Mechanisms of coexistence : a multi-disciplinary investigation of sympatric juvenile reef sharks

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PSL
UNIVERSITÉ PARIS

THÈSE DE DOCTORAT
DE L'UNIVERSITÉ PSL

Préparée à l'École Pratique des Hautes Études

Les mécanismes de la coexistence: une étude multidisciplinaire sur des requins de récif juvéniles vivant en sympatrie

Mechanisms of coexistence: A multi-disciplinary investigation of sympatric juvenile reef sharks

Soutenue par

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Le 11 Juin 2020

École doctorale n° 472

École doctorale de l'École Pratique des Hautes Études

Spécialité

Biologie des populations, génétique et éco-éthologie

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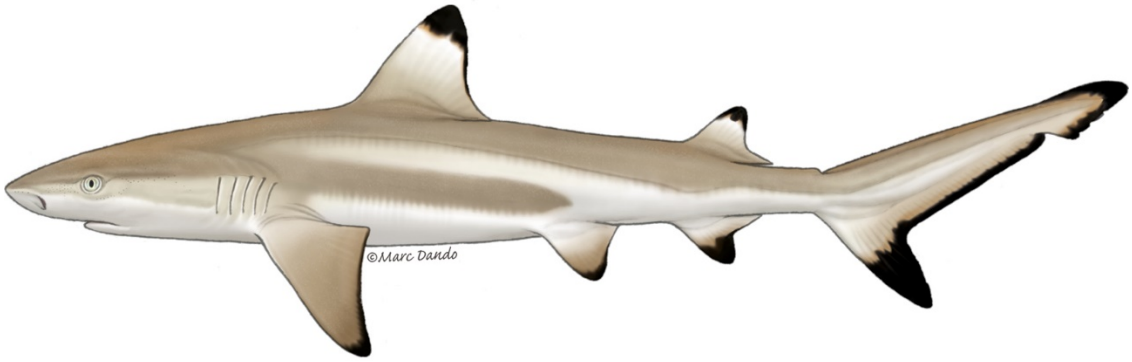
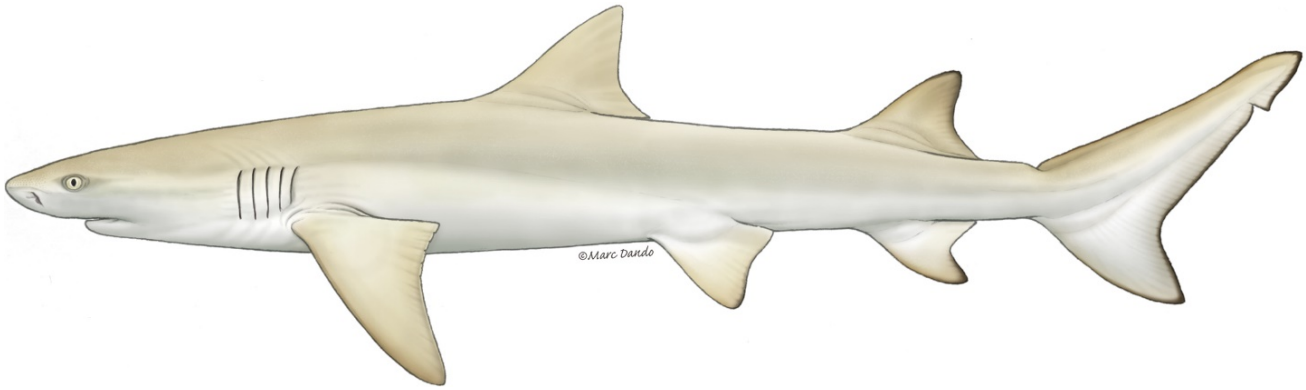


Blacktip Reef Shark, *Carcharhinus melanopterus* (Quoy & Gaimard 1824)



Sicklefin Lemon Shark, *Negaprion acutidens* (Rüppell 1837)

Illustrations of the shark species examined in this PhD thesis.

Artwork by Marc Dando

This thesis is dedicated to my dear parents, Heidi and Heinz Weideli-Zwingli, who have loved and supported me from the moment I was born. For this, and much more I am forever thankful.

Acknowledgements

This PhD thesis would have never been possible without a large number of outstanding people who deserve specific thanks for their scientific, logistical, emotional, physical, and financial support. Numerous individuals are acknowledged after each chapter, but the list of people mentioned below (in no specific order) deserve a special thanks:

First, I would like to express my gratitude to my principal supervisor Serge Planes and my co-supervisor Yannis Papastamatiou. Serge, thank you for your continuous support and above all for taking the chance on accepting me as your PhD student. You gave me such an important opportunity, which helped me to develop new skills, extend my expertise, and expand my collaborative network. Also, thank you for your generosity and for giving me the opportunity to remain independent during my intensive field and laboratory work. Likewise, I'm forever grateful to you, Yannis for being my co-supervisor, excellent role model, and mentor. What started with an email conversation about blacktip reef sharks from Palmyra ended with you becoming one of the most influential people regarding my PhD and my scientific career. There has never been a question you weren't able to answer! Second, I would like to thank Michael Berumen, Nadia Bruyndonckx, Laurent Dagorn, Mireille Harmelin-Vivien, Dean Grubbs, and Johann Mourier for contributing their knowledge as part of my PhD committee. Likewise, I'm sincerely grateful to my co-authors Ian Bouyoucos, Ryan Daly, Mike Heithaus, Jeremy Kiszka, Phil Matich, Yannis Papastamatiou, Lauren Peel, Serge Planes, Jodie Rummer, and Mahmood Shivji for your time, endless support, and for sharing your knowledge and expertise with me.

As I was based in several laboratories and field stations during my PhD, I would like to express my gratitude to a diverse group of people that supported me in one way or another: At NOVA Southeastern University, USA, I wish to acknowledge Mahmood Shivji for hosting me at your lab, Cassandra Ruck Calhoun and Cristin Keelin for being the best teachers I could have asked for, and to Igbal Elhassan for your friendship, giggles, and your kind heart. At Florida International University, Miami, USA, I'm thankful to Mike Heithaus for hosting me at your lab and to Kirk Gastrich for your immense support. Likewise, I thank Maria Sabando and John Harris for processing my blood samples. A total of nine visits to the SOSF-D'Arros Research Centre allowed me to meet and work alongside a vast number of people and I would like to express my deepest gratitude to the SOSF-DRC staff Clare & Ryan Daly, Chris Boyes, Kerryn Bullock, and Rainer von Brandis and to the group of assistants that greatly supported my fieldwork: Katherine, Nadia, Sacha, Bridgette, Morgan, Chantel, James, Cheryl and Eric. Also,

D'Arros wouldn't have been the same without Dorthy, Magdeline, the Danah Divers, Chelonia, and Jeanne Mortimer. Likewise, I would like to acknowledge Karl Fleischmann and Edith Mueller for your hospitality and friendship, and to Mike Anacoura and Terence Vel for your support whenever I got back to Mahe. My first steps at the CRIOBE in Perpignan were not easy, as my French was rather rusty. Therefore, thanks to Nathalie Tolou and Aurélie Mariotti for your immediate help. I'd further like to thank Caroline Dubé, Emile Boissin, Jeanine, Ryan & Maya Almany, Rita Sahyoun, Alex Mercier, and Guillaume Iwankow for your friendship and for showing me the fantastic French way of life. I also acknowledge Yu Xiang for your help in the lab, Johann Mourier for your support and advice with data analysis, and the most patient IT person, Peter Esteve. At the CRIOBE in Moorea I wish to express my deepest gratitude to Ian Bouyoucos and Jodie Rummer. Thank you for your hard work, good laughs, endless conversations, trust, and support. The evenings in the field and our 'UruFara' spirit will stay with me forever. Just as importantly, I thank Lizzie Duncan and Eva Jacquesson for your hard work and dedication, as well as the whole CRIOBE team, especially Elina, Simon, Cécile, and Pascal. Also, thank you Philippe and the sunset team for your amazing hospitality, the best dinners, and for providing tuna for our babies.

Even though I spent most of my time outside of Switzerland, my PhD allowed me to come back on multiple occasions. I don't know what I would have done without the never-ending love, laughter, fun, and support of my friends. I like to specially recognize Laura Erismann, Catherine Rimann, Manuela Vogel, Marika, Christoph, Jim, 'Godson' Jack and Jeffrey Kiener, Julia Künzi, Thomas Schläppi, Benjamin Wegmüller, Nino Mürset, Adrienne Roggo, Janick and Nicole Gfeller, Christian Lehmann, Claudia Bernoulli, Corina Risch and Malini Pittet. Getting stuff ready and organized on my laptop wouldn't have been possible without you, Pascal Megert. Also, I wouldn't have studied biology without Heinz Stöckli, my former high-school teacher and the best biology teacher I have ever had. Malini Pittet, Patrick Meier, Corina & Lorenz Risch thank you for your friendship, inspiring discussions, support, trust and for offering office spaces at InnFlow in Regensdorf and the Medizinisches Zentrum Dr. Risch in Kloten. Special thanks also to my former bosses Daniel Bernoulli and Patrick Schaad for accepting an 18 year-old version of me that wasn't even able to properly open a bottle of wine. You always believed in me and have told me to follow my dreams and for this, and much more, I'm forever thankful.

I'm also very fortunate that my path has crossed with people from all over the globe and that some of them have become friends that have all something in common with me: The Ocean

and the sharks. I'm deeply thankful to Nadia Bruyndonckx and Michael Scholl for always listening to my questions, answering my (sometimes too) long emails and above all for your endless support, your trust, and the motivation you have always given me. Likewise, my deepest thanks to my two 'adoptive' American families, Doc and Marie Gruber, and Jeff Trotta and Missy Belsito for taking care of me and for always giving me a loving welcome whenever I was back in Florida. Also, a massive thank to my friend Hannah Medd for being such a dear friend, sistar, great support, and dive buddy and to hubby aka Ivan Rutzen, who I, after eight years slowly start to understand. Even though we don't see each other often enough, Ruth aka 'Bruce' Harris, thank you for always being my friend and a shining human being. Starting as roommates in 2009 in Bimini, Elena Salim Haubold, you have become a dear friend and I feel overly honoured that I was able to be one of your 'bridesmates'. Kerstin Glaus, my Swiss companion, I'm so glad we met at EEA in 2013 and stayed in contact since. You are a true inspiration. Further, thanks to Steve Kessel for being an incredible teacher and role model when I took my first steps in the scientific shark world.

Last but not least, I wish to express my thankfulness to my parents, Heidi and Heinz Weideli-Zwingli, who have always been there for me, despite the distances between us. I know you would have loved if I had studied fish ecology in the lake of Thun or became the new director of the oceanium in Basel. But the ocean, the sharks, and the tropical adventures just kept on luring me away from Switzerland. I'll always be grateful that you accepted my decisions and always loved and appreciated our reunions at Zurich Airport (with luckily no cowbells involved!). I'm also thankful for my entire, yet small and precious family (the Weidelis, the Zwinglis, and the Balsigers) that always welcomed me back with open arms. Finally, a very special thanks to my partner Tobias Hossmann. Thanks for your love, trust, support, and for sometimes knowing me better than I do. Also, thank you for expanding my passion from under the water up to the sky!

During this research I was generously supported by the Save Our Seas Foundation (Keystone Grant SOSF 290), the SOSF-D'Arros Research Centre, the Basler Stiftung für biologische Forschung, and the CRIOBE. Also, massive thanks to Tauchsport Käser (TSK), Mares, and Fourth Element for providing me with dive equipment, and to Inn Flow and Medizinisches Zentrum Dr. Risch for generously offering office spaces in your amazing companies.

Publications arising during this candidature

Peer-reviewed articles included in this thesis

This thesis is submitted as a series of manuscripts, which are to be submitted, or published in a peer-reviewed journal. The data chapters 2 – 5 are formatted according the journal of interest.

Weideli OC, Kiszka JJ, Matich P, Heithaus MR (2019) Effects of anticoagulants on stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) of shark blood components. *Journal of Fish Biology*. 95:1535-539. https://doi.org/10.1111/jfb.14164 **(Chapter 2)**

Weideli OC, Papastamatiou, YP, Daly, R, Peel L, Shivji, M, Heithaus, M, Planes, S. Mechanisms of coexistence with predator-induced niche overlap in sympatric juvenile sharks. In preparation for *Oecologia* (**Chapter 3)**

Weideli, OC, Papastamatiou, YP, Planes S (2019) Size frequency, dispersal distances and variable growth rates of young sharks in a multi-species aggregation. *Journal of Fish Biology*. 94:789–797, http://doi: 10.1111/jfb.13968 **(Chapter 4)**

Weideli OC, Bouyoucos IA, Papastamatiou YP, Mescam G, Rummer J, Planes, S (2019) Same species, different prerequisites: investigating body condition and foraging success in young reef sharks between an atoll and an island system. *Scientific Reports. 9:13447* https://doi.org/10.1038/s41598-019-49761-2 **(Chapter 5)**

Peer-reviewed articles not included in this thesis

Matich, P, Plumlee, J, **Weideli, OC**, Fisher, M (*In Review*) New insights into the trophic ecology of blacktip sharks (*Carcharhinus limbatus*) from a subtropical estuary in the western Gulf of Mexico. *Journal of Fish Biology*.

Weideli, OC, Papastamatiou, YP (2020) Intestinal eversion in a pelagic shark evokes chase and bite attempts by large-bodied carangid fish. *Journal of Ethology*. https://doi.org/10.1007/s10164-020-00664-0.

Bouyoucos IA, Morrison PR, **Weideli OC**, Jacquesson E, Planes S, Simpfendorfer CA, Brauner CA, Rummer JL (2020) Thermal tolerance and hypoxia tolerance are associated in blacktip reef shark (Carcharhinus melanopterus) neonates. *Journal of Experimental Biology, ournal of Experimental Biology*. doi: 10.1242/jeb.221937.

Bouyoucos, IA**, Weideli, OC**, Planes, S, Simpfendorfer, CA, & Rummer, JL (2018). Dead tired: evaluating the physiological status and survival of neonatal reef sharks under stress. *Conservation Physiology*, *6*, coy053; doi:10.1093/conphys/coy053.

Hussey NE, DiBattista JD, Moore, JW, Ward, EJ, Fisk, AT, Kessel, S, Feldheim, KA, Gruber, SH, Guttridge, TL, **Weideli, OC**, and Chapman DD (2017) Risky business for a juvenile marine predator? Testing the influence of foraging strategies on size and growth rate under natural conditions. *Proc. R. Soc. B.* 284:20170166. http://dx.doi.org/10.1098/rspb.2017.0166.

Weideli OC, Mourier J, Planes S (2015) A massive surgeonfish aggregation creates a unique opportunity for reef sharks. *Coral Reefs*, 34(3):835, doi: 10.1007/s00338-015-1290-2.

Publication in preparation

Weideli OC, Medd H, Kessel TS Abnormal skin patterns in provisioned adult lemon sharks, *Negaprion brevirostris*. In preparation for *Coral Reefs*.

Matich P, Shipley ON, **Weideli OC** Accounting for shifting isotopic baselines in ecotones using a model estuarine predator, the bull shark *Carcharhinus leucas.* In preparation for *Marine Biology*.

Conference and meeting presentations during candidature

Weideli OC, Bouyoucos IA, Papastamatiou YP, Mescam G, Rummer JL, Planes, S (2019) Same species, different prerequisites: investigating body condition and foraging success in young reef sharks between an atoll and an island system. *American Elasmobranch Society (AES)*. Snowbird, Utah, USA (*Oral presentation - English*)

Weideli OC, Papastamatiou YP, Planes S (2018) Size frequency, dispersal distances and variable growth rates of shark in a multi-species nursery. *Rencontres de l'ichtyologie en France* (*RIF*). Paris, France (*Oral presentation - French*)

Weideli OC (2017) Home is where the shark is. *Indo-Pacific Fish Conference*. Tahiti, French Polynesia (*Oral Presentation - English*)

Weideli OC, Papastamatiou YP, Planes S (2017) Safe haven: A mark recapture study reveals fast growth rates and long-term residency patterns in an oligotrophic shark nursery. *Indo-Pacific Fish Conference*. Tahiti, French Polynesia (*Oral presentation - English*)

Bouyoucos IA, **Weideli OC**, Simpfendorfer CA, Planes S, Rummer JL (2017) Dead tired: physiological exhaustion in neonatal reef sharks (*Carcharhinus melanopterus* and *Negaprion acutidens*) in nursery areas. *Indo-Pacific Fish Conference*. Tahiti, French Polynesia (*Oral presentation - English*)

Weideli OC, Papastamatiou YP, Shivji MS, Heithaus MR, von Brandis R, Planes S. (2016) Resource partitioning and competition in a mutually used shark nursery. *American Elasmobranch Society (AES)*. New Orleans, Louisiana, USA. (*Oral Presentation - English*)

Weideli OC, Papastamatiou, YP, Shivji, MS, Heithaus, MR, von Brandis, R, Planes, S. (2016) Sharing or competing? Ecological niches of juvenile sharks within a mutual shark nursery. *International Coral Reef Symposium*. Honolulu, Hawai'i, USA. (*Oral Presentation - English*)

Statement of contribution of others

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Author contribution

This PhD thesis represents the collective work of myself and numerous others that are co-authors of the manuscripts associated with the data chapters of this thesis. My contribution and the co-author's contributions are listed at the end of each thesis chapter.

Résumé substantiel

Les espèces qui coexistent dans un même habitat, surtout si elles sont étroitement liées et partagent des caractéristiques morphologiques et écologiques similaires, peuvent être en compétition pour les mêmes ressources naturelles. Si celles-ci sont limitées, les espèces coexistantes présenteront une ségrégation de niche (ségrégation spatiale, temporelle ou alimentaire) pour réduire la concurrence interspécifique, car des concurrents complets ne peuvent pas coexister. Bien que la ségrégation de niche soit généralement considérée comme un mécanisme permettant d'atténuer la concurrence, elle est difficile à étudier car le lien direct entre ségrégation de niche et concurrence est controversé. En outre, comme les ségrégations de niche sont rarement symétriques, les espèces dominantes et subordonnées sont censées être influencées différemment par ce type de ségrégation. Bien que les requins aient fait l'objet de nombreuses études portant sur les modèles de niches spatiales, temporelles ou trophiques des espèces coexistantes, seuls quelques-uns ont évalué plus d'une dimension de niche. À ce jour, aucune étude n'a établi de lien entre le modèle de niche et les effets de la concurrence, les caractéristiques de population et les traits liés à la fitness des requins; en conséquence, l'origine et les effets potentiels sur les traits liés à la fitness du modèle de niche ne sont pas clairs.

