin, bone, liver oil: China (SEAFDEC 2006, 2017)	Meat: 20 (Dent and Clarke 2015)	

Appendix S3

Categories of reported elasmobranch catch from Southeast Asian countries to the FAO as reported in software FishStatJ.

Country	General categories	Family categories	Species categories
Brunei	1	0	0
	Rays, stingrays, mantas nei		
Cambodia	0	0	0
Indonesia	3	8	2
	-Rays, stingrays, mantas nei -Stingrays, butterfly rays nei -Sharks, rays, skates etc. nei	-Dogfish sharks nei -Eagle rays nei -Mantas, devil rays nei -Guitarfish etc nei Hammerhead sharks etc nei -Mackerel sharks, porbeagles nei -Requiem sharks nei -Sawfishes -Thresher sharks nei	-Blue sharks -Whitespotted wedgefish
Malaysia	2	0	0
	-Rays, stingrays, mantas nei -Sharks, rays, skates etc. nei		
Myanmar	0	0	0
Philippines	2	0	2

	-Rays, stingrays, mantas nei -Sharks, rays, skates etc. nei		-Blue shark -Shortfin mako
Singapore	2	0	0
	-Rays, stingrays, mantas nei -Sharks, rays, skates etc. nei		
Timor-Leste	0	0	0
Thailand	2	0	0
	-Rays, stingrays, mantas nei -Sharks, rays, skates etc. nei		
Vietnam	0	0	0

Appendix S4.

Interview questions for the private supplier on blackspot sharks

Regarding the general fishery:

1. What depth is the longline usually set at (if a range, please indicate)
2. How long is the longline and how many hooks does each longline have
3. How far from shore is the longline set (if a range, please indicate)
4. What bait is used on the longline
5. What is the target animal for this fishery or is the fishery mix-species (any species are targeted as every species has a use)
6. How long (hours/days) does the fishing boat go out for at a time & what time are sharks usually caught
7. Do you practice any catch and release with any animals currently

Regarding blackspot shark populations:

1. Have you noticed a decline in their numbers over the years
2. When the longline is pulled in, is the blackspot shark alive or dead
3. Have you observed any seasonality in when you catch them and what do you attribute this too (e.g. change of fishing location? Monsoon?)
4. Have you observed a month(s)/season when particularly small animals are pulled in (e.g. young animals)
5. How valuable or important is this species to your business:
 - a) *This species is important for my business and not catching it would impact me*
 - b) *This species is fairly important for my businesses and not catching it would impact me somewhat*
 - c) *This species is not important for my business and not catching it would not impact me*

Regarding the supply chain:

1. Is the catch aggregated at any island before being imported into Singapore
2. Is the catch 'landed' at JFP and then driven to your warehouse

Regarding the market:

1. Who are your main buyers of blackspot sharks in Singapore
2. How much do you sell them for in Singapore
3. Has the market demand for blackspot sharks changed over the years
4. Has the market demand for sharks in general changed over the years
5. Why was this species in particular favoured for their meat

Regarding solutions:

1. Do you think blackspot sharks could benefit from improved fishery management to help their numbers?
 - a) *Yes*
 - b) *No*

2. What do you think would be suitable and feasible measure to help blackspot shark populations (tick all that apply)

- a) No measures needed – the species seems to be doing fine with current rates of fishing*
- b) Release of all blackspot shark caught (e.g. removing species from the fishery)*
- c) Release of certain blackspot shark and retention of others (e.g. releasing animals over or under a certain size)*
- d) Release of blackspot shark during a particular season only (e.g. release animals for a few months of the year)*
- e) Set a quota to limit that number of animals that can be caught per month or year*
- f) Set up more Protected Areas to better protect particular habitats from fisheries*
- g) Other*

3. If you could not catch/sell or could only catch/sell fewer of blackspot sharks than currently, would you replace this species with another one (e.g. become more reliant on another species?)