Pour combler ces lacunes, les recherches menées dans le cadre de cette thèse ont utilisé de nombreuses méthodes complémentaires (échantillonnage par marquage-recapture, suivi actif, analyse des isotopes stables et analyse des contenus stomacaux) qui ont permis une évaluation initiale de la population suivie d'une étude de niche multidimensionnelle de juvéniles de deux espèces de requins sympatriques : le requin de récif à pointe noire, *Carcharhinus melanopterus*, et le requin citron faucille, *Negaprion acutidens*. En outre, des expériences sur le comportement de dominance en captivité ont été menées pour mieux comprendre comment les effets de la concurrence peuvent contribuer aux modèles de ségrégation des niches. Des échantillons et des données ont été recueillis sur deux sites - l'atoll de Saint-Joseph, République des Seychelles (principal site d'étude) et Moorea, Polynésie française (site d'étude supplémentaire) - sur une période de quatre ans (2014-2018), ce qui a permis d'obtenir des informations écologiques et comportementales détaillées sur plus de 640 requins de récif juvéniles.

Dans le **Chapitre 2**, j'ai testé les effets potentiels des anticoagulants sur les isotopes stables du sang, car les composants sanguins ont été utilisés pour les études trophiques dans le cadre de cette thèse (**Chapitre 3**). Plus précisément, j'ai testé les effets potentiels de l'anticoagulant

EDTA et de l'héparine sodique sur les valeurs isotopiques stables du carbone $\delta^{13}C$ et de l'azote $\delta^{15}N$ des globules rouges et du plasma sanguin chez *C. melanopterus* juvénile. Le plasma conservé avec des anticoagulants n'était pas isotopiquement distinct du plasma dans des tubes de contrôle sans additif, mais les valeurs des globules rouges $\delta^{15}N$ présentaient de faibles enrichissements lorsqu'ils étaient conservés avec de l'EDTA et de l'héparine sodique. Les résultats suggèrent que l'EDTA et l'héparine sont des anticoagulants viables pour les analyses isotopiques stables des fractions sanguines, mais il est conseillé de mener des études complémentaires pour valider ces résultats. Par conséquent, et par précaution, seuls des échantillons de sang sans additifs ont été utilisés pour les analyses isotopiques du **Chapitre 3**.

Dans le **Chapitre 3**, j'ai établi un lien entre une étude de niche multidimensionnelle (ségrégation spatiale, temporelle ou alimentaire) et des expériences de comportement de dominance chez *C. melanopterus* et *N. acutidens* coexistant dans l'atoll de Saint-Joseph, afin d'améliorer notre compréhension des facteurs sous-jacents qui affectent les modèles de niche. Les expériences sur le comportement de dominance ont révélé que *C. melanopterus* a cédé dans 93% des interactions observées ($p < 0,001$) et n'a réussi à manger aucune des proies présentées, ce qui suggère que *N. acutidens* est dominant dans les nurseries. Par conséquent, des *C. melanopterus* subordonnés occupaient de plus larges parts des microhabitats disponibles, y compris dans des zones plus profondes et plus exposées. De même, la ségrégation trophique s'est avérée asymétrique, *C. melanopterus* atténuant les effets de la compétition en incluant des proies plus diverses et de contenu énergétique plus faible. L'utilisation quotidienne de l'espace par les requins dans les zones de distribution mixte présentait un chevauchement entre les espèces, peut-être en raison des effets de risque (c'est-à-dire l'évitement des grands prédateurs) et des fluctuations des marées. Les effets de risque et les fluctuations des marées peuvent donc considérablement exacerber la concurrence, car la grande majorité des individus des deux populations se trouvent dans la même zone, spatialement limitée. Dans l'ensemble, le **Chapitre 3** fournit des preuves irréfutables que la concurrence peut façonner le modèle de niche d'espèces sympatriques similaires, et que, selon l'axe de niche étudié, des facteurs autres que la concurrence doivent être pris en compte.

Le **Chapitre 4**, porte sur une enquête de marquage-recapture sur trois ans qui a permis de marquer et de mesurer des *C. melanopterus* juvéniles ($n = 333$) et des *N. acutidens* juvéniles ($n = 302$) coexistant dans l'atoll de Saint-Joseph. Les deux espèces ont montré une synchronisation saisonnière de la reproduction et des tailles à la naissance relativement grandes. Malgré les périodes de liberté prolongées (> 2,5 ans), la majorité des recaptures ont

été trouvées à proximité du lieu de marquage initial (< 500 m). Les taux de croissance annuels de *C. melanopterus* (*n* = 24) et de *N. acutidens* (*n* = 62) ont varié de 6,6 à 31,7 cm/an (moyenne ± SE ; 16,2 ± 1,2 cm/an) et de 0,2 à 32,2 cm/an (11,8 ± 1 cm/an), respectivement, et sont les plus variables jamais enregistrés chez les requins juvéniles sauvages. Les abondances élevées et les recaptures répétées sur le long terme pour les deux espèces sont les signes d'un habitat favorable aux juvéniles pendant leurs premières années de vie. Cependant, la grande variabilité des taux de croissance annuels des deux espèces semble suggérer une forte concurrence intra- et interspécifique induite par un habitat isolé et peut-être limité en ressources. Pris dans son ensemble, le **Chapitre 4** a mis en évidence des perspectives nouvelles et essentielles sur les caractéristiques de ces deux populations de requins coexistantes pour lesquelles il n'existait pas de données préalables. Bien que l'on ait pu s'attendre à des conséquences variables des schémas de ségrégation (**Chapitre 1**), les caractéristiques des populations et les traits liés à la fitness étaient similaires d'une espèce à l'autre, c'est pourquoi un lien entre le schéma de ségrégation et les traits d'adaptabilité n'a pu être mis en évidence.

Dans le **Chapitre 5**, mon objectif était de comprendre si les investissements énergétiques maternels varient entre deux populations de *C. melanopterus* et dans quelle mesure les variations d'investissements maternels pourraient influencer la condition physique et le début de leur recherche de nourriture. Au total, 546 jeunes requins ont été capturés dans l'atoll de Saint-Joseph et à Moorea, et les indices de condition physique et le pourcentage d'estomacs contenant des proies ont été mesurés. L'investissement maternel s'est avéré être spécifique à chaque site, avec des individus significativement plus grands, plus lourds et en meilleure condition physique à Moorea. Malgré ces avantages, au fil du temps, les requins de Moorea ont montré une diminution significative de leur condition physique et ont été plus lents à initier leur recherche de nourriture. En conclusion, le **Chapitre 5** suggère que le succès de la recherche de nourriture des jeunes requins est indépendant de la qualité des ressources énergétiques maternelles, et que d'autres facteurs, tels que la disponibilité et qualité des proies, et/ou les facteurs de stress anthropiques sont probablement responsables des différences observées entre les sites. Enfin, en utilisant Moorea comme site d'étude supplémentaire, j'ai fourni de nouvelles perspectives sur les différences intraspécifiques dans le développement précoce de l'alimentation des requins, soutenant des stratégies de gestion spécifiques aux sites pour les jeunes requins des habitats proches des côtes.

Bien que des recherches ultérieures seront nécessaires pour établir une image complète des mécanismes sous-jacents de la coexistence chez les requins, cette thèse améliore et approfondit les recherches précédentes sur les niches des requins sympatriques. Dans l'ensemble, les recherches suggèrent qu'une diminution des ressources en proies est susceptible d'accroître la concurrence entre les jeunes requins coexistants, ce qui souligne la nécessité de stratégies spécifiques aux sites pour la gestion des zones littorales.

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Figure 3.4 Kernel utilisation distributions (KUDs) of manually tracked juvenile blacktip reef sharks *Carcharhinus melanopterus* (A-E), and sicklefin lemon sharks *Negaprion acutidens* (F-K) at St. Joseph Atoll, Seychelles. All individuals were tracked on a different day. Drone image © Save Our Seas Foundation.

Figure 3.5 Fine-scale movements and location of area restricted searching (ARS) determined by first passage time (FPT) for juvenile blacktip reef sharks *Carcharhinus melanopterus* (A) and juvenile sicklefin lemon sharks *Negaprion acutidens* (D, G) at St. Joseph Atoll, Seychelles. White triangle indicates the start of the track, white square represent the end of the track, and different coloured dots represent GPS locations at corresponding tidal stages. ARS was calculated from the peak in log variance FPT and the time of the maximum FPT values (B, E, H). Time and tidal stage when ARS was found are highlighted by red squares (C, F, I). Note that on the intertidal reef flats the tides were slightly lagged compared to the location of the water pressure logger (indicated by white star). Drone image © Save Our Seas Foundation.

Figure 3.6 Non-metric multidimensional scaling (nMDS) plot of percentage numeric contribution (%N) to the diets of juvenile blacktip reef sharks *Carcharhinus melanopterus* and juvenile sicklefin lemon sharks *Negaprion acutidens*. A two-dimensional Bray-Curtis dissimilarity index was used and the seven major prey categories (Mugilidae, Gerreidae, Labridae, other teleosts, unidentified teleosts, crustaceans, and cephalopods). Each point of the plot represents the pooled mean for groups of five individuals.

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Figure 4.1 Location of the Republic of Seychelles and St. Joseph Atoll and map of the study location with the neighbouring D'Arros island. Permanent landmasses are shown in black. Map created by Ryan Daly | SOSF.

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Chapter 1 | General Introduction

1.1 The coexistence of similar species

The coexistence of closely related species with similar sizes, morphologies, and behaviours has important implications for marine ecosystems, because similar species may rely on the same natural resources (i.e., food and habitat). Depending on the abundance and availability of such resources, competition can occur (MacArthur, 1958). On an individual basis, competition can influence an animal's energy budget by a reduced food intake with negative impact on growth or survival (Munday, 2001; Eccard & Ylönen, 2003). At the population level, competition can reduce the abundance of the less competitive species (Ellis et al., 1996; Dulvy et al., 2000), or in the most extreme case, competition can result in competitive exclusion of the subordinate species (Hardin, 1960).

Classical niche theory predict that stable coexistence of competing species can be achieved through niche segregation to reduce interspecific competition (MacArthur, 1958; Hutchinson, 1959; Schoener, 1974). Niche segregations can involve some or all of the niches of the *n*-dimensional niche hypervolume (Hutchinson, 1957) and can be accomplished by consuming different types of food, foraging in different areas, or at different times of the day (Wilson, 2010; Jeglinski et al., 2013; Wakefield et al., 2013; Correa & Winemiller, 2014). These segregation effects are asymmetric with one of the interacting species being more affected than the other (Connell, 1983; Schoener, 1987), because species differ in their competitive abilities through differences in dominance hierarchies, prey handling, and search times (Holmgren, 1995). Under conditions with variable dominance hierarchies between unequal competitors (ideal free distribution [IFD] for unequal predators; Holmgren 1995), predictions include the dominant competitor using qualitatively better habitats, while the subordinate competitor using both, higher and lower quality habitats, or solely occurring in lower quality habitats (Holmgren, 1995; Smallegange & van der Meer, 2009). Analogously, predictions with unequal competitors also include the dominant competitor consuming more energy efficient and preferable food resources, while subordinate competitor consuming prey from a wider range, including prey of lower energy content (Davis et al., 2015; Droege et al., 2017). For example, as a result of spatial overlap on several reef sites in the northern Gulf of Mexico (GOM), red snappers, *Lutjanus campechanus* and vermillion snappers, *Rhomboplites aurorubens* partition their diet. The dominant red snapper feeds on high calorie prey, while the subordinate vermillion snapper consumes 39% less fish, and increases its

consumption of amphipods, a lower quality prey (Davis et al., 2015). Ultimately, if such asymmetric segregation patterns persist over time, they may have varying consequences on population characteristics and fitness-related traits (e.g., growth, survival and reproductive output) for the dominant, as well as for the subordinate species (Martin & Martin, 2001; Munday, 2001; Eccard & Ylönen, 2003).

Understanding the competitive abilities of sympatric species therefore provides fundamental information that help predicting segregation patterns. Additionally, knowing how niche segregations affect population characteristics and fitness-related traits may bridge gaps between population biology and effects of competition on sympatric species. Going out from these perspectives, studying resource use patterns in conjunction with competitive abilities, population characteristics and fitness-related traits in sympatric species is important, especially in the marine realm, where deteriorating environmental conditions (Lotze et al., 2006) may result in an increase of competition for diminishing prey resources.

1.2 The coexistence of similar shark species

Among the marine taxa that is severely threatened by anthropogenic stressors are elasmobranchs (sharks, rays and skates), which are a taxonomic subclass within the Chondrichthyes. As of 2019, 18.1% of elasmobranchs are threatened (VU, EN, CR), while 41.5% are classified as data-deficient (https://www.iucnssg.org/global-analyses.html). Over 500 of the known species of elasmobranchs belong to the superorder Galeomorphii and Squalomorphii, which are characterized by a cylindrical shape, five to seven paired gill openings on the side of their head, and pectoral fins that are not attached to the head (Compagno et al., 2005). Sharks possess, notwithstanding some variation among species, conservative K-selected life history traits including slow growth, long gestation periods, small litter sizes, and late sexual maturity (Myers & Worm, 2003; Frisk et al., 2005). These characteristics coupled with cumulative impacts from overfishing, incidental by-catch, habitat degradation, and pollution have reduced shark populations worldwide (Stevens et al., 2000; Myers & Worm, 2003; Ferretti et al., 2010). The ecological implications of declining population numbers are likely profound for apex predators (e.g., large-bodied sharks) because they may play critical roles in shaping community interactions, food web structure, and the flow of

energy and biomass among ecosystems (Heithaus et al., 2008; Ferretti et al., 2010; Estes et al., 2011; McCauley et al., 2012). The ecological implications of declining mesopredatory shark populations are also likely to have cascading effects on biotic assemblages and food webs, however, as opposed to apex predators, these might be more species-specific and depending on the habitat studied (Heupel et al., 2014; Frisch, Ashley et al., 2016; Roff et al., 2016; Rasher et al., 2017).

While sharks occur in all major oceans, the greatest diversity is found in the tropics, where they also face the largest anthropogenic threats (Dulvy et al., 2014). The mutual affinity for tropical coastal areas results in the occupation of similar ecological niches by multiple species of sharks. Despite this co-occurrence, there is relatively little knowledge on how coastal sharks affect the resource use of other sympatric shark species, although this has important implications for the abundances and distributions of other marine communities (Estes et al., 2011), as well as for creating ecosystem-based management plans (Pikitch et al., 2004). Studies that have investigated resource partitioning of coastal sharks have either examined dietary overlap (e.g., Bethea et al., 2004; White et al., 2004; Kinney et al., 2011; Tillett et al., 2014; Shaw et al., 2016; Matich et al., 2017c; Shiffman et al., 2019), habitat overlap (Cartamil et al., 2003; Speed et al., 2011; Heupel et al., 2018b), or have used a combination of these two approaches (Papastamatiou et al., 2006; Bethea et al., 2011; Speed et al., 2012; Matich & Heithaus, 2014). Such niche investigations, especially multi-dimensional approaches (i.e., including more than one niche dimension) improve the ecological understanding and conservation and management needs of sympatric shark species. However, in many cases, logistical challenges related to mobile and large-bodied sharks prevent insights on additional data, such as data on competitive abilities, population characteristics, and fitness-related traits. Taken together, a multi-disciplinary approach composed of *i*) a multi-dimensional (trophic, spatial, and temporal) niche investigation, *ii*) insights on competitive abilities, and *iii*) baseline data on the biology and ecology would considerably further our understanding about the key factors that influence the underlying ecological mechanisms of sympatric sharks, and how asymmetric segregation patterns may differently impact unequal competitors in the long-term.

1.3 Multi-disciplinary methods to unveil mechanisms of coexistence

1.3.1 Dietary investigations

There is a variety of ways to measure and compare a sharks' diet and resource use; from dissecting and visually observing stomach items to the use of biochemical tracers. Each method has its limitations, which can bias data collection and its interpretation. The traditional approach of dissection an animal's digestive tract is an efficient and thorough method, as it allows the collection of the entire amount of ingested prey items (Bangley et al., 2013). While dissections can be useful for the investigation of small-bodied sharks with no urgent conservation concern (Bethea et al., 2004) or for commercially landed species (McElroy et al., 2006; Biton Porsmoguer et al., 2014), this method is not applicable for mobile and large-bodied sharks with conservation concern, although this is contentious (Heupel & Simpfendorfer, 2010; Hammerschlag & Sulikowski, 2011). As an alternative approach, ingested prey items can also be sampled via non-lethal stomach content analyses (SCA), such as gastric lavages or stomach eversions. These techniques enable considerable insights into a species' diet (Newman et al., 2010), and when coupled with genetic analyses such as DNA barcoding, the accuracy of prey species identification is significantly increased (Barnett et al., 2010; Dunn et al., 2010). Although recent improvements in genetic approaches (Matley et al., 2018), there are several limitations and considerable concerns inherent to SCA: the dependence of the digestive stage (soft tissues are digested faster than bony tissues; Wilson et al., 1985), the 'snapshot' view of the diet (only the most recent prey consumption is apparent; Bearhop et al., 2004), the invasive and time consuming sampling collection (Maia et al., 2006), and the high percentages of empty stomachs (Cortés, 1997).

To compensate for these limitations, biochemical tracers, such as stable isotopes, are an increasingly used and powerful approach in ecology to study diet and food web dynamics (Hussey et al., 2012; Layman et al., 2012; Munroe et al., 2018). Stable isotope analysis (SIA) delineates the isotopic niche of animal by making use of the fact that consumer tissues integrate information on resource use, as well as foraging habitats (Bearhop et al., 2004; Newsome et al., 2007). Because the ratio of heavy-to-light isotopes of carbon ($\delta^{13}C$) only depicts minimal changes with trophic positions, carbon can be used to estimate baseline ecosystem signatures, foraging strategies and locations (Hobson, 1999). The ratio of heavy-to-light isotopes of nitrogen ($\delta^{15}N$) increases in a

predictable way and can therefore be used to estimate the consumer's trophic position (e.g., Post, 2002). Together, $\delta^{13}C$ and $\delta^{15}N$ can be used as a proxy for trophic niches (Newsome et al., 2007; Jackson et al., 2011). Since the isotopic turnover rate (e.g., the duration of time it takes to integrate the prey tissue into the consumer tissue) is influenced by metabolic activity, a variety of body tissues can be used for elucidating temporal trends (Hussey et al., 2012). For example, the turnover rate of stable isotopes in metabolically inert tissues (e.g., fins) represents food intake over the long term (months to years; MacNeil et al., 2006), while active tissues such as blood fractions represent more recent dietary patterns (weeks to months; MacNeil et al., 2006; Kim et al., 2012). The use of blood fractions, specifically red blood cells (RBC) and blood plasma, has increased over recent years (Vander-Zanden et al., 2015), because they provide insights into very recent trophic interactions, which may be needed if the early foraging abilities of slow growing marine species are to be investigated (McMeans et al., 2009; Vaudo et al., 2010; Matich et al., 2019). The main limitation of SIA is that it does not provide the taxonomic resolution of stomach contents, and multiple trophic pathways can lead to similar stable isotope values despite different diets (Martinez del Rio et al., 2009; Kinney et al., 2011). Taken together, SIA is a considerable and frequently used method to investigate trophic niche patterns of sharks (Hussey et al., 2012; Layman et al., 2012; Munroe et al., 2018), but should, whenever feasible, be validated with stomach content data (Kinney et al., 2011).

1.3.2 Movement and spatial investigations

To fill the knowledge gap regarding multi-dimensional niche investigations, insights from additional niche axes are required, most importantly, data on movements and spatial pattern. There are multiple methodical approaches to study sharks' spatial pattern, but methods can roughly be categorized into three categories: broad-scale investigations on large spatial areas, broad-scale investigations on small spatial areas, and fine-scale investigations on small spatial areas. Broad-scale studies on large spatial scales are used for highly mobile species with long-distance movements that can span entire coast lines or ocean basins (Kessel et al., 2014b; Hussey et al., 2015; Lea et al., 2015a). Such studies often rely on satellite telemetry (Lea et al., 2015a) and passive acoustic telemetry (Kessel et al., 2014b), because both methods can reveal data on very large scale (i.e., in kilometers). While satellite telemetry prevents insights into fine-scale movement pattern, acoustic telemetry only registers tagged animals within the range of fixed

receivers. Hence, the further away acoustic receivers are installed from each other, the less accurate are spatial location measurements and the longer the duration of time where no data is collected (Hussey et al., 2015). If, however, the acoustic receivers are moored in closer proximity from each other, long-term data on residency and space use can be collected (e.g., Filmalter et al., 2013; Munroe et al., 2016). This method is especially useful for species with residential movement patterns or for species with high reliance on specific ecosystems for survival (Roff et al., 2016; Heupel et al., 2018b).

However, if a study's objective is to receive higher resolution data, passive acoustic telemetry may not deliver the accuracy needed. In this case, and also if the study's objectives does not aim at a long-term investigation, active acoustic telemetry and manual tracking may be accurate methods. Active acoustic tracking, although arduous and time consuming, provides some of the highest spatial resolution on sharks' movements (Towner et al., 2016) and can also be used to track small-bodied or juvenile sharks (Morrissey & Gruber, 1993b; George et al., 2019). Active tracking has also been used to investigate activity space and fine-scale movements of various elasmobranchs (Papastamatiou et al., 2009b; McCauley et al., 2014; Towner et al., 2016; George et al., 2019), and can further be used to identify different modes of movements, e.g. area restricted searching (ARS; Fauchald & Tveraa, 2003). In shallow and turbid waters where the detection range of acoustic receivers may be too limited (Kessel et al., 2014a), towed floats, similar to what has been used for eagle rays and stingrays (Riding et al., 2009; Martins et al., 2019), may provide an additional approach to gather fine-scale movement data of sharks. This methodology, although primitive when compared to the above mentioned and elaborated tracking technologies, allows real-time tracking in very shallow and potentially turbid waters. This method may particularly be useful for small-bodied animals occurring in areas where acoustic tracking may not be applicable.

1.3.3 Competitive abilities

To better understand if niche patterns are driven by competition, in-depths knowledge about species' competitive abilities are needed. There are two main approaches on how to assess the competitive abilities of sympatric species. One is to remove one of the sympatric species and see if its absence causes changes in the foraging pattern of the other species. This traditional experimental manipulation is effective to investigate competitive abilities across small and

abundant species with fast reproduction cycles, such as small birds, barnacles, and small reef fishes (Connell, 1961; Alatalo et al., 1987; Martin & Martin, 2001; Munday et al., 2001). However, for relatively small-bodied animals that cannot be removed or dislocated from their habitat (e.g., due to conservation concern), competitive interactions can also be tested through aquaria- or mesocosm experiments (Munday et al., 2001; Davis et al., 2015). Such captive studies can for example measure habitat choice or consumption rate during a specific time when a competing individual is present or absent (Munday et al., 2001; Davis et al., 2015).

Insights about a species' competitive abilities are subsequently useful in models that predict the distribution of sympatric species across habitats of varying resource quality. Such habitat distribution models base their assumptions on the ideal free distribution (IFD), which suggests that sympatric species have equal access to all habitats, and thus distribute themselves optimally across a landscape (Fretwell & Lucas, 1970). However, since interference competitive abilities (e.g., dominance hierarchies) are rarely equal in sympatric species, the IFD can be adjusted so that it also considers unequal competitors. Competitor models for unequal dominance predict one of two general distributions: (1) a (semi) truncated distribution, where high-quality habitats are occupied by dominant competitors while subordinates are either mixed between high- and low-quality habitat or only occur in low-quality habitats (Holmgren, 1995); or (2) dominants and subordinates are equally distributed across high- and low quality habitats, resulting in a mixed distribution (Smallegange & van der Meer, 2009; Pilfold et al., 2014). A recent study has further suggested that an animal's competitive advances may only exist in certain habitats, therefore habitat-specific competitive advantages should additionally be considered in species distribution models (Papastamatiou et al., 2018). Overall, while habitat distribution models with large-bodied predators rely on estimates of a species' competitive ability (Pilfold et al., 2014; Papastamatiou et al., 2018), studies on small-bodied predators can integrate exclusion or mesocosm experiments to measure a species' competitive ability prior to applying spatial distribution models.

1.3.4 Population characteristics and fitness-related traits

In order to understand if niche pattern have the ability to influence population characteristics and fitness-related traits in the long-term, comprehensive knowledge on species-specific biology and ecology are essential. Population characteristics and fitness-related traits describe a species'

characteristics in terms of its survival, growth, and reproduction, and are particularly critical for understanding the status and susceptibility of species or populations to environmental changes and exploitation (Grime & Pierce, 2012). Traditionally, such data have been collected through lethal sampling, which is the most-effective way to gather these type of data (reviewed by Heupel & Simpfendorfer, 2010). Lethal sampling is especially effective, when specimens are used as completely as possible, when unused body tissues are shared among collaborators, or if they are archived for future scientific research (Heupel & Simpfendorfer, 2010). However, if lethal sampling is not an option to collect baseline data - due to species-specific conservation status or habitat-specific regulations - scientist must rely on non-lethal methods, such as mark-recapture techniques. Though traditional mark-recapture approaches have used external tags such as Rototags (Stevens, 1984), a growing number of studies applies internal chips, such as passive integrated transponder (PIT) tags, due to their long retention time (up to 17 years; Feldheim et al., 2013). Despite these technological advances, the innate limitations of the mark-recapture sampling approach to gather data on population characteristics remains the same: time, habitat and population size, and dispersal of the focal species. Mark-recapture in large-bodied and slow growing species may take several years (Bradley et al., 2017b), and can result in only very small numbers of recaptures (Barker et al., 2005; Hodgkiss et al., 2017). Similarly, if the habitat is not spatially restricted and the population consists of a large number of also dispersing individuals, the likelihood of recapturing already tagged individuals is considerably small (Barker et al., 2005). If, however, the study species occurs over a very small spatial range, shows limited dispersal, and the size of the population is relatively limited, the mark-recapture technique can be a useful method to estimate growth rates (Freitas et al., 2006; DiBattista et al., 2007), population size (Bradley et al., 2017b), reproduction cycles (Feldheim et al., 2013) and dispersal distances (Merson & Pratt, 2001). Despite the fact that lethal sampling provides the highest amount of biological and ecological data per specimen, the mark-recapture technique is a considerable method in areas, where lethal sampling is not an option, or where threatened species are excluded from any scientific sampling. Taken together, mark-recapture techniques are an essential approach to gather population characteristics and fitness-related traits, especially because additional data (e.g., resource use pattern and competitive abilities) can simultaneously be estimated from captured (and released) individuals.

1.4 Thesis approach

1.4.1 Studying sympatric juvenile reef sharks as model species

In this thesis, I apply the abovementioned methods to further our understanding on how multi-dimensional niche pattern may be linked with competitive abilities, population characteristics, and fitness-related traits by using two sympatric juvenile reef shark populations. I've chosen blacktip reef sharks, *Carcharhinus melanopterus* (Quoy & Gaimard 1824) and sicklefin lemon sharks, *Negaprion acutidens* (Rüppell 1837) as my two model species. *Carcharhinus melanopterus* are one of the most abundant high-level mesopredators (maximum length of 2 m) at many atolls, islands and coastal areas in the Indo-Pacific (Hobson, 1963; Stevens, 1984; Mourier et al., 2013b). *Negaprion acutidens* on the other hand, are considered as rare, data deficient, and threatened (assessed as Vulnerable by the IUCN [International Union for the Conservation of Nature] Red List with local extinctions in South East Asia [IUCN 2003]) apex predators (maximum length of 3.5 m) with a fragmented distribution range and small breeding populations (Compagno et al., 2005; Schultz et al., 2008; Mourier et al., 2013a). Despite these distinctions, females of both species use shallow nearshore areas of islands, atolls and coastal bays as partition sites and nursery habitats (Stevens, 1984; Speed et al., 2011; Oh, 2016; Matich et al., 2017c). In areas of sympatry, simultaneous parturition cycles can result in high numbers of morphologically similar sharks (size at birth 55 – 66 cm for *C. melanopterus*; 33 – 52 cm total length for *N. acutidens;* Mourier et al. 2013a, 2013b). Both species likely face unique foraging challenges when they aggregate in nearshore areas, because they are inexperienced, they typically have restricted home ranges and extended periods of residency, and may be frequented by large predatory sharks and teleosts (Heupel & Hueter, 2002; Bethea et al., 2004; Kinney et al., 2011; Tillett et al., 2014; Oh, 2016). Moreover, several concerns have been raised that nearshore nursery areas may not provide enough food resources (Duncan & Holland, 2006; Kinney, 2011; Kinney et al., 2011), thus competition for mutual prey resources is likely. Indeed, sympatric juvenile sharks were found to either forage on different trophic levels (as inferred by variable $\delta^{15}N$; Matich et al., 2017b), use sub-habitats at different times (DeAngelis et al., 2008), or apply different dietary modes (specialised versus opportunistic foragers; Kinney et al., 2011; Oh, 2016) likely as a means to avoid (or as a result of) competition in an areas where resources are limited. Cumulatively, while such studies estimate ecological niches, they do not conclude if niche patterns originate from competitive processes, or

arise from different habitat- and prey preferences, because competition was not tested. Moreover, small sample sizes and the lack of long-term recaptures (> 90 days) prevent insight on how asymmetric niche pattern may influence population characteristics and fitness-related traits in the long-term (Matich et al., 2017c). Consequently, my small-bodied and morphologically similar model species that demonstrate simultaneous parturition cycles and are amenable to captivity (Chin et al., 2015; Bouyoucos et al., 2018) are ideal study species for addressing these knowledge gaps.

1.4.2 Study locations

I chose two study locations for my thesis: the St. Joseph Atoll and Moorea. The majority of the research outlined in this thesis was conducted in the shallow, nearshore areas at the uninhabited and near-pristine St. Joseph Atoll in the Republic of Seychelles (05°26′S, 53°20′E; Figure 1.1, top figure). St. Joseph Atoll is part of the island chain that makes up the Amirantes Bank in the outer islands of Seychelles, and is composed of a ring of seven small islands that surround a central oval shaped lagoon. Multiple threatened and ecologically important species inhabit the atoll, including three species of stingrays (cowtail ray *Pastinachus sephen*, porcupine ray *Himantura granulate*, and mangrove ray *Urogymnus asperrimus*) (Elston, 2018), as well as two species of sea turtles (green turtle *Chelonia mydas* and hawksbill turtle *Eretmochelys imbricate*) (Mortimer et al., 2011). Most importantly, unpublished field observations hypothesized that juvenile *C. melanopterus* and juvenile *N. acutidens* coexist within the atoll's shallow and protected intertidal reef flats. Therefore, St. Joseph Atoll offers an ideal experimental site for a multi-disciplinary study in an uninhabited habitat with relatively little human interference.

I further chose Moorea, French Polynesia (17°30′S, 149°51′W) as an additional study location for my thesis (Figure 1.1, bottom figure). As opposed to St. Joseph Atoll, Moorea is populated, has a substantial infrastructure, and has an increasing number of visiting tourists each year. Juvenile *C. melanopterus* and juvenile *N. acutidens* are predominantly found across at least nine putative nursery sites (Mourier & Planes, 2013; Mourier et al., 2013b, 2013a), which are within or adjacent to a network of eight marine protected areas (MPA) with different levels of protection (de Loma et al., 2008). Importantly and similar to St. Joseph Atoll, juvenile sharks occur in very shallow habitats close to the shore where they possibly seek protection from adult sharks and predatory

fish (Mourier et al. 2013, personal observation, Weideli OC). Moorea thus not only offers an additional natural experimental habitat, but more importantly, the island's Centre de Recherches Insulaires et Observatoire de l'Environnement (CRIOBE) has laboratory facilities that allow captive dominance behaviour experiments to be conducted. Together, these two study locations and study species will allow me to further our understanding on the underlying mechanisms of coexistence.

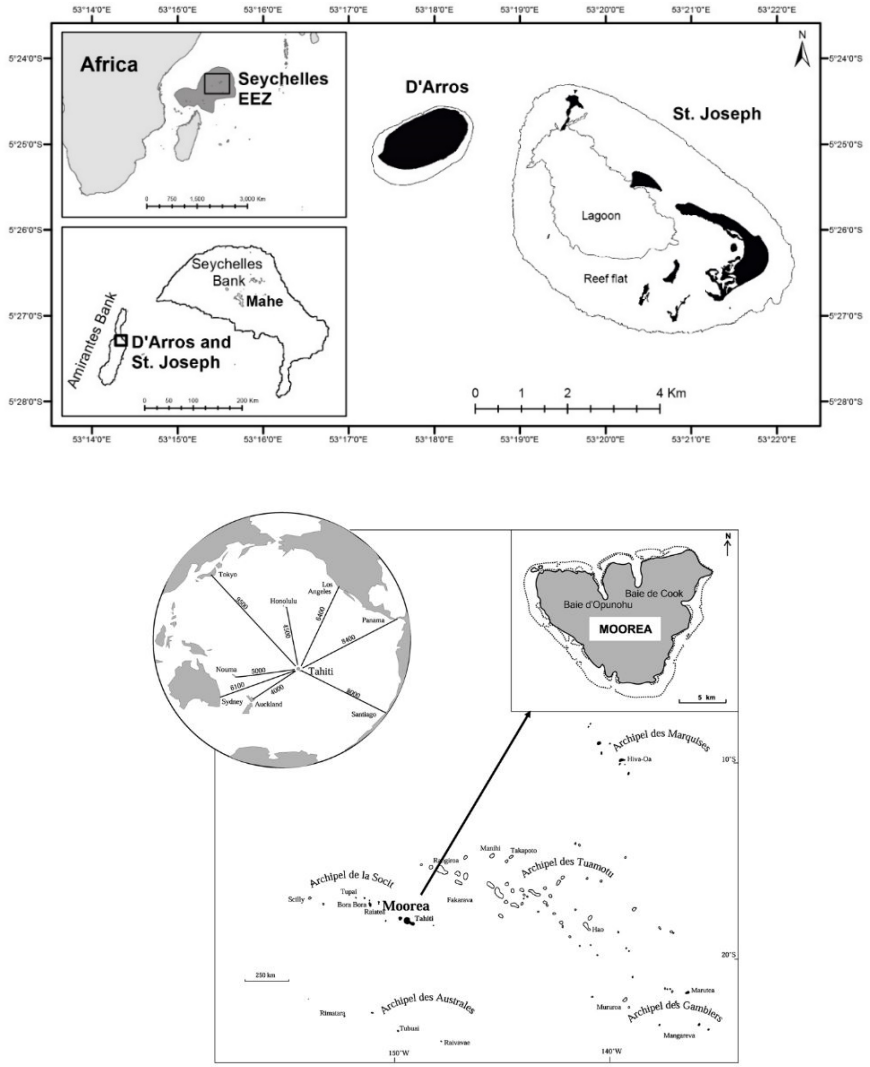

Figure 1.1 Study sites for this thesis. **Top**: Location of the Republic of Seychelles and St. Joseph Atoll and map of the study location with the neighbouring D'Arros island. Permanent landmasses are shown in black. Map created by Ryan Daly | SOSF. **Bottom**: Location of French Polynesia and map of Moorea within the Society Islands. Permanent landmasses are shown in grey. Map adapted from Mourier 2011.

1.5 Aims and outline of thesis

The overarching aim of this thesis is to improve our understanding of the underlying mechanisms of coexistence and to enable new insights on how niche pattern may be linked to competitive interactions, population characteristics, and fitness-related traits. To ensure accurate assessment, data from St. Joseph Atoll were collected during three parturition seasons between November 2014 and April 2017, while data from Moorea were gathered during two parturition seasons in the years 2016/2017 and 2017/2018. The data obtained from this comprehensive sampling approach were analysed and structured into four, research-based and complete manuscripts that are either published (**Chapter 2, 4, 5**) or to be submitted (**Chapter 3**) in a peer-reviewed journal. As a result, each chapter is written as a stand-alone work that includes an independent introduction that reviews relevant literature and identifies the overall aims of the chapter, as well as the methods, results and discussion related to that chapter. While data for **Chapters 2** and **4** were solely collected at St. Joseph Atoll, **Chapters 3** and **5** include data that originate from St. Joseph Atoll, as well as from Moorea (Figure 1.2). Each data chapter is formatted according to the requirements of the journal and is written in the first person plural. On the contrary, the general introduction (**Chapter 1**) as well as the general discussion (**Chapter 6**) are composed in the first person singular.

Figure 1.2 Schematic representation of the distribution of thesis chapters. Data for chapter 2 and 4 were solely collected at St. Joseph Atoll, while data for chapter 3 and 5 were collected at both, St. Joseph Atoll and Moorea. Map adapted from www.everycrsreport.com.

This thesis is structured as follows:

Chapter 2 addresses the question if the preservation of red blood cells (RBC) and blood plasma with the anticoagulants (EDTA and SH) bias stable isotope values. Determining if blood anticoagulants bias stable isotope values in blood components is crucial for **Chapter 3**, because trophic interactions were inferred (besides other methods) from blood stable isotope ratios. Knowing if anticoagulants bias blood stable isotope ratios has also broader implications, because the use of blood fractions for isotopic investigations has increased in recent years (Vander-Zanden et al., 2015), due to its wide application covering trophic interactions, spatial studies as well as individual foraging specializations (Matich et al., 2011, 2017c; Speed et al., 2011).