Appendix S5.

Interview questions for the private supplier on bluespotted maskrays

Regarding the general fishery (traps):

1. What depth is the trap usually placed (if a range, please indicate)
2. How big is the trap
3. How many traps are deployed in an area at one time and what is the distance between them
4. How far from shore are the traps placed (if a range, please indicate)
5. What bait is used in the trap
6. What is the target animal for this fishery or is the fishery mix-species (any species are targeted as every species has a use)
7. How long (hours/days) is the trap deployed for & what time are rays usually caught
8. Do you practice any catch and release with animals currently

Regarding the general fishery (longlines):

1. What depth is the longline usually set at (if a range, please indicate)
2. How long is the longline and how many hooks does each longline have
3. How far from shore is the longline placed (if a range, please indicate)
4. What bait is used on the longline
5. What is the target animal for this fishery or is the fishery mix-species (any species are targeted as every species has a use)
6. How long (hours/days) does the boat go out for at a time
7. Do you practice any catch and release with animals currently

Regarding the difference between longline & traps:

1. Have you noticed a difference in the size of maskrays caught in longlines vs traps (and what do you attribute this to)
2. Have you noticed a difference in the numbers of maskrays caught in longlines vs traps (and what do you attribute this to)

Regarding bluespotted maskrays:

1. Have you noticed a decline in their numbers over the years
2. When the trap and longline are pulled in, is the animal alive or dead
3. Have you observed any seasonality or peak season in when you catch them and what do you attribute this too (e.g. change of fishing location? Monsoon?)
4. Have you observed a month(s)/season when particularly small animals are pulled in (e.g. young animals)
5. How valuable or important is this species to your business:
 - a) *This species is important for my business and not catching it would impact me*
 - b) *This species is fairly important for my businesses and not catching it would impact me somewhat*
 - c) *This species is not important for my business and not catching it would not impact me*

Regarding the supply chain:

1. Is the catch aggregated at any island/port before being imported into Singapore

2. Is the catch landed at JFP and then driven to your warehouse

Regarding the market:

1. Who are your main buyers of *Neotrygon* in Singapore
2. How much do you sell them for
3. Has the market demand for *Neotrygon* changed over the years
4. Has the market demand for rays in general changed over the years
5. Why is this species in particular favoured for their meat

Regarding solutions:

1. Do you think *Neotrygon spp.* could benefit from improved fishery management to help their numbers?
a) Yes
b) No
2. What do you think would be suitable and feasible measure to help *Neotrygon spp.* populations (tick all that apply)
a) No measures needed – the species seems to be doing fine with current rates of fishing
*b) Release of all *Neotrygon spp.* caught (e.g. removing species from the fishery)*
*c) Release of certain *Neotrygon spp.* and retention of others (e.g. releasing animals over or under a certain size)*
*d) Release of *Neotrygon spp.* during a particular season only (e.g. release animals for a few months of the year)*
e) Set a quota to limit that number of animals that can be caught per month or year
f) Set up more Protected Areas to better protect particular habitats from fisheries
g) Other
3. If you could not catch/sell or could only catch/sell fewer of *Neotrygon spp.* than currently, would you replace this species with another one (e.g. become more reliant on another species?)

Appendix S6.

Parameter estimates and performance of models for age-growth analysis for bluespotted maskrays (*Neotrygon* spp.). MCMC analysis was used to assess model performance. Best performing models are indicated with a **, and models where output is unreliable due to limited data input indicated with a *. Where models had the same Looic values, the VB model was used as it is the most widely used for life-history analysis. Species composition includes (a) all bluespotted maskrays (n=252) regardless of species, (b) mahogany maskrays (n=45) categorised using 'criteria' method (outlined in Table 3.3, section 3.2.4), females could not be analysed due to limited data, (c) oriental bluespotted maskrays (n=104) categorised using 'criteria' method (outlined in Table 3.3, section 3.2.4), (d) undetermined species of bluespotted maskray, categorised using 'criteria' method (outlined in Table 3.3, section 3.2.4), (e) mahogany maskray (n=69) (categorised using 'mitochondrial' method, outlined in section 3.2.2), and (f) oriental bluespotted maskrays (n=135) (categorised using 'mitochondrial' method, outlined in section 3.2.2). Numbers in parentheses after length at birth (L_0), asymptotic length (L_∞) and the K-value are the standard error. For the MCMC analysis, priors were set as: for all bluespotted maskrays grouped together (L_0 : 120 mm DW (se: 12), L_∞ : 370 mm (se: 37)), for mahogany maskrays (L_0 : 120 mm DW (se: 12), L_∞ : 330 mm (se: 33)), for oriental bluespotted maskrays ((L_0 : 120 mm DW (se: 12), L_∞ : 380 mm (se: 38))), for undetermined species of bluespotted maskray (L_0 : 120 mm DW (se: 12), L_∞ : 370 mm (se: 37)).

Model	Model estimate			Model performance (AIC)			Model performance (LooIC) with MCMC		
	L_0 (mm)	(L_∞) (mm)	K (year-1)	AICc	AIC diff	AICc weight	LooIC	LooIC SE	LooIC Weight
(a) <i>Neotrygon</i> spp. (all groups) males and females									

Von Bertalanffy	184.7 (SE: 15.61)	524.5 (SE: 173.1)	0.05909 (SE:0.06437)	2323	0.34	0.3	2328**	21.63	0.54
Logistic	188.4 (SE: 12.23)	407.6 (SE: 75.25)	0.1687 (SE: 0.06618)	2323	0	0.36	2330	21.85	0.19
Gompertz	186.7 (SE: 13.7)	442.6 (SE: 119.9)	0.114 (SE: 0.06518)	2323	0.17	0.33	2329	21.77	0.27
(a1) Neotrygon spp. (all groups) females									
Von Bertalanffy	157.3 (se: 33)	378.1 (se: 51.13)	0.168 (se: 0.091)	1123	0.48	0.29	671.1**	13.1	0.44
Logistic	169.7 (se: 22.42)	355.8 (se: 29.46)	0.289 (se: 0.1009)	1123	0	0.37	672.4	12.97	0.23
Gompertz	164.3 (se: 26.46)	364.3 (se: 36.96)	0.229 (se: 0.096)	1123	0.22	0.33	671.1	13.01	0.33

(a2) <i>Neotrygon</i> spp. (all groups) males									
Von Bertalanffy	182.1 (se: 33.4)	289.7 (se: 35.23)	0.257 (se: 0.2247)	1176	003	0.33	1176**	13.4	0.45
Logistic	185.5 (se: 27.59)	283.2 (se: 25.31)	0.3559 (se: 0.2431)	1176	0	0.34	1176	13.37	0.25
Gompertz	183.9 (se: 30.12)	286 (se: 29.36)	0.3067 (se: 0.2336)	1176	0.01	0.33	1177	13.34	0.3
(b) Mahogany maskray (<i>Neotrygon varidens</i>) males and females									
Von Bertalanffy	184 (SE: 10.71)	13386708 9 (SE: 1.15) *	9.31e (SE: 8.03) *	406.8	0.49	0.28	412.1**	9.749	0.51
Logistic	190.5 (SE: 14.24)	503110 (SE:2.078) *	0.05104 (SE: 0.106) *	406.3	0	0.36	414	10.13	0.19
Gompertz	190.3 (SE:	1.905e (SE:	0.001 (SE:0.10)	406.3	0.01	0.36	413.1	9.935	0.3