In **Chapter 3**, I study segregation and overlap pattern in sympatric *C. melanopterus* and *N. acutidens*. I use a multi-disciplinary approach that includes gillnet sampling, active tracking, stomach content analysis, and stable isotope analysis (SIA). For the SIA part of **Chapter 3**, I apply the findings from the **Chapter 2**. Further, dominance behaviour experiments were applied to validate patterns of resource use, a pilot approach that has, to the best of my knowledge, never been applied in sharks. Such multi-dimensional approaches in combination with competition experiments are needed to better understand processes underlying niche segregation, a strategy that provides to a wider understanding of the ecology of coexisting species.

In **Chapter 4**, I measure population characteristics (neonatal sizes, annual growth rates, long-term recaptures, and recapture distances) of juvenile *C. melanopterus* and *N. acutidens*. Besides reporting new insights of the biology and ecology of these juvenile sharks, I document the largest variation of growth rates in wild caught juvenile sharks. In line with previous findings (Martin & Martin, 2001; Munday, 2001), I investigate if sharks' competition effects (**Chapter 3**) influence population characteristics and fitness-related traits in the long-term.

In **Chapter 5**, I compare population characteristics such as size, weight, and body condition of newborn *C. melanopterus* from St. Joseph Atoll and Moorea. Further, to follow the sharks early development, body condition as well as foraging success are measured and compared among different early life-stages. Overall, this approach makes it possible to determine whether

population characteristics and foraging development in *C. melanopterus* vary across sites with different habitat characteristics.

Chapter 6 consists of the general discussion of my thesis, in which the main findings are synthesized, and the ecological and management implications and future research opportunities are summarized and discussed.

This thesis offers an extensive multi-disciplinary investigation on the underlying mechanisms of coexistence. The combination of a multi-dimensional niche investigation, dominance behaviour experiments, and long-term mark-recapture sampling have, to the best of my knowledge, never been performed in sympatric shark species. A greater knowledge of how similar sympatric shark species coexist and what consequences this coexistence may have for each of the sympatric species is needed, especially also because many of the nearshore areas used by multiple juvenile shark species are in close proximity to human developments (DeAngelis et al., 2008; Dale et al., 2011; Matich et al., 2017c, 2017b; Vierus et al., 2018). Anthropogenic exploitation can lead to declining prey resources (Lotze et al., 2006; Jennings et al., 2008), which may eventually increase competition for mutual prey resources.

Author contribution

O.C.W. wrote the original draft; O.C.W. edited subsequent drafts with support from, and under the supervision of Y.P.P. and S.P.

Chapter 2 | Effects of anticoagulants on stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) of shark blood components

This chapter is published as:

Weideli, O.C., Kiszka, J.J., Matich, P., Heithaus, M.R. (2019) Effects of anticoagulants on stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) of shark blood components. *Journal of Fish Biology*. 95(6):1535-1539. https://doi.org/10.1111/jfb.14164

Effects of anticoagulants on stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) of shark blood components

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Received: 09 May 2019 | **Accepted**: 15 October 2019 | **Published online**: 16 October 2019

Key words: Blood plasma, blood preservatives, *Carcharhinus melanopterus*, $\delta^{13}C$, $\delta^{15}N$, red blood cells (RBC)

2.1 Abstract

The effects of anticoagulant ethylenediamine tetraacetic acid (EDTA) and sodium heparin (SH) on stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic values of red blood cells (RBC) and blood plasma in juvenile blacktip reef sharks *Carcharhinus melanopterus* were analysed. Plasma preserved with anticoagulants was not isotopically distinct from plasma stored in no-additive control tubes. RBC $\delta^{15}N$ values exhibited small enrichments when preserved with EDTA and SH. Results suggest EDTA and SH are viable anticoagulants for stable isotopic analyses of blood fractions, however additional studies are encouraged to validate results.

2.2 Introduction

Investigating the trophic ecology of marine predators, such as sharks is crucial for understanding their ecological roles and importance (Heithaus et al., 2008; Speed et al., 2012; Roff et al., 2016). Using naturally occurring carbon and nitrogen stable isotopes as chemical tracers provides the opportunity to examine the trophic ecology of sharks and other taxa over various time scales (Matich et al., 2017a). Stable isotope ratios of carbon 13C:12C ($\delta^{13}C$) and nitrogen 15 N:14 N

($\delta^{15}N$) can depict the food webs in which consumers are foraging and their relative trophic position (Hobson, 1999), respectively. Stable isotope analysis (SIA) can also provide further insight into ontogenetic shifts in trophic interactions, long and short-term movements and individual foraging specializations (Papastamatiou et al., 2010; Matich et al., 2011, 2017c; Speed et al., 2012; Kiszka et al., 2015), but interpretation of isotopic data must be made cautiously (Thomson et al., 2018).

Depending on the study questions and species of interest, a variety of body tissues are used for SIA in sharks (Hussey et al., 2012). Metabolically inert (e.g., bones, fin) or active (e.g., liver, muscle, blood) tissues can be sampled independently, or in combination they allow for the investigation of potential temporal dietary changes (Bearhop et al., 2004; Matich & Heithaus, 2014; Matich et al., 2019). The use of blood fractions, specifically red blood cells (RBC) and blood plasma, has increased over recent years (Vander-Zanden et al., 2015). RBC and plasma are especially useful to assess trophic positions and foraging behaviours of juvenile sharks (Kinney et al., 2011; Matich et al., 2015, 2017c; Hussey et al., 2017), because they incorporate trophic interactions over different time scales and can be collected non-lethally. RBC stable isotope values reflect energy sources (e.g., foraging and maternal provisions) over extended periods (multiple months), while plasma stable-isotope values represent more recent trophic interactions (weeks to months; McMeans et al., 2009; Vaudo et al., 2010; Matich et al., 2019).

Despite the advantages of using blood for SIA, one of the major limitations of its use is its rapid coagulation. To obtain accurate isotope values for RBC and plasma, they need to be separated immediately but remote and logistically challenging field conditions may impede such rapid centrifugation. Therefore, alternative solutions that prevent tissue degradation are needed. Blood preservatives, such as anticoagulants, provide a potential solution. Anticoagulants inhibit the coagulation process and extend the time before centrifugation is needed. Common preservatives for blood collection and storage include EDTA and sodium heparin (SH). EDTA prevents the coagulation cascade through its binding ability of calcium and magnesium as a chelator (Rand et al., 1996; Banfi et al., 2007) and SH stimulates the production of antithrombin III to inactivate thrombin (Shuman & Majerus, 1976).

In order to correctly interpret stable isotope values in blood tissues of young sharks, it is critical to know if blood anticoagulants bias these values. Recent investigations in leopard sharks *Triakis semifasciata* (Girard 1855) showed that RBC and plasma collected in tubes coated with lithium heparin (an anticoagulant) were not isotopically distinct from blood collected in no-additive tubes (Kim & Koch, 2012). In contrast, studies on birds and sea turtles revealed that stable-isotopic signatures are influenced by anticoagulants and the magnitude and nature of shifts in isotopic values varied among taxa, blood fractions and anticoagulants (Bugoni et al., 2008; Lemons et al., 2012). Consequently, by investigating the effects of a different assortment of anticoagulants on blood components in another species of sharks, we aim to extend the currently limited knowledge of blood preservative effects on isotopic values in shark blood. In this study, we investigated whether EDTA and SH preservation modified $\delta^{13}C$ and $\delta^{15}N$ values in RBC and plasma of blacktip reef sharks *Carcharhinus melanopterus* (Quoy & Gaimard 1824).

2.3 Material and methods

Juvenile sharks were captured with gillnets (20.0 m × 1.5 m, 5.0 cm mesh) at St. Joseph Atoll, Republic of Seychelles (05°26’ S,53°20’ E) from November to December 2014. After the insertion of PIT tags (Biomark; www.biomark.com) and total length measurements (L_T), blood was collected from the caudal vein using 5 ml syringes (BD Plastipak; www.bd.com) with 18 gauge needles (BD PrecisionGlide). Each 5 ml blood sample was split into three tubes: 2 ml in EDTA coated tubes (BD), 2 ml in SH coated tubes (BD) and 1 ml in no-additive (control) tubes. To avoid coagulation, the latter sample was immediately spun at 1500 *g* by a hand-powered centrifuge (Hettich; www.hettich.ch) for 30 s and resulting blood components (RBC and plasma) were separated and kept in no-additive tubes. Together with the EDTA and SH treated samples, all tubes were kept on ice for a maximum duration of 6 h.

At the laboratory, blood samples preserved in anticoagulants were spun with a mini-centrifuge (Mini Fuge, STARLAB; www.starlabgroup. com) at 2000 *g* for 1 min and then frozen with control samples at -18°C. After 7 days, blood samples were placed in a drying oven (60° C) for 72 h and homogenised with a mortar and pestle. Subsequently, to determine the abundances of carbon (13C:12C) and nitrogen (15N:14N), 500–800 μg of the homogenised powder was loaded into tin

capsules and analysed by a continuous-flow isotope-ratio mass spectrometer [Finnigan Delta C EA-IRMS (with temperature conversion element analyser; TC-EA), Thermo Fisher Scientific; www.thermofisher. com] with bovine liver, international Atomic Energy Authority (I-AEA)-N-1, I-AEA-C-6 and glycine used as standards. Variation among laboratory standard for samples were 0.15‰ for $\delta^{13}C$ and < 0.10‰ for $\delta^{15}N$. Lipid extractions were not conducted, because C:N for RBC (mean ± SD; 2.8 ± 0.08) and plasma (1.8 ± 0.03) were below those recommended for extraction or mathematical correction (Hussey et al., 2012).

Where parametric assumptions were met (assessed with Shapiro-Wilk tests), we used paired t-test to determine whether $\delta^{13}C$, $\delta^{15}N$ and C:N isotopic values from treated samples (EDTA and SH) were statistically different from non-additive control samples. Where assumptions were not met for $\delta^{13}C$, $\delta^{15}N$ and C: N, we applied a Wilcoxon signed rank test to compare differences between treated and non-additive control samples. Also, power analyses were run for comparisons. All the statistical analyses were performed in R (version 3.5.3; R Core Team 2017; www.r-project.org) within the RStudio interface 1.0.153 (RStudio Team 2016) and the level of statistical significance α was set at 0.05.

2.4 Results and discussion

Eleven juvenile *C. melanopterus* ranging from 54.6 - 78.0 cm L_T (mean ± SD; 62.8 ± 7.7 cm L_T) were collected (mean $\delta^{13}C$, $\delta^{15}N$ and C:N values in Table 2.1). For RBC, there was no significant difference in $\delta^{13}C$ values nor in C:N values between no-additive control and treated samples. The difference in mean $\delta^{15}N$ values for no-additive control and EDTA and SH treated samples was small (0.0873‰ and 0.2018‰, respectively), but significant (Figure 2.1 and Table 2.2). No-additive plasma $\delta^{13}C$, $\delta^{15}N$, or C:N were not different from treated plasma. Mean differences in $\delta^{13}C$ and $\delta^{15}N$ were considerably higher in plasma than RBC, except for $\delta^{13}C$ between control and EDTA treated samples (mean difference -0.0854‰; Table 2.2).

Table 2.1 *Carcharhinus melanopterus* captured at St. Joseph Atoll, Seychelles. Mean ± SD of $\delta^{13}C$, $\delta^{15}N$ and C:N values for control (no-additive) and blood components treated with anticoagulants (EDTA and SH).

Tissue	n	$\delta^{13}C_{CON}$	$\delta^{13}C_{EDTA}$	$\delta^{13}C_{SH}$	$\delta^{15}N_{CON}$	$\delta^{15}N_{EDTA}$	$\delta^{15}N_{SH}$	$C:N_{CON}$	$C:N_{EDTA}$	$C:N_{SH}$
Carcharhinus melanopterus										
RBC	11	-9.04 ± 0.84	-9.15 ± 0.86	-9.08 ± 0.83	10.89 ± 0.45	10.88 ± 0.6	10.77 ± 0.59	2.83 ± 0.08	2.79 ± 0.13	2.79 ± 0.06
Plasma	11	-8.95 ± 0.75	-8.87 ± 0.83	-9.20 ± 0.82	10.84 ± 0.75	11.35 ± 0.78	10.63 ± 0.74	1.80 ± 0.12	1.76 ± 0.05	1.83 ± 0.33

Abbreviations: CON, control; EDTA, ethylenediamine tetraaceitic acid; SH, sodium heparin.

Table 2.2 Statistical results from paired analyses between control (no-additive) and blood components treated with EDTA or SH.

Test	Tissue	Parameter	Mean difference	n	t	Two-tailed p-value	Comments
Control vs. EDTA	RBC	$\delta^{13}C$ values	0.1118	11	1.4347	0.1819	
		$\delta^{15}N$ values	0.0873	11	2.6966	**0.02244***	
		C:N	0.0349	11	43 (v)	0.4131	Wilcoxon test
	Plasma	$\delta^{13}C$ values	-0.0854	11	-0.6803	0.5118	
		$\delta^{15}N$ values	-0.5064	11	-1.8205	0.0987	
		C:N	0.0305	11	1.0121	0.3354	
Control vs. SH	RBC	$\delta^{13}C$ values	0.0445	11	1.1276	0.2858	
		$\delta^{15}N$ values	0.2018	11	7.3136	**< 0.001***	
		C:N	0.0369	11	1.0102	0.3362	
	Plasma	$\delta^{13}C$ values	0.2482	11	0.7918	0.4469	
		$\delta^{15}N$ values	0.2164	11	1.007	0.3377	
		C:N	-0.0367	11	43 (v)	0.4131	Wilcoxon test

Abbreviations: EDTA, ethylenediamine tetraaceitic acid; SH, sodium heparin. * denotes significant difference

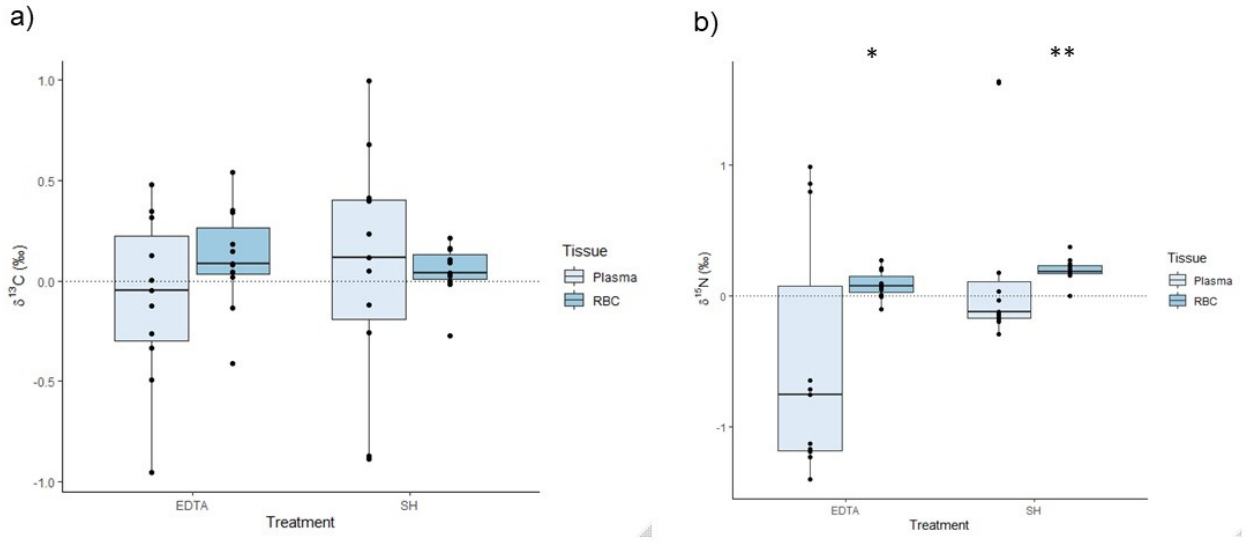

Figure 2.1 Individual and mean differences in $\delta^{13}C$ and $\delta^{15}N$ values between no-additive control and treated samples for all blood components. Boxes indicate the interquartile range with the median shown by horizontal lines, minimum and maximum values shown by whiskers, and black dots represent outliers. Horizontal dotted lines denote zero difference between control and treated samples. Significant difference from control, where * = $p < 0.05$, ** $p < 0.001$ (see Table 2 for details). EDTA: ethylene diaminetetraaceitic acid SH: sodium heparin; RBC: red blood cells.

To our knowledge, the present study is the first characterization of the effects of EDTA and SH on stable isotope values in RBC and plasma in sharks. Our results revealed that RBC and plasma isotope values were highly similar in no-additive control samples and samples treated with anticoagulants. The only significant differences between control and treated samples were found in RBC $\delta^{15}N$ values treated with either EDTA or SH. Despite statistical significance, differences among treatments were small, particularly for EDTA (< 0.1 ‰; Table 2.2), suggesting minimal effects of these anticoagulants on isotopic values in blood components.

Overall, our study confirms and extends the findings provided by Kim and Koch (2012), where blood components treated with lithium heparin yielded accurate isotopic data for shark blood. This is a promising outcome for the increasing number of isotopic studies on blood components (Vander-Zanden et al., 2015), but interpretation must be made cautiously. Our limited sample size may have resulted in smaller treatment effects and mean differences between control and treated plasma samples showed high variability for both anticoagulants (Figure 2.1 and Table 2.2). This

observation is further supported by low statistical power (0.2) and there remains a potential for type II error, particularly for plasma samples. Further research should therefore aim at larger sample sizes and potentially include multiple shark species in order to validate if results are comparable and applicable for isotopic studies on a wide range of sharks.

Acknowledgements

The authors wish to thank C. Boyes, R. von Brandis, K. Bullock and numerous SOSF-DRC assistants for their support in the field and J. Harris, K. Gastrich and M. Sabando for their assistance at Florida International University. This is contribution #169 from the Center for Coastal Oceans Research within the Institute of Water and Environment at Florida International University.

Author contribution

O.C.W. designed and coordinated the study; O.C.W. collected field data; O.C.W. analysed the data and wrote the manuscript with support from J.J.K, P.M. and M.R.H.; all authors gave approval for publication. O.C.W. secured the funding to conduct this study.

Chapter 3 | Mechanisms of coexistence with predator-induced niche overlap in sympatric juvenile sharks

This chapter is in preparation for submission to *Oecologia* as:

Weideli, O.C., Papastamatiou, Y.P., Daly, R., Peel, L.R., Shivji, M.S., Heithaus, M.R., Planes, S. Mechanisms of coexistence with predator-induced niche overlap in sympatric juvenile sharks.

Mechanisms of coexistence with predator-induced niche overlap in sympatric juvenile sharks

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Keywords: *Carcharhinus melanopterus*, competition, dominance hierarchies, *Negaprion acutidens*, risk effects, niche overlap, niche segregation, risk effects.

3.1 Abstract

To alleviate the effects of interspecific competition, subordinate competitors may avoid dominant competitors in space, time, or through variable prey selection. In the presence of a common predator, the ability to partition resources may, however, be reduced for sympatric species and niche overlap can be increased. Shark nursery areas, where coexisting juvenile species are forced to use shallow waters to avoid predation, provide an environment to investigate the mechanisms maintaining coexistence. We study sympatric juvenile shark species (blacktip reef shark,

Carcharhinus melanopterus and sicklefin lemon shark, *Negaprion acutidens*) that co-occur in the intertidal reef flats of St. Joseph Atoll, Seychelles, likely as the threat of predation in the deeper lagoon is very high. Although blacktip reef sharks were captured over a broader range of available microhabitats than lemon sharks, within areas where both species are found, daily space use of actively tracked sharks showed a high degree of overlap between species. Stomach content and stable isotope analyses indicated asymmetric segregation patterns, with blacktip reef shark diet including a broader range of prey items with lower energy content, albeit a smaller isotopic niche. Behavioural experiments concurrently conducted in Moorea, French Polynesia, revealed that lemon sharks displayed dominance over blacktip reef sharks based on give-way interactions and feeding rates. Overall, our results match predictions of competition models with a subordinate avoiding the dominant competitor through variable prey selection, and highlight that predatory risk effects may impede spatial segregation and consequently intensify interspecific competition across juvenile shark populations.

3.2 Introduction

Coexisting species, especially those that are closely related and morphologically similar, may compete for the same natural resources (Hutchinson, 1957; MacArthur, 1958). If resources are limited, niche segregation may reduce interspecific competition (Gause, 1934; Pianka, 1974; Schoener, 1974), because complete competitors cannot coexist (Hardin, 1960). Niche segregation can be revealed in any dimension of the *n*-dimensional niche hypervolume (Hutchinson, 1957), for example along spatial, temporal, or dietary niche axes (Schoener, 1968; Alatalo et al., 1987; Barlow et al., 2002; Wilson, 2010). Segregation effects are asymmetric with one of the interacting species being more affected than the other (Connell, 1983; Schoener, 1987), because even morphologically and ecologically similar species differ in their competitive abilities through differences in dominance hierarchies, prey handling, and search times (Holmgren, 1995). This can make it challenging to unveil the driving force behind niche segregation, particularly in light of the contentious issue of drawing a direct link between niche segregation and competition (e.g., Wilson, 2010; Papastamatiou et al., 2018).

Ideal free distribution (IFD) models can predict the spatial distribution of competing species, dependent on how differences in competitive capabilities manifest themselves (e.g., dominance hierarchies; Holmgren 1995). Under condition of differences in dominance, the IFD models predict across a two patch (high and low quality habitat) system either: (1) a (semi) truncated distribution, where high-quality habitats are occupied by dominant competitors, while subordinates are either mixed between high- and low-quality habitats or only occur in low-quality habitats; or (2) high- and low-quality habitats are equally inhabited by dominants and subordinates (Holmgren, 1995; Smallegange & van der Meer, 2009). Alternatively, animals may select different prey either because they forage in different habitats (where different prey are found) or because they reduce interspecific competition within shared habitats (Davis et al., 2015).

Space use and patch selection by sympatric species will, however, be driven by other factors in addition to competition and resource distribution, such as predator avoidance. By avoiding the threats of predation, a prey's behaviour may be altered, including changes in habitat and foraging pattern (Heithaus, 2007; Lester et al., 2020). While such behaviours can be costly for a single prey species (Heithaus & Dill, 2002), these ecological costs of behavioural adaptations ('risk effects') may also result in sympatric species being forced to have higher niche overlap because they will likely have limited options for reducing overlap. In areas where spatial avoidance is impeded, risk effects may be more pronounced for the subordinate, because the dominant competitor is selectively feeding on more energy efficient and preferable food resources, while the subordinate species forages on a wider range of prey, including prey of lower energy content (Davis et al., 2015; Droege et al., 2017).

Several species of reef sharks use shallow nearshore habitats for parturition or as nursery areas, resulting in spatially-restricted areas with high numbers of juvenile sharks of multiple species (Simpfendorfer & Milward, 1993; Bethea et al., 2004; Kinney et al., 2011; Tillett et al., 2014; Vierus et al., 2018). Nearshore nursery areas are microhabitats commonly characterized by very shallow intertidal reef or sand flats, likely as they provide juvenile sharks with safety from predators (e.g., Morrissey & Gruber, 1993a; Simpfendorfer & Milward, 1993; Heupel & Hueter, 2002; Heithaus, 2007; Heupel et al., 2007; Guttridge et al., 2012; George et al., 2019). In areas with considerable tidal fluctuations, juvenile shark movements are often synchronized with tidal

cycles (George et al., 2019), which further limits the potential for spatial separation among species. Juvenile sharks may therefore reduce interspecific competition via dietary partitioning, with the larger species feeding at a higher trophic level (Matich et al., 2017c), or exhibiting different foraging strategies (opportunists vs. specialists; Kinney et al., 2011; Tillett et al., 2014). However, no study has yet investigated the mechanisms by which juvenile sharks of different species within a restricted nursery area may coexist, and/or the role of competition and risk effects in driving niche segregation patterns.

Juvenile blacktip reef sharks (*Carcharhinus melanopterus*) and juvenile sicklefin lemon sharks (*Negaprion acutidens*, hereafter referred to as "lemon sharks") coexist on islands, atolls, and in coastal areas throughout the Indo-Pacific (Stevens, 1984; Mourier et al., 2013b; Oh et al., 2017). Both species also coexist at St. Joseph Atoll in the Republic of Seychelles where juveniles occupy shallow reef flats with considerable tidal fluctuations (**Chapter 4**), likely as a refuge from the large numbers of predators found in deeper lagoon waters (Filmalter et al., 2013; Lea et al., 2016, 2020). Both species are born during the same time of the year, are morphologically similar despite lemon sharks being slightly larger, and their distribution patterns are restricted to the atoll's intertidal reef flats during at least the first 2.5 years of life (**Chapter 4**). We assess how coexistence between these two species occurs by quantifying the degree of spatial and dietary overlap using a combination of gillnet sampling, active tracking, stomach content, and stable isotope analysis at St. Joseph Atoll. Due to availability of experimental facilities, captive experiments we conducted in parallel in Moorea, French Polynesia. Since dominance hierarchies in sharks appear size-based (Myrberg & Gruber, 1974), we: *i*) hypothesize that the larger lemon shark will be dominant over the blacktip reef shark, and test this prediction using captive dominance behavioural experiments. We *ii*) predict, based on the unequal competitor IFD (based on dominance) that dominant lemon sharks either use different space than subordinate blacktip reef sharks (truncated distribution), or only use a portion of available space used by blacktip reef sharks (a semi-truncated distribution). If space use patterns overlap due to risk effects, we *iii*) predict dietary segregation to occur, or dominant lemon sharks having a more specialized diet than blacktip reef sharks (i.e., lemon sharks have a reduced trophic niche).

3.3 Material and methods

3.3.1 Captive dominance behaviour experiments

Captive studies were used to quantify dominance hierarchies between lemon sharks and blacktip reef sharks using individuals captured in the nearshore areas of Moorea (S 17°30'; W 149°50'), French Polynesia (Mourier & Planes, 2013; Mourier et al., 2013b, 2013a) in December 2016. After capture with gillnets (50.0 m × 1.5 m, 5.0 cm mesh), sharks were returned to the research facility of the Centre de Recherches Insulaires et Observatoire de l'Environnement (CRIOBE) and habituated to laboratory conditions for one week in 1250 L circular tanks (1.7 m diameter). Sharks were fed 5% of their body mass every other day with fresh tuna (*Thunnus spp*.). A circular test tank (4 m diameter) filled to approximately 50 cm depth was set adjacent (< 4 metres) to the holding tanks. All tanks were set out-door in a covered and calm area, and temperature, salinity, and pH levels were monitored twice per day. The sharks were fasted for 48-hours before the start of trials to ensure a standardized fasted level (Chin et al., 2015; Bouyoucos et al., 2018). Sharks were transferred from holding tanks to experimental tanks 12 hours prior to commencing the trial and were paired with an individual they had no prior contact with. Transfer-related stress was assumed to be negligible because the process was quick, and the experimental tank was adjacent to the holding tanks.

The behaviour experiments consisted of two treatments: 1) one blacktip reef shark and one lemon shark without food, 2) one blacktip reef shark and one lemon shark with food. In trials without food (Test 1), the sharks' behaviour was recorded for 10 min with a GoPro Hero3 camera mounted 2.5 meters above the center of the tank (Supplementary material S1). In trials with food (Test 2), sharks' behaviours were monitored by the ceiling camera, and a GoPro Hero3 camera mounted on a Plexiglas frame also used as a feeding plate. This feeding plate was equipped with 5 × 10g of fresh *Thunnus spp*. in view of the camera. The plate was randomly placed in the circular tank and the camera recorded the feeding activity for 10 min (Supplementary material S1). Following the trials, the video sequences from the camera installed above the centre of the tank were analyzed to monitor when one species gave way to the other (1 for the dominant species, 0 for the species that gave way). The feeding plate video sequences were used to estimate interference competition (e.g., the dominance interactions of one species alters the feeding rates of the other species) by counting

the number of prey items eaten per species. Data were analyzed with Pearson's Chi^2-tests to determine if the ratio of dominant interactions and number of prey items eaten between the species were significantly different. After cessation of experiments, sharks were returned to the holding tanks for observation and then released at the site of capture. No mortality occurred during experiments.

3.3.2 Field sampling and capture locations

Fieldwork surveys were conducted at St. Joseph Atoll, Republic of Seychelles (05°26' S, 53°20' E, Figure 2.1), an uninhabited and near-pristine habitat. Juvenile blacktip reef and lemon sharks were captured during the parturition months in austral summer (October – April) from 2014 until 2017. Sharks were captured using gillnets (20.0 m × 1.5 m, 5.0 cm mesh) deployed on the intertidal reef flats during high, low, incoming and outgoing tides. No fishing was conducted during extreme spring high tides or during the night due to logistical constraints. For each captured shark, GPS coordinates at capture locations were recorded.

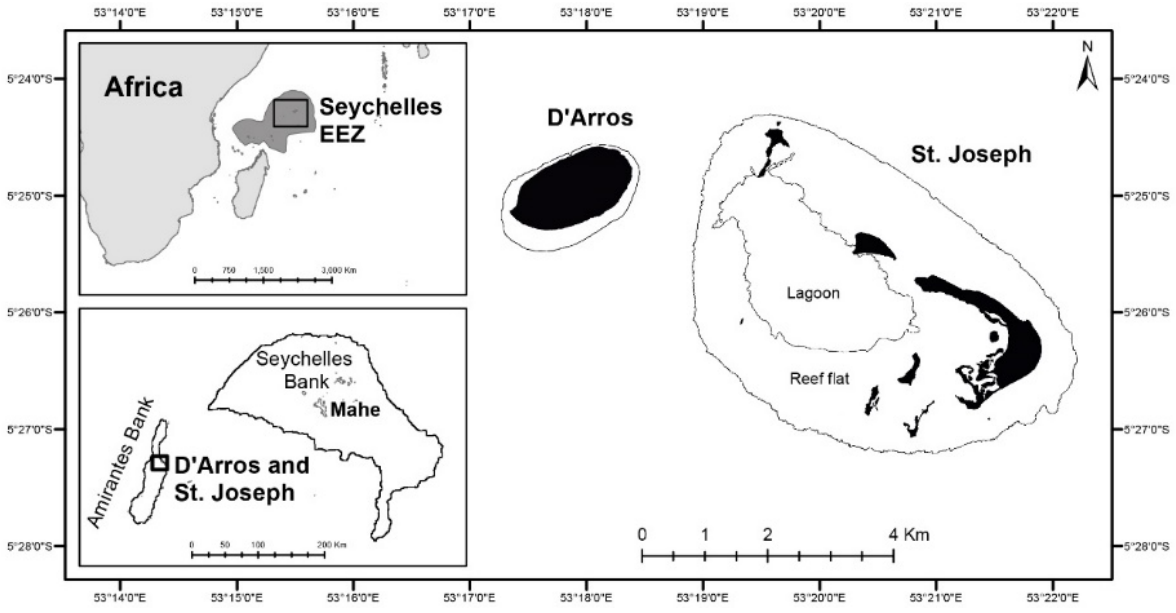


Figure 3.1 Location of the Republic of Seychelles and St. Joseph Atoll and map of the study location with the neighbouring D'Arros island. Permanent landmasses are shown in black. Map created by Ryan Daly | SOSF.

Upon capture, young sharks were sexed by noting the presence or absence of claspers, sized (precaudal length, PCL; cm), weighed using a hand-held spring scale (Pesola®), and tagged with passive integrated transponder (PIT) tags (Biomark; www.biomark.com) for identification in case of recapture. In order to categorize individual age classes and to discriminate between neonates and juveniles, a quantitative three-point umbilical scar stage (USS) system was applied following details in **Chapter 5**.

3.3.3 Space use and movement patterns

Active tracking was used to investigate sharks' daily habitat use and fine-scale movements. Cone-shaped foam fishing floats (5g) were attached to the first dorsal fin of the shark with a 1.0 m long monofilament fishing line of 0.41 mm diameter, a galvanic timed release (GTR) and a padded wire (similar to methods in Speed et al., 2013; Supplementary material S2). Handling time for each individual was less than five minutes and sharks were released at the site of capture. Sharks were visually tracked on foot or from small kayaks, with observers remaining at least 10 m away from the animal at all times. GPS locations were recorded every 20 seconds (Garmin GPSMAP 78SC), depth was recorded every five minutes, and water temperature was measured every minute by a HOBO ® temperature logger (U22-001, Onset Computer Corporation, Bourne, MA, USA). Sharks were tracked during the day (07.00 h – 18.00 h) and a single track lasted a maximum of 9h 45 min. All GTRs were set to release after 24 hours, however, sharks were recaptured at the end of each track to remove the floating device. A pressure logger (HOBO Water Level Data Logger, Onset, Bourne, USA) was used to measure tidal height during days when manual active tracking was conducted. Tidal cycles were also modelled using the Oregon State University Tidal Model Driver (Egbert & Erofeeva, 2002) based on the dynamics for St. Joseph Atoll, outputting predicted tidal heights in meters every 10 mins (Lea, 2017). As the tidal heights of the logger and model only differed by 1.96%, the tidal heights predicted from the model were used to estimate tidal heights for every GPS location of the manual active tracks (Lea, 2017).

We compared the depth and water temperatures experienced during tracks between species using Wilcoxon matched-pairs tests. To estimate the size of the daily space use of manually tracked sharks, we calculated kernel utilisation distributions (UD) for each individual using the package '*adehabitatHR*' (Calenge, 2019; version 0.4.16) in R and ArcMAP (ESRI, version 10.7). The core

activity space (50% UD) and the total activity space (95% UD) were calculated separately for each individual (e.g.; Munroe et al., 2016). In addition, daily space use overlap for core (50% UD) and total activity space (95% UD) between species was calculated by combining all blacktip reef and all lemon sharks tracks, respectively.

To investigate the fine-scale habitat use of tracked sharks, we quantified the locations of 'area restricted searching' (ARS), and how this varied by tidal stage. We used first passage time (FPT) analysis to identify the spatial scale and geographic location of ARS behaviour (Fauchald & Tveraa, 2003). FPT is based on the time required for an animal to cross a circle with a given radius. FPT was log transformed to make the variance independent of the magnitude of the mean FPT (Fauchald & Tveraa, 2003), and FPT was calculated using a circle with radius *r* ranging from 20 to 500 m at 10 m sampling intervals, using the package '*adehabitatLT*' (Calenge 2019, version 0.3.24) in R.

3.3.4 Stomach content analysis

Sharks' stomachs were flushed using non-lethal gastric lavages, as described in **Chapter 5**. A transparent and beveled acrylic tube was inserted in the sharks' stomach to flush its content. The stomach items were captured in a sieve, washed and preserved with 70% ethanol. This procedure was conducted only on sharks deemed to be in good condition (e.g. exhibited no open wounds) and was kept to a maximum of three consecutive procedures per individual. Upon return to the laboratory, the 70% ethanol was replaced by 100% ethanol, and samples were stored in the fridge at 4°C for a maximum of six months.

Stomach items were separated into non-teleost and teleost tissues. While the former were visually identified to the lowest possible taxonomic level, muscle tissues of teleost samples (~25 mg) were collected for DNA analysis. For each sample, total genomic DNA was extracted using the Qiagen DNeasy Blood & Tissue Kit (QIAGEN Inc. Valencia, CA, USA). An approximately 600 base pair (bp) region from the 5'-end of the cytochrome oxidase 1 (COI) gene was amplified using the barcode primers FishF1 and FishR1 (Ward et al., 2005). Amplifications were performed in 50-µLvolumes, containing: 1 µL of extracted genomic DNA, 10 pmol of each primer, 10X PCR buffer (Qiagen Inc.), 50 µM dNTPs mix, 1 unit of HotStarTaq DNA Polymerase kit (Qiagen Inc.), and

33.3 μL water. Amplifications were carried out in a MJ Research Inc. PTC-100™ Programmable Thermal Controller using the conditions: initial denaturation step of 95°C for 15 min; 35 cycles of 94°C for 30 s, 52°C for 40 s, and 72°C for 60 s, followed by an extension at 72°C for 10 min. PCR products were purified using the Qiagen PCR Purification Kit (QIAGEN Inc. Valencia, CA, USA) and sequenced bidirectionally using the Applied Biosystems BigDye Terminator v3.1 Cycle Sequencing Kit on an Applied Biosystems 3130 Genetic Analyzer. DNA sequences were aligned and edited using the software Geneious v.9.1.18 (https://www.geneious.com), and consensus sequences for each sample compared with the National Center for Biotechnology Information (NCBI) nucleotide collection using BLAST. A top species match was identified with a sequence similarity of at least 99.8%.

All prey items were identified to the lowest level possible, but family levels were used for subsequent calculations. Despite being an efficient dietary method, gastric lavage does not always allow stomachs to be emptied entirely (Bangley et al., 2013). Therefore, diets were quantified on the basis of percentage occurrence (%O), which was calculated as: (the number of stomachs containing prey taxon/total number of stomachs containing prey) × 100 (Hyslop, 1980). Prey diversity and niche breadth were calculated using the Shannon-Wiener (H') index and Levin's standardized niche breath (B_A), respectively (Krebs, 1999). For further comparisons of dietary compositions across species, prey items were classified into seven major prey categories: (1) Mugilidae, (2) Gerreidae, (3) Labridae, (4) other teleosts, (5) unidentified teleosts (e.g. prey items of distinctive teleost origin, such as fish scales and parts of vertebrae), (6) crustaceans, and (7) cephalopods. Dietary data were visualized using non-metric multidimensional scaling (nMDS) ordination plots and Permutational Multivariate Analysis of Variance (PERMANOVA; 999 permutations) used to examine whether there was a significant difference in the diet composition of the two shark species based on the seven prey categories. To overcome issues associated with individual stomachs with zero values, dietary data were pooled into groups of five randomly selected stomachs for each species and mean values were used (Platell & Potter, 2001; White et al., 2004; Marshall et al., 2008). Data were log-transformed prior to analysis and a Bray-Curtis dissimilarity matrix was constructed (Platell & Potter, 2001). Analyses were conducted with the package '*vegan*' (Oksanen, 2019) in R.