	15.02)	2.97e) *							
(b1) Mahogany maskray (<i>Neotrygon varidens</i>) males									
Von Bertalanffy	183.2 (se: 35.38)	264.4 (se: 113.7)	0.1917 (se: 0.5087)	243.3	0.07	0.33	244.9**	8.408	0.41
Logistic	182.9 (se: 31.95)	255.3 (se: 69.05)	0.287 (se: 0.517)	243.4	0.04	0.33	245.4	8.754	0.31
Gompertz	182.9 (se: 33.74)	258.6 (se: 84.25)	0.2423 (se: 0.5134)	243.4	0	0.34	245.7	9.032	0.28
(c) Oriental bluespotted maskray (<i>Neotrygon orientalis</i>) females									
Von Bertalanffy	189 (SE: 15.72)	1106714 (SE: 5.106e) *	1.523e (SE: 0.07027) *	942.7	0.38	0.29	945**	14.95	0.62
Logistic	199.8 (SE: 18.27)	4519 (SE: 109755) *	0.06496 (SE: 0.1054)	942.3	0	0.35	948.3	15.66	0.12

Gompertz	199.7 (SE: 20.07)	15545309 4 (SE: 4.793e) *	0.004604 (SE: 0.1073) *	942.3	0	0.35	956.7	15.42	0.26
(c1) Oriental bluespotted maskray (<i>Neotrygon orientalis</i>) females									
Von Bertalanffy	182.3 (se: 35.57)	1072 (se: 4415) *	0.02327	527.2	0.12	0.32	526.1**	11.93	0.55
Logistic	184.8 (se: 27.18)	482.1 (se: 265.2) *	0.1604 (se: 0.1325)	527.1	0	0.34	528.7	13.42	0.15
Gompertz	183.7 (se: 30.77)	578.3 (se: 578.3) *	0.0917 (se: 0.131)	527.1	0.06	0.33	527.3	12.88	0.3
(c2) Oriental bluespotted maskray (<i>Neotrygon orientalis</i>) males									
Von Bertalanffy	0.3024 (se:101 5)	266 (se: 18.57)	0.7961 (se: 1.447)	411	0.03	0.33	407**	6.635	0.37
Logistic	83.05 (se:	264.3 (se: 13.61)	1.043 (se: 1.668)	411	0	0.34	407.4	6.649	0.3

	266.1)								
Gompertz	41.44 (se: 351.8)	264 (se: 14.1)	1.002 (se: 1.652)	411	0.01	0.33	407.3	6.649	0.3
(d) Undetermined species of bluespotted maskray (<i>Neotrygon</i> spp.) males and females									
Von Bertalanffy	186.7 (SE: 32.19)	359.9 (SE: 60.37)	0.1568 (SE: 0.1203)	950.7	0	0.33	945.2**	14.88	0.61
Logistic	194.9 (SE: 23.98)	345.5 (SE: 40.04)	0.2419 (SE: 0.1277)	950.7	0.01	0.33	948.4	15.6	0.12
Gompertz	191.2 (SE: 27.39)	351.3 (SE: 47.73)	0.1998 (SE: 0.1242)	950.7	0	0.33	946.9	15.36	0.27
(d1) Undetermined species of bluespotted maskray (<i>Neotrygon</i> spp.) females									
Von Bertalanffy	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Logistic	24.95 (se:	311.6 (se: 8.799)	1.231 (0.818)	n/a	n/a	n/a	n/a	n/a	n/a

	56.82)								
Gompertz	0.561 (se: 7.557)	312.5 (se: 9.556)	1.072 (se: 0.7137)	n/a	n/a	n/a	n/a	n/a	n/a
(d2) Undetermined species of bluespotted maskray (<i>Neotrygon</i> spp.) males									
Von Bertalanffy	182.7 (se: 53.67)	283.3 (se:18.64)	0.3981 (se: 0.3159)	502.7	0	0.34	501.8**	11.31	0.46
Logistic	190.4 (se: 42.37)	281.6 (16.35)	0.476 (se: 0.35)	502.8	0.04	0.33	503.3	11.15	0.22
Gompertz	187 (se: 46.96)	282.3 (se: 17.37)	0.437 (se: 0.332)	502.8	0.02	0.33	502.5	11.2	0.32
(e) Mahogany maskray (<i>Neotrygon varidens</i>) males and females									
Von Bertalanffy	173 (SE: 24.43)	792 (SE: 1870)	0.03182 (SE: 0.1119)	670.9	0.21	0.32	671.1**	13.1	0.44
Logistic	174.7	427.7	0.1704 (SE:	670.6	0	0.34	672.4	12.97	0.23