3.3.5 Stable isotope analysis

Stable isotope analysis (SIA), an indirect tool that provides a means to examine trophic roles sharks (Hussey et al., 2012; Munroe et al., 2018), was additionally used to investigate the juvenile sharks' dietary pattern. The isotopic ratios of nitrogen ($^{15}N/^{14}N$, hereafter $\delta^{15}N$) and carbon ($^{13}C/^{12}C$, hereafter $\delta^{13}C$) were analysed in blood plasma, because plasma has a relatively fast turnover rate, thus avoiding the effect of maternal influence on stable isotope signatures in young animals (Hussey et al., 2010; Olin et al., 2011; Kim et al., 2012). Following **Chapter 2**, 3 ml blood was collected from the caudal vein using 5 ml syringes (Plastipak ™) with 18 - gauge needles (BD PrecisionGlide ™) and was immediately spun into blood components at 1500 *g* by a hand-powered centrifuge (Handzentrifuge, Hettich®) for 30 seconds. The resulting blood components were separated, and plasma samples were stored in no-additive vials on ice. On return to the laboratory (< six hours between sampling and storage), the plasma samples were immediately frozen (-18°C). After seven days, they were placed in a drying oven (60° C) for 72 h, and homogenized with a mortar and pestle. Finally, to determine the abundances of carbon and nitrogen isotopes in collected plasma samples, 500-800 µg of the homogenized powder was loaded into tin capsules and analyzed by a continuous-flow isotope-ratio mass spectrometer [Finnigan Delta C EA-IRMS (with TC/EA)] with bovine liver, I-AEA-N-1, I-AEA-C-6, and glycine used as standards. Variation among lab standard for samples were 0.15‰ for $\delta^{13}C$ and < 0.10‰ for $\delta^{15}N$. Lipid extractions were not conducted, because C:N ratios for RBC (mean ± SD; 2.8 ± 0.08) and plasma (1.8 ± 0.03) were below those recommended for extraction or mathematical correction (Hussey et al., 2012).

Trophic niche comparisons between species based on stable isotope data were analyzed using Mann Whitney U-test as these data were not normally distributed (Shapiro-Wilk test, $p < 0.05$). The relationships between shark PCL and isotopic values of carbon and nitrogen were examined using linear regression models. We further calculated $\delta^{13}C$ range (CR), $\delta^{15}N$ range (NR), and total convex hull (TA, Layman et al., 2007). Standard ellipse areas (SEA, Jackson et al., 2011) were calculated using the package '*SIBER*' (Jackson et al., 2011; Jackson & Parnell, 2019) in R. Estimates of the core isotopic niche widths were calculated using maximum likelihood and Bayesian approaches based on approximately 40% of the data (Jackson et al., 2011) resulting in three metrics: SEA, SEA_C and the Bayesian standard ellipses (SEA_B), which represent the

'average' isotopic niche breadth of the population (Jackson et al., 2011). Differences in SEA_B size were considered significant if the 95% credibility intervals of posterior draws did not overlap. Bayesian standard ellipses were used to estimate and visualize total trophic niche overlap among species as implemented in the package '*nicheROVER*' (Lysy et al., 2015; Swanson et al., 2015) in R. This approach draws probable isotopic overlap estimates from the posterior distribution using a Bayesian framework.

All statistical analyses were conducted in R version 3.5.3. (R Core Team, 2016) within the RStudio interface ver. 1.0.153 (RStudio Team 2016). Where applicable, data were checked for normality using Shapiro-Wilk tests prior to analyses, and the level of statistical significance α was set at 0.05.

3.4 Results

3.4.1 Captive dominance behaviour experiments

Due to technical problems regarding the use of the aquarium system, only three replicates of the non-food (Test 1) and three replicates of the food trials (Test 2) were completed. Lemon sharks (n = 4) used for trials were larger (mean ± SD, 53.4 ± 7.0 cm PCL) and heavier (1600 ± 80 g) than blacktip reef sharks *(*41.0 ± 5.0 cm PCL; 900 ± 20 g, n = 3), which is typical of the natural size difference at Moorea for sharks at this life-stage (Mourier et al., 2013b, 2013a; Matich et al., 2017b) and is similar to the size differences of sharks captured at St. Joseph Atoll. During trials without food (Test 1), an individual giving way to another individual was observed on 11 occasions. In 55% of these interactions, blacktip reef sharks gave way to lemon sharks, which was not significantly different from unity ($\chi^2 = 0.05$, $p > 0.05$, Figure 3.2). During trials with prey items present (Test 2), 41 interactions were observed in which one individual would give way to another. In 93% of these cases, blacktip reef sharks gave way to lemon sharks leading to a significant difference from unity ($\chi^2 = 20.9$, $p < 0.001$, Figure 3.2). This one-sided interaction was further corroborated by the amount of prey consumed. In all three replicates of Test 2, lemon sharks succeeded in eating all of the prey items (n = 15, 100%) leading to a significant difference from unity ($\chi^2 = 10$, $p < 0.001$). Direct aggressive interactions between species were not observed in any trials, but blacktip reef sharks would approach the feeding plate only after all prey items were gone, likely avoiding any potential competition for food (Supplementary material S3).

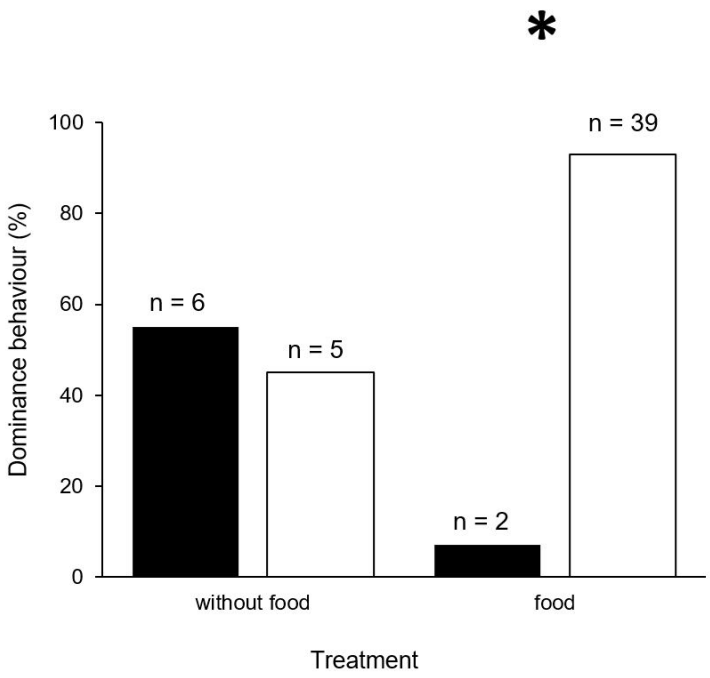


Figure 3.2 Frequency histogram of dominance behaviours observed between blacktip reef sharks *Carcharhinus melanopterus* (black) and sicklefin lemon *Negaprion acutidens* (white) during competition experiments. Dominance behaviours were noted when one individual gave way to the other (1 for dominant individual, 0 for individual that gives way). Significant difference between species is indicated by *.

3.4.2 Field sampling and capture locations

Between November 2014 and April 2017, a total of 640 individual sharks were captured at St. Joseph Atoll. Blacktip reef sharks were significantly smaller (mean ± SD, 42.5 ± 6.8 cm PCL, n = 333) and lighter (1079 ± 555 g) than lemon sharks (52.0 ± 6.2 cm PCL; W = 13444, $p < 0.001$; 1475 ± 591 g; W = 20539, $p < 0.001$, n = 307). Capture locations for both species were mostly mixed throughout the sampling area (Figure 3.3A). Lemon sharks, however, occurred in a smaller portion of available space than blacktip reef sharks (a semi-truncated distribution; Figure 3.3 B & C), leading to 96% and 72% overlap of lemon shark with blacktip distribution, and blacktip reef shark with lemon shark distribution, respectively.

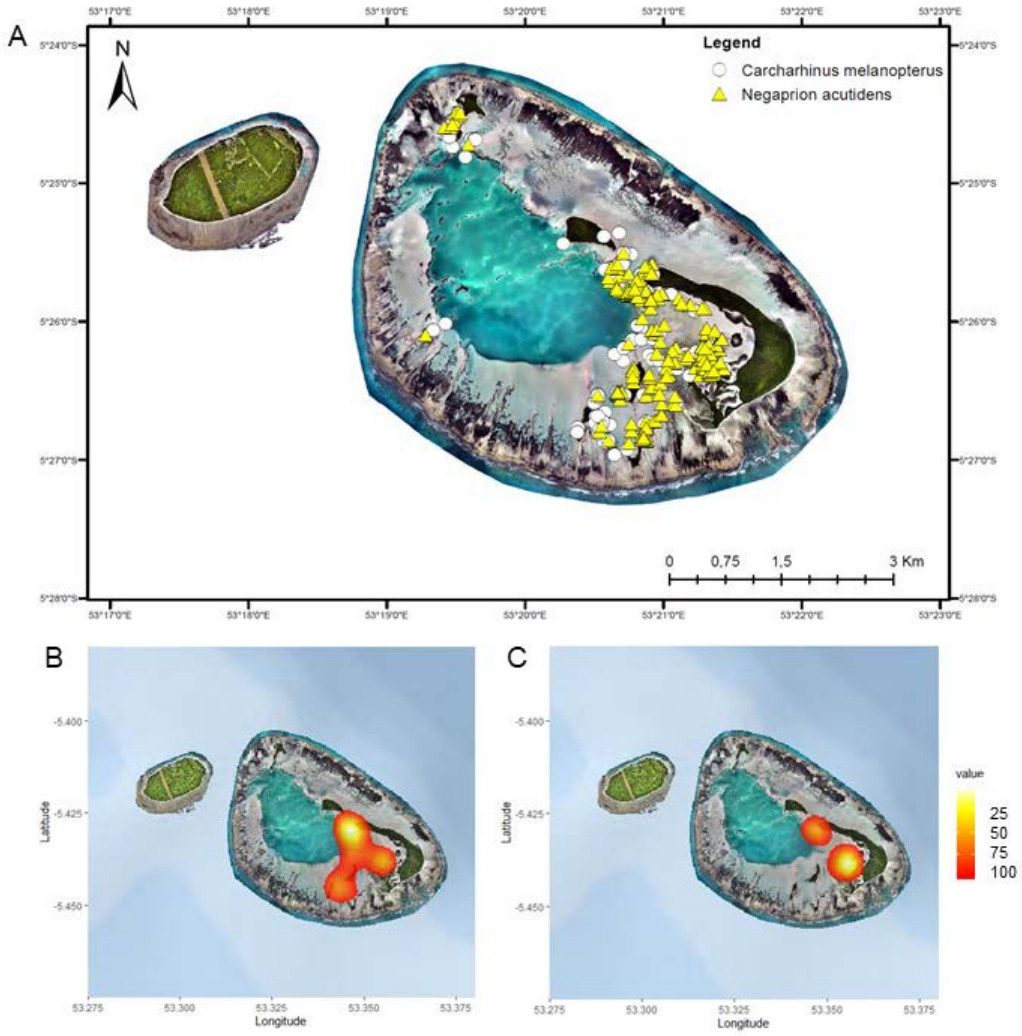


Figure 3.3 Distribution of juvenile blacktip reef sharks *Carcharhinus melanopterus* and juvenile sicklefin lemon sharks *Negaprion acutidens* captured at St. Joseph Atoll from between November 2014 and April 2017. Capture locations for *Carcharhinus melanopterus* (black triangles) and for *Negaprion acutidens* (white circles; A). Kernel utilisation distribution (KUD) of capture locations for *Carcharhinus melanopterus* (B) and for *Negaprion acutidens* (C). Drone image © Save Our Seas Foundation.

3.4.3 Space use and movement pattern

We actively tracked 11 juvenile sharks (*blacktip reef shark*, 45.56 ± 5.1 cm PCL, n = 5; *lemon shark*, 52.3 ± 3.9 cm PCL, n = 6; Table 3.1). Continuous tracking periods ranged from 07:10 to 09:45 h, and all tracking occurred during daylight hours (from 06.00 to 18.00 hours, Supplementary material S4). Tracks revealed all sharks remained on the shallow intertidal reef

flats. At high tide, sharks were predominately on the outskirts of the intertidal reef flats towards the islands. During low tides, when large parts of the reef flats were exposed to air, sharks were forced to move towards the deeper central lagoon (Supplementary material S5). Tracked sharks, and simultaneously observed free swimming juvenile sharks, were never seen to enter the deeper central lagoon (Weideli, OC, personal observation). Mean depth (*blacktip reef shark*: 27.7 ± 12.5 cm, *lemon shark*: 27.2 ± 9.8 cm) and temperature (*blacktip reef shark*: 32.3 ± 2.1° Celsius, *lemon shark*: 32.2 ± 2.4° Celsius) measured during tracks were similar for both species (*depth*: W = 476610, $p = 0.254$, *temperature*: W = 13239000, $p = 0.3593$, Supplementary material S6 and S7, respectively).

The 50% UDs for tracked blacktip reef sharks ranged from 0.01 to 0.50 km^2 (0.19 ± 0.21 km^2) and from 0.15 to 0.30 km^2 (0.20 ± 0.05 km^2) for lemon sharks (Figure 3.4). The 95% UDs for tracked blacktip reef sharks ranged from 0.15 to 2.08 km^2 (0.91 ± 0.74 km^2) and from 0.71 to 1.21 km^2 (0.91 ± 0.17 km^2) for lemon sharks (Figure 3.4). Neither the 50% UDs (Wilcoxon Mann-Whitney U-test, W = 12, $p = 0.66$) nor 95% KUDs (W = 14, $p = 0.93$) differed between species. When data from manual tracks were combined for each species, the 50% and 95% UDs were 0.29 km^2 and 1.74 km^2 for blacktip reef sharks, and 0.65 km^2 and 2.66 km^2 for lemon sharks, respectively. The combined daily spatial areas used by blacktip reef sharks largely overlapped with areas used by lemon sharks (93% of 95% contour, and 84% of 50% contour). In contrast, lemon sharks spatial areas overlapped only 61% and 40% (for 95% and 50% contours) of the area used by blacktip reef sharks.

According to FPT analysis, five individuals (*blacktip reef sharks,* n = 3, *lemon sharks,* n = 2) from the total of eleven tracked sharks showed evidence of area restricted searching (ARS), at spatial scales of 50 to 200 m (Figure 3.5). ARS behaviour in both species occurred in shallow and sheltered waters close to islands during high tides, and in more exposed areas during early incoming tides. Note that ARS behaviour in exposed areas was observed during early incoming tides when the raising water level had not yet reached the areas where tracking was conducted (Figure 3.5).

Table 3.1 Overview of samples collected from juvenile blacktip reef sharks *Carcharhinus melanopterus* and sicklefin lemon sharks *Negaprion acutidens* at St. Joseph Atoll. Tissues collected, sample sizes (n), range of precaudal lengths, L_{PC} (cm) and mean precaudal length, L_{PC} (cm) of sampled individuals. Numbers in parentheses indicate the percentage of empty stomachs. Note that size data was only taken from individuals with full stomachs (*) and for stable isotope data size measurements were only taken from sharks at umbilical scar stage 3 (**).

Species	**Tissue and individuals**	**n**	**L_{PC} range (cm)**	**mean L_{PC} (cm)**
	No. manual tracks	5	39.3 – 51.7	45.56 ± 5.1
Carcharhinus melanopterus	No. stomachs investigated	115 (21%)	37.3 – 65.5*	47.57 + 5.5 *
	No. stable isotope samples	61	39.0 – 63.0**	48.8 ± 5.3**
	No. manual tracks	6	47.5 – 58.9	52.3 ± 3.9
	No. stomachs investigated	188 (54%)	46.0 – 76.5*	54.95 + 7.4 *
Negaprion acutidens	No. stable isotope samples	42	49.0 – 85.0**	58.49 ± 8.4 **

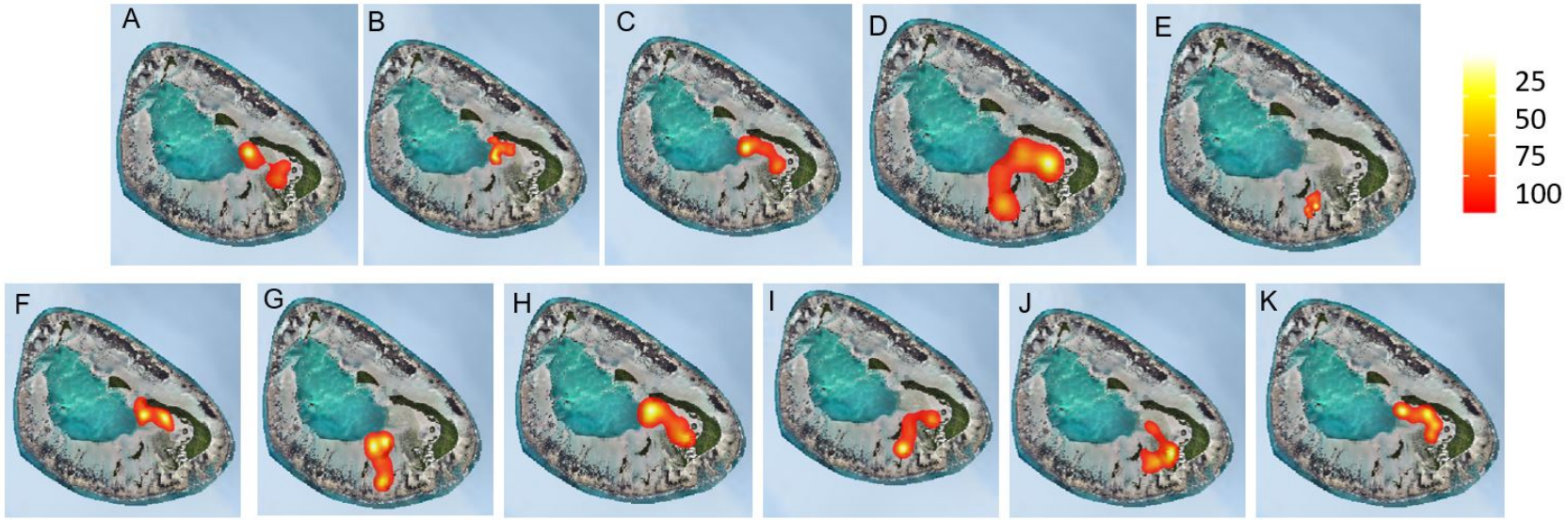


Figure 3.4 Kernel utilisation distributions (KUDs) of manually tracked juvenile blacktip reef sharks *Carcharhinus melanopterus* (A-E), and sicklefin lemon sharks *Negaprion acutidens* (F-K) at St. Joseph Atoll, Seychelles. All individuals were tracked on a different day. Drone image © Save Our Seas Foundation.