	(SE: 19.8)	(SE: 180.2)	0.1163)						
Gompertz	173.9 (SE: 21.86)	499.2 (SE: 361.2)	0.1011 (SE:0. 1137)	670.8	0.1	0.33	671.1	13.01	0.33
(e1) Mahogany maskray (<i>Neotrygon varidens</i>) females									
Von Bertalanffy	163.1 (se: 50.84)	380.7 (se: 125.1)	0.149 (se: 0.1727)	313.8	0.13	0.32	501.9**	11.31	0.45
Logistic	169.5 (se: 39.39)	349.2 (se: 63.27)	0.283 (se: 0.196)	313.7	0	0.34	503.3	11.15	0.23
Gompertz	166.5 (se: 44.09)	360.8 (se: 83.36)	0.216 (se: 0.183)	313.7	0.06	0.33	502.6	11.22	0.32
(e1) Mahogany maskray (<i>Neotrygon varidens</i>) males									
Von Bertalanffy	173 (se: 52.27)	277.7 (se: 92.81)	0.241 (se: 0.483)	355.2	0.09	0.33	501.8**	11.27	0.46

Logistic	174.2 (se: 44.25)	267.6 (se: 55.73)	0.368 (se: 0.505)	355.1	0	0.34	502.6	11.21	0.32
Gompertz	174 (se: 47.93)	271.7 (se: 69.83)	0.304 (se: 0.493)	355.1	0.05	0.33	503.3	11.14	0.23
(f) Oriental bluespotted maskray (<i>Neotrygon orientalis</i>) males and females									
Von Bertalanffy	169.5 (SE: 26.48)	398.6 (SE: 86.12)	0.1286 (SE: 0.9105)	1318	0.12	0.32	1318**	12.95	0.6
Logistic	179 (SE: 18.83)	365.3 (SE: 44.87)	0.2402 (SE: 0.09686)	1318	0	0.34	1321	13.01	0.13
Gompertz	174.8 (SE: 21.9)	377.6 (SE: 58.36)	0.1849 (SE: 0.09399)	1318	0.05	0.33	1319	13.01	0.13
(f1) Oriental bluespotted maskray (<i>Neotrygon orientalis</i>) females									
Von Bertalanffy	182.3 (se: 35.57)	1072 (se: 4415)	0.0232 (se: 0.1306)	527.2	0.12	0.32	502.2**	11.29	0.43

Logistic	184.8 (se: 27.18)	482.1 (se: 265.2)	0.1604 (se: 0.1325)	527.1	0	0.34	503.3	11.17	0.24
Gompertz	183.7 (se: 30.77)	578.3 (se: 578.3)	0.0917 (se: 0.131)	527.1	0.06	0.33	502.7	11.22	0.33
(f2) Oriental bluespotted maskray (<i>Neotrygon orientalis</i>) males									
Von Bertalanffy	187.6 (se: 47.36)	286.6 (se: 28.54)	0.3067 (se: 0.2956)	665	0.01	0.33	502.3**	11.29	0.42
Logistic	192.4 (se: 38.81)	283.2 (se: 23.15)	0.3892 (se: 0.3229)	665	0	0.33	503.5	11.14	0.24
Gompertz	190.2 (se: 42.47)	284.7 (se: 25.52)	0.348 (0.308)	665	0	0.33	502.8	11.24	0.33