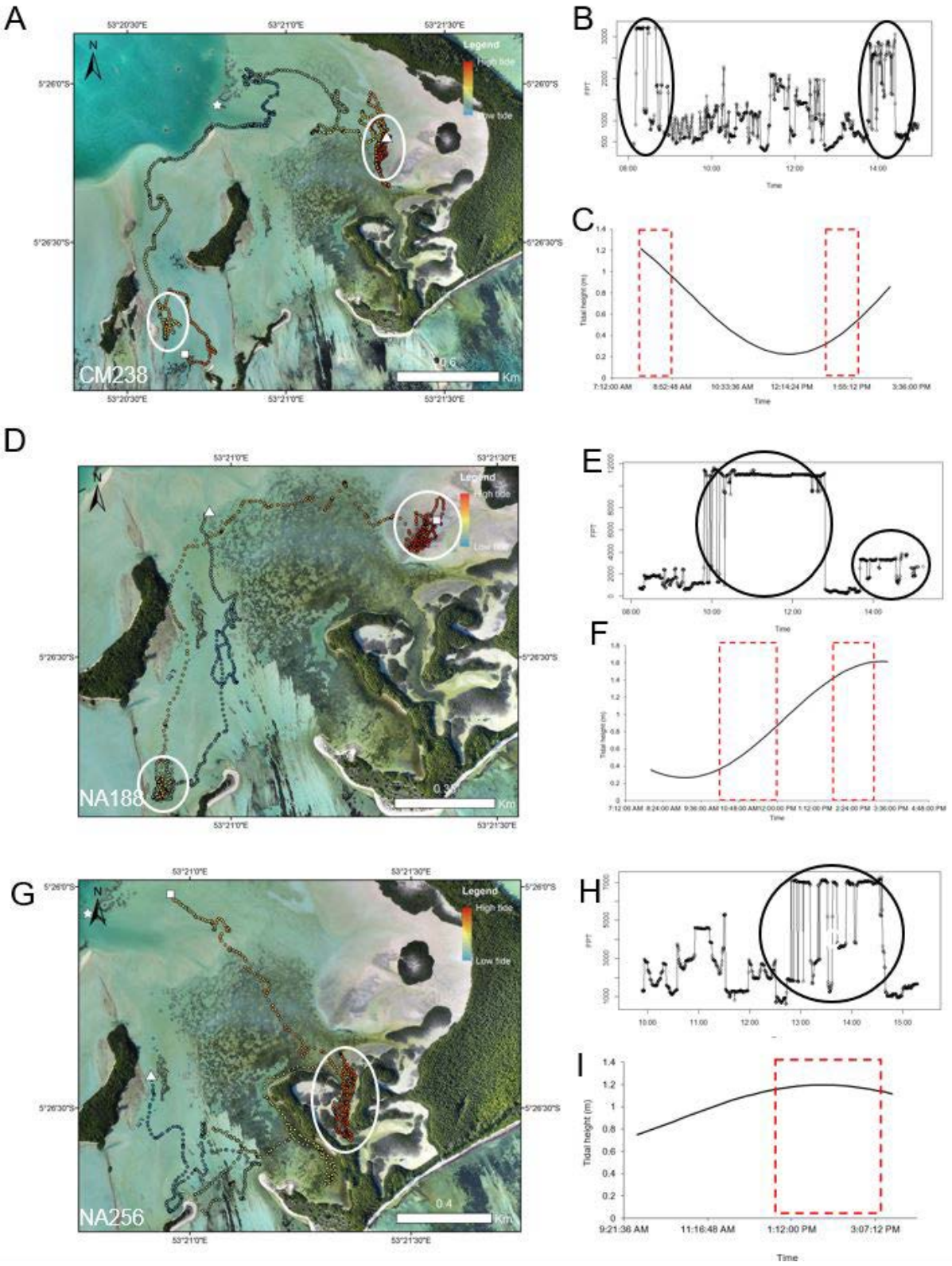

A
B
C
D
E
F
G
H
I
CM238
NA188
NA256
Legend
High tide
Low tide
Km
Time
Tidal height (m)
FFT

Figure 3.5 Fine-scale movements and location of area restricted searching (ARS) determined by first passage time (FPT) for juvenile blacktip reef sharks *Carcharhinus melanopterus* (A) and juvenile sicklefin lemon sharks *Negaprion acutidens* (D, G) at St. Joseph Atoll, Seychelles. White triangle indicates the start of the track, white square represent the end of the track, and different coloured dots represent GPS locations at corresponding tidal stages. ARS was calculated from the peak in log variance FPT and the time of the maximum FPT values (B, E, H). Time and tidal stage when ARS was found are highlighted by red squares (C, F, I). Note that on the intertidal reef flats the tides were slightly lagged compared to the location of the water pressure logger (indicated by white star). Drone image © Save Our Seas Foundation.

3.4.4 Stomach content analysis

Gastric lavages were performed on 115 blacktip reef sharks and 188 lemon sharks of which 91 blacktip reef sharks (47.57 ± 5.5 cm PCL) and 86 lemon sharks (54.95 ± 7.35 cm PCL) contained prey items (Table 3.2). The number of stomachs containing prey was significantly greater in blacktip reef sharks (79%) than in lemon sharks (46%; $\chi^2 = 31.38$, df = 1, $p < 0.001$). Overall, blacktip reef sharks were piscivorous (84.62 % Occurrence) with crustaceans (6.59 %O) and cephalopods (8.79 %O) accounting for the remainder of stomach contents (Table 3.2). The diet of lemon sharks was similar to that of blacktip reef sharks with a higher occurrence of teleosts (94.19 %O) and minor contributions of crustaceans and cephalopods (Table 3.2). Within teleost prey, the diet of blacktip reef sharks was composed of 32 species from 23 families, while 17 species from 13 teleost families were found in lemon sharks (Supplementary material S8). Largescale mullet (*Planiliza macrolepis*) was the most abundant teleost in both species (*blacktip reef sharks*: 8.79 %O, *lemon sharks*: 30.23 %O), followed by strong-spine pursemouth (*Gerres longirostris,* 7.69 %O and 6.89 %O in blacktip reef sharks and lemon sharks, respectively, Supplementary material S8). Highly digested teleost tissues (e.g., solid tissues such as scales and vertebrae) with no muscle tissues couldn't be identified to species level, but they contributed a high proportion to the diet in both species (unidentified teleosts: 35.16 %O and 38.37 %O in blacktip reef sharks and lemon sharks, respectively, Table 3.2). The slightly more opportunistic diet of blacktip reef sharks was supported by a broader dietary breadth ($B_A = 0.21$, H' = 0.75; Table 3.2), than lemon sharks ($B_A = 0.11$, H' = 0.58; Table 3.2). Although not completely distinct, the nMDS ordination plot revealed a significant difference in the diets between blacktip reef and lemon sharks (PERMANOVA $F_{1,34} = 5.64$, $p < 0.01$; Figure 3.6).

Table 3.2 Summary of the percentage of occurrence (%O) of prey families from the diet of blacktip reef sharks *Carcharhinus melanopterus* and sicklefin lemon sharks *Negaprion acutidens* from St. Joseph Atoll. Levin's and Shannon-Wiener indices are given at the bottom of the table.

Prey items	***C. melanopterus* (n = 91)**	***N. acutidens* (n = 98)**
Teleosts	**84.62**	**94.19**
Acanthuridae	1.10	-
Alustomidae	1.10	-
Apogonidae	1.10	-
Atherinidae	1.10	-
Balistidae	1.10	-
Blenniidae	2.20	-
Bothidae	-	1.16
Carangidae	2.20	2.33
Chanidae	-	1.16
Clupeidae	1.10	1.16
Fistulariidae	2.20	-
Gerreidae	7.69	6.98
Gobiidae	2.20	3.49
Holocentridae	-	1.16
Labridae	6.59	-
Lethrinidae	2.20	2.33
Lutjanidae	2.20	2.33
Mugilidae	8.79	30.23
Mullidae	3.30	-
Muraenidae	4.40	1.16
Ophichthidae	1.10	1.16
Plotosidae	2.20	-
Pomacentridae	1.10	-
Scorpaenidae	1.10	-
Synodontidae	1.10	-
Terapontidae	-	2.33
Zanclidae	1.10	-
Unidentified teleost	35.16	38.37
Crustacean	**6.59**	**3.49**
Unidentified crab	6.59	3.49
Cephalopoda	**8.79**	**1.16**
Unidentified cephalopod	8.79	1.16
Bivalvia	**-**	**1.16**
Unidentified bivalvia	-	1.16
Miscellaneous	**-**	**1.16**
Unidentified seagrass	-	1.16
Levin's index	0.21	0.12
Shannon-Wiener index	0.75	0.58

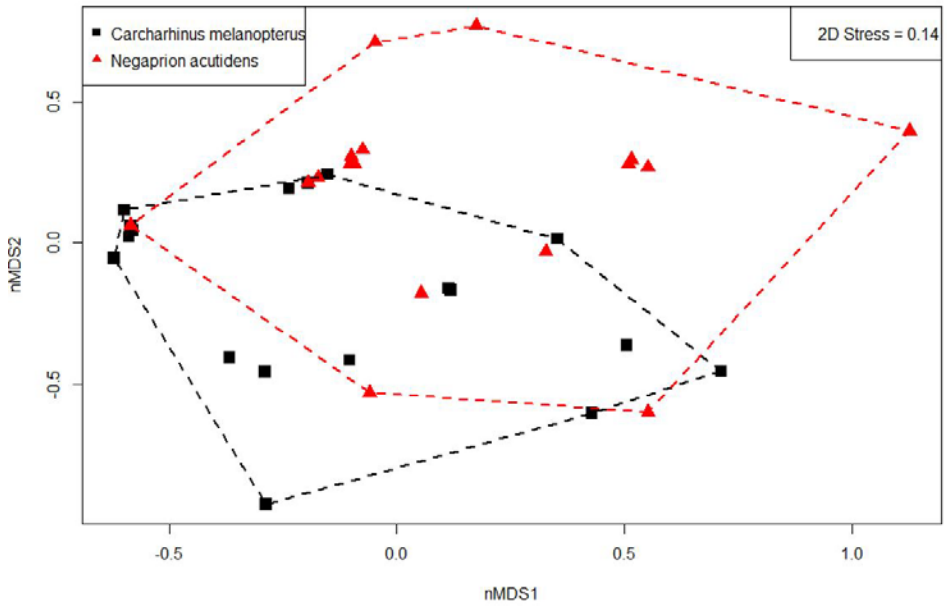


Figure 3.6 Non-metric multidimensional scaling (nMDS) plot of percentage numeric contribution (%N) to the diets of juvenile blacktip reef sharks *Carcharhinus melanopterus* and juvenile sicklefin lemon sharks *Negaprion acutidens*. A two-dimensional Bray-Curtis dissimilarity index was used and the seven major prey categories (Mugilidae, Gerreidae, Labridae, other teleosts, unidentified teleosts, crustaceans, and cephalopods). Each point of the plot represents the pooled mean for groups of five individuals.

3.4.5 Stable isotope analysis

Blood plasma was collected from 61 blacktip reef sharks (48.8 ± 5.3 cm PCL) and 42 lemon (54.5 ± 8.4 cm PCL, Table 3.1) for carbon and nitrogen isotopic comparisons. All sharks sampled were individuals of USS3 to avoid maternal isotopic ratios (Olin et al., 2011). Blacktip reef and lemon sharks exhibited wide ranges of $\delta^{13}C$ values in plasma (-12.5 to -6.4‰, and -11.4 to -6.3‰, respectively) indicating a broad range of carbon sources, as well as a wide range in $\delta^{15}N$ (9.6 – 14.8‰, and 10.2 – 17.5‰, respectively; Figure 3.7, Table 3.3). Lemon sharks demonstrated a significantly higher mean $\delta^{13}C$ signatures (-7.72 ± 1.06) than blacktip reef sharks (-8.74 ± 1.17; $W = 592$, $p < 0.001$), while mean $\delta^{15}N$ signatures (*blacktip reef sharks*: 12.19 ± 1.29, *lemon sharks*: 12.69 ± 1.84) showed no statistical difference between species ($W = 1192.5$, $p > 0.05$). There was no significant relationship between PCL and isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) for either of the two species (linear regression, for all comparisons: $p > 0.05$, Supplementary material S9).

Table 3.3 Summary of isotopic niche metrics including $\delta^{13}C$ and $\delta^{15}N$ (mean values ± SD), range of samples, total area of convex hull (TA), maximum likelihood estimate of the core trophic niche standard ellipse area (SEA), small sample size corrected SEA (SEA_C), Bayesian standard ellipse area (SEA_B) ($‰^2$), and total trophic overlap estimates. The overlap is based on ellipses encompassing 95% of the data (Lysy et al. 2015, Swanson et al. 2015).

Species	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	CR	NR	TA	SEA	SEA_C	SEA_B	Total trophic overlap (%)
Carcharhinus melanopterus	-8.74 (1.2)	12.22 (1.3)	-12.5: -6.4	9.6: 14.8	19.64	4.62	4.7	4.71	82.99
Negaprion acutidens	-7.71 (1.5)	12.69 (1.8)	-11.4: -6.3	10.2: 17.5	21.33	5.8	5.95	5.95	76.24

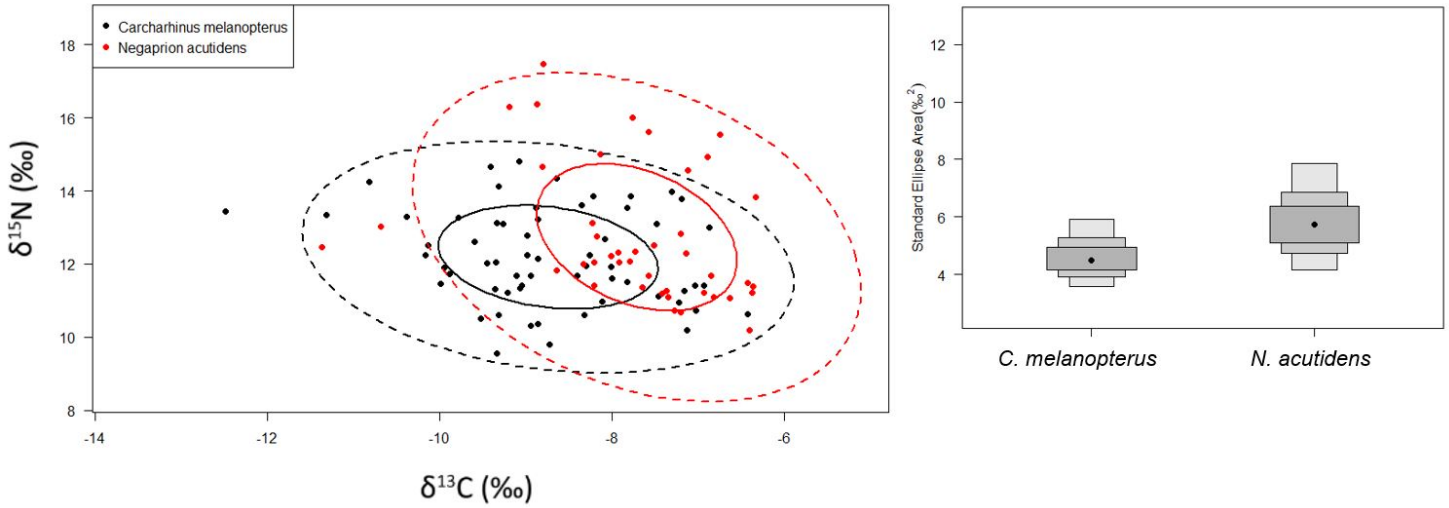


Figure 3.7 Left) Bivariate plot with standard ellipse areas (40% of data [SEA], solid coloured lines) and total trophic niche (95% of data [used for niche overlap calculations], dashed coloured lines) are estimated for $\delta^{13}C$ and $\delta^{15}N$ of coexisting blacktip reef sharks *Carcharhinus melanopterus* and sicklefin lemon sharks *Negaprion acutidens*. Right) Boxplots are Bayesian derived estimates of standard ellipse area (SEA_B, with 50%, 75% and 95% credible intervals) for *Carcharhinus melanopterus* and *Negaprion acutidens*.

Core trophic niche areas (SEA, SEA_C, SEA_B) yielded similar estimates of niche sizes for both species, with lemon sharks exhibiting a slightly larger core trophic niche width compared to blacktip reef sharks (Figure 3.7, Table 3.3). This finding was further supported by a larger total area of convex hull (TA) in lemon sharks compared to blacktip reef sharks (Table 3.3). Considerable total tropic niche overlap was found between the species, of which blacktip reef sharks had 82.99% total trophic overlap with lemon sharks, while lemon sharks overlapped the trophic niche of blacktip reef sharks by 76.24% (Figure 3.8, Table 3.3).

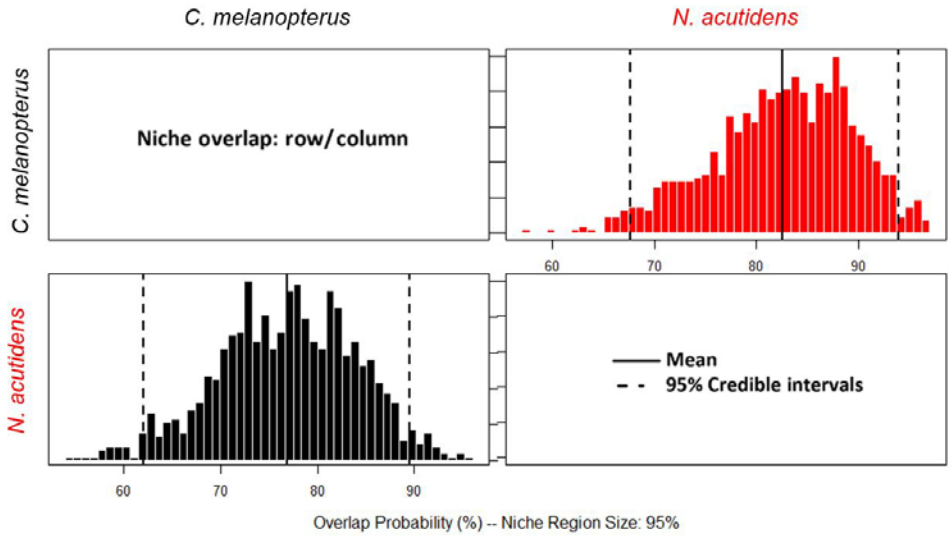


Figure 3.8 Posterior density distributions from Bayesian niche overlap estimates for blacktip reef sharks *Carcharhinus melanopterus* and sicklefin lemon sharks *Negaprion acutidens*. Dashed lines represent 95% credible intervals and solid lines represent means overlap estimates.

3.5 Discussion

The multi-species shark nursery at St. Joseph Atoll matches predictions of Ideal Free Distribution (IFD) models, with subordinate blacktip reef sharks alleviating competition effects by occurring in a larger portion of available space and by consuming a more generalized diet including prey of lower energy content. Within the areas where both species co-occur, daily space use, however, overlapped considerably between species. Overall, our study provides evidence that sympatric

juvenile sharks at St. Joseph Atoll coexist despite substantial predator-induced spatial overlap that likely increases interspecific competition.

We first tested the assumption that larger shark species are dominant by directly measuring dominance hierarchy in competition experiments *in vivo*. During trials without prey items, juvenile blacktip reef and lemon sharks did not show evidence of dominance hierarchy and were often observed to 'follow' and 'circle' each other. Grouping behaviour in juvenile sharks is known from the wild (Gruber et al., 1988; Reyier et al., 2008) and has been observed to occur between heterospecific pairs (Guttridge et al., 2009). During trials with prey items, however, lemon sharks showed a clear dominance over blacktip reef sharks, with the latter giving way in 93% of all interactions and not obtaining a single prey item. These findings are similar to a recent study where larger grey reef sharks (*Carcharhinus amblyrhynchos*) were found to be dominant over smaller blacktip reef sharks (Papastamatiou et al., 2018). Consequently, the interactions observed in our behaviour experiments support our hypothesis that lemon sharks are dominant over blacktip reef sharks and are capable of weak competitive interference without agonistic interaction.

Though many marine species spatially segregate in areas of sympatry (Wilson, 2010; Ratcliffe et al., 2014; Matley et al., 2016), the two shark species considered here were captured on the shallow areas of the intertidal reef flats and capture locations were mixed throughout most of the sampling area (Figure 3.3A). Lemon sharks, however, occurred in a smaller portion of available space than blacktip reef sharks (Figure 3.3 B&C). This spatial segregation over a very small spatial scale (approximate total area where sharks were capture: ~5 km^2) matches our hypothesis and the predictions of the IFD with unequal competitors that differ in dominance, and reveal a semi-truncated distribution (Holmgren 1995). While dominant lemon sharks primarily occur in areas that are sheltered by larger islands, subordinate blacktip reef sharks also occur in microhabitats that are more exposed and dominated by higher tidal fluctuations (personal observation, Weideli OC). An alternative explanation for the semi-truncated distribution is the potential for competition for pupping sites. At St. Joseph Atoll, females of both species concurrently seek the shallow reef flats for parturition (**Chapter 4**) and may compete for the safest areas to give birth. Adult lemon sharks are larger (Lea et al., 2016) and possibly more competitive than blacktip reef sharks in attaining these more protective areas for parturition. Capture numbers in the areas where blacktip

reef sharks were predominantly captured were, however, too limited to conclude if this fine-scale spatial segregation was exclusively driven by dominance hierarchies between juveniles and/or adults, or because juvenile blacktip reef sharks naturally use a wider variety of habitats and display broader movements than lemon sharks (Chin et al., 2013a; Oh et al., 2017).

Daily movements across species were similar with regards to their area, depths, temperature, and behaviour. Indeed, active tracking revealed that sharks occur in the shallowest possible waters by moving closer to the islands during high tide, and approaching the central lagoon during low tide. Moreover, we found that both species exhibited straight movements during low and outgoing tides, while tortuous movements and frequent changes in directions (ARS behaviour) were predominantly observed during high and early incoming tides, notably during times when there was limited water movement. Although ARS behaviour is commonly associated with foraging activities (Weimerskirch et al., 2007; Weng et al., 2008; Edwards et al., 2013), ARS behaviour at St. Joseph Atoll may imply resting behaviour in shallow and sheltered areas during tidal stages with limited water flow. The most likely explanation for the ARS behaviour is that risk effects force some degree of spatial overlap across species. Indeed, sub-adult and adult blacktip reef and lemon sharks are abundant at St. Joseph Atoll and occur within reef habitats around the atoll, and in the central lagoon adjacent to the shallow intertidal reef flats (Filmalter et al., 2013; Lea et al., 2016). With the incoming tides, adult sharks gain access to these shallow areas (Filmalter et al., 2013; Lea et al., 2020), thus juvenile sharks may seek the shallowest areas of the atoll for safety (Heupel & Hueter, 2002; Heithaus, 2007; George et al., 2019). Such tidal dependent movements are not unique to St. Joseph Atoll and have previously been documented in juvenile sharks and rays in nursery areas within both the Indo-Pacific and Atlantic (Knip et al., 2011; Guttridge et al., 2012; George et al., 2019; Martins et al., 2020). Overall, predatory risk effects may force sharks' daily movements within the mixed areas to overlap, suggesting a reinforced potential for interspecific competition.

As predicted, dietary segregation was found between species, as seen with previous studies of multi-species aggregations of juvenile sharks (Kinney et al., 2011; Tillett et al., 2014; Matich et al., 2017c). Stomach content data show significantly different dietary compositions between species, with a slightly wider dietary breadth and larger contribution of lower energetic prey items

(e.g., crustaceans and cephalopods) found in blacktip reef sharks. This observation is similar to a study on sympatric snappers where subordinate vermilion snapper (*Rhomboplites aurorubens)* consume 39% less fish and more than twice as many amphipods at sites where they coexist with a dominant competitor (Davis et al., 2015). Optimal foraging predicts that with increasing competition (or through food scarcity) animals will diversify their diet and thus widen their niche breadth and become more generalists (Stephens & Krebs, 1986; Newman et al., 2010). We hypothesize that due to their subordinate status, blacktip reef sharks become more opportunistic by including prey of lower energetic content, and as a consequence may be forced to forage more frequently (79% of stomachs contain prey items) as opposed to lemon sharks that feed less frequently (46% of stomachs contain prey items). In line with these findings, it was also expected that dominant and larger lemon sharks would feed at a higher trophic level, as recently shown for juvenile lemon sharks at Moorea (Matich et al., 2017c). However, both species were found to forage at the same trophic level, assuming $\delta^{15}N$ is an accurate indicator of trophic level (Post, 2002). Further, $\delta^{13}C$ signatures differed across species, with lemon sharks depicting higher $\delta^{13}C$ signatures and wider isotopic niche breadths compared to blacktip reef sharks. While the higher $\delta^{13}C$ signatures in lemon sharks' diet are likely impacted by the high contribution (30.23 %O) of prey items from the family Mugilidae ($\delta^{13}C$: – 7.9 ± 1.4; Weideli OC, unpublished data), a wide isotopic breadth may result from a diet composed of relatively few prey items that are isotopically dissimilar (i.e., foraging in different microhabitats; Kinney et al., 2011; Hussey et al., 2017). Overall, we confirm that data from SCA and SIA do not provide comparable estimates for dietary niche and dietary breadths, but rather complement trophic information by providing additional insight on 'isotopic niches' (Hette-Tronquart, 2019; Petta et al., 2020). Finally, we suggest that the asymmetric dietary segregation of these species is likely influenced by differences in dominance, and highlight that this segregation may help alleviating interspecific competition in nursery areas where levels of risk effects are high.

Our approach of combining behavioural experiments with multi-dimensional niche investigations has allowed us to further our understanding of the role that competition may play in shaping patterns of niche use. Although the sample sizes used in our behavioral experiments and for our active tracking were small and we were not able to assess the degree to which food resources for blacktip reef and lemon sharks are, or were, limited at St. Joseph Atoll, this study provides an

important framework for future multi-faceted research of this kind. The highly variable growth rates found in both species (**Chapter 4**) support the claim that prey at St. Joseph Atoll is somehow limited for juvenile sharks. Our data reveal promising evidence that competition effects can help explain niche patterns in wild marine predators. Additionally, our findings further suggests that complete niche segregation may not always be possible for coexisting species, as risk effects (or other external factors) may have a stronger influence on coexisting juvenile shark populations than competitive ones. Finally, being able to understand the drivers of niche separation/overlap also has important implications for conservation measures. Juvenile sharks occupying nearshore areas are likely vulnerable to local (Jennings et al., 2008; Stump, 2013) and global (Bangley et al., 2018; Bouyoucos et al., 2018) anthropogenic stressors. If such stressors lead to declining prey abundance and habitat restriction or loss, interspecific competition is likely to increase and may impact unequal competitors differently. Therefore, to ensure the coexistence of potentially unequal juvenile competitors, we need to maintain intact and healthy nearshore ecosystems that support prey and habitat resources for all inhabiting species.

Ethical approval

Research on sharks at St. Joseph Atoll was approved by, and conducted with the knowledge of Ministry of Environment, Energy, and Climate Change, Seychelles. Animal handling and tagging methods were conducted in accordance with the approved guidelines of S. Planes by the Autorisation de pratiquer des expériences sur les animaux n° 006725 (1995) from the ministry of Agriculture. Permission to work with sharks in French Polynesia was obtained from the Ministère de l'Environnement (Arrete N° 9524).

Acknowledgements

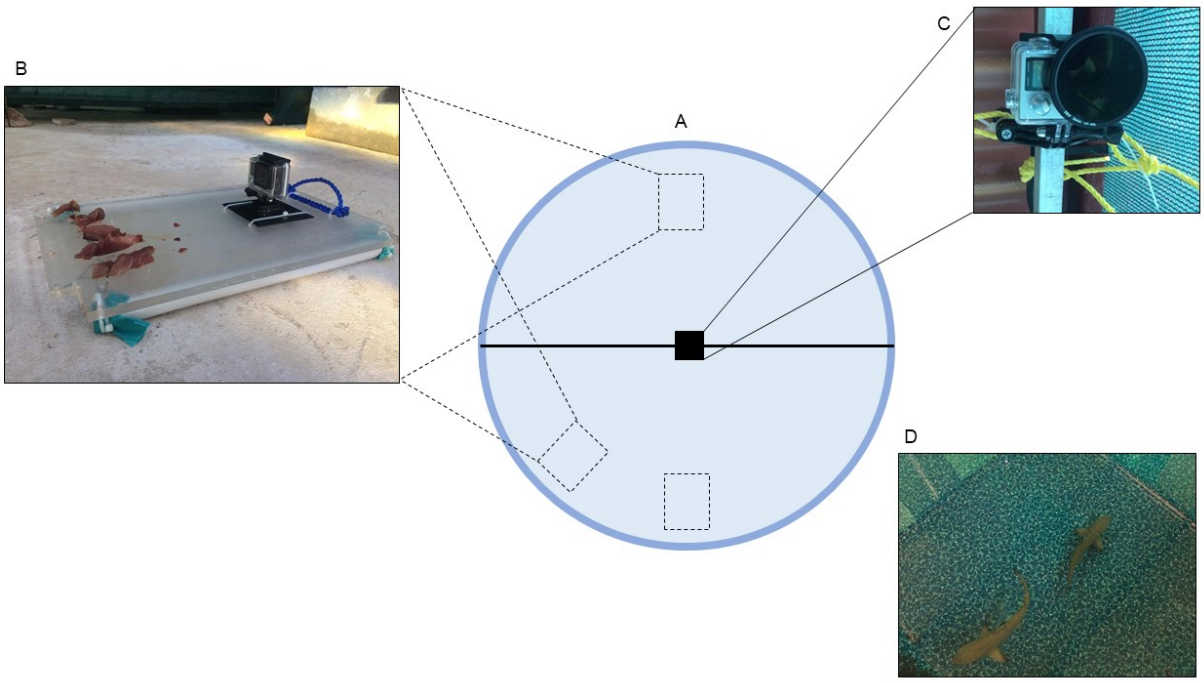
This research was gratefully funded by the Save Our Seas Foundation (SOSF Keystone Grant 290) awarded to OCW and SOSF Grant 157 to MSS (genetic analyses). The authors wish to thank the staff and volunteers involved in the study, especially C. Daly, C. Boyes, R. von Brandis, and K. Bullock from the SOSF D'Arros Research Centre (SOSF-DRC), and I. A. Bouyoucos, E. Jacquesson, E. Duncan and J.L. Rummer at the CRIOBE. We also thank C. Ruck Calhoun, C. Keelin Fitzpatrick at the Save Our Seas Shark Research Center, Nova South Eastern University for support with genetic analyses, K. Gastrich, J. Harries, M. Sabando at FIU for the grateful

assistance with stable isotope analyses, J. Mourier for support with spatial analyses, J. Lea for providing tidal models, and Drone Adventures® for creating high resolution drone maps. We thank D. Grubbs and M. Harmelin-Vivien, part of OCW's thesis committee, for their for their valuable feedback on a previous version of this manuscript.

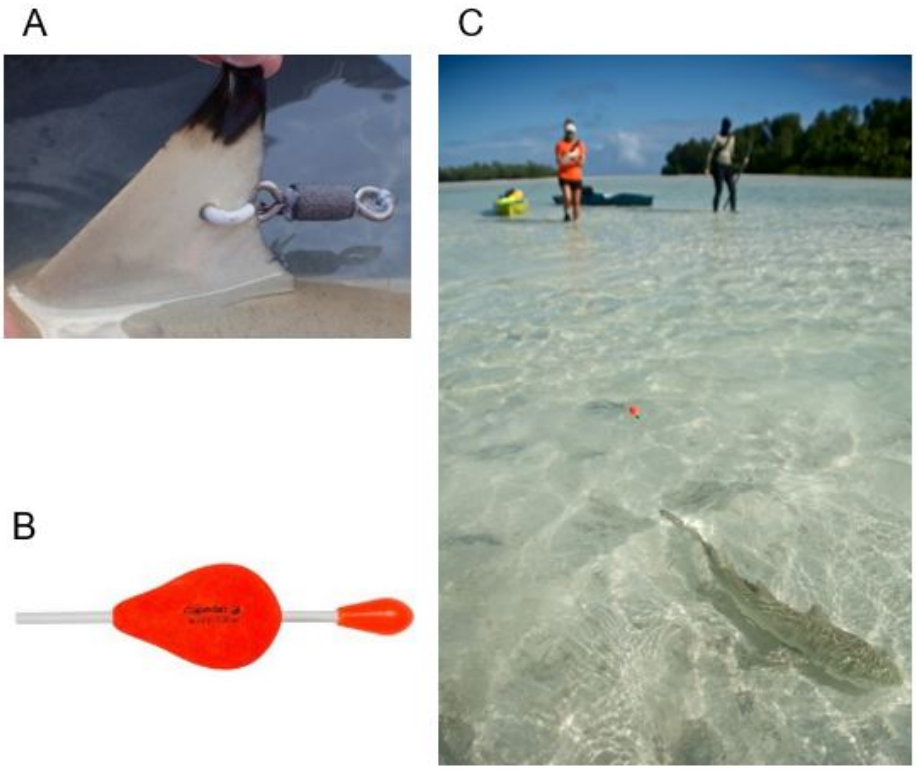
Author contribution

O.C.W. designed and coordinated the study; O.C.W. and R.D. collected field data; O.C.W. analysed and interpreted the data with inputs from Y.P.P., R.D. and L.R.P. O.C.W. wrote the manuscript with support from Y.P.P., R.D., and S.P. O.C.W. secured the funding to support this study. The remaining co-authors L.R.P., M.S.S., and M.R.H. have not yet proofread this draft.

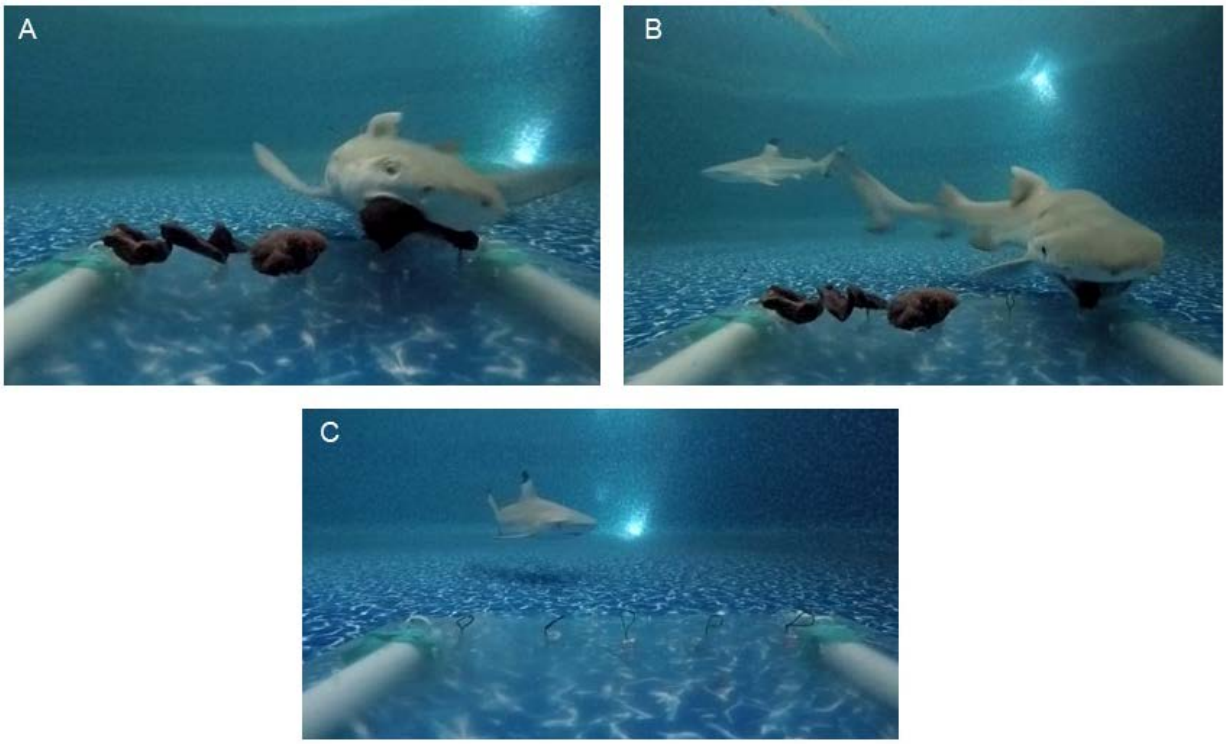
Supplementary information



Supplementary information S1. Schematic setup of competition experiment. A) Circular experimental tank system of 4.0 m diameter with 50 cm of water. B) Feeding plate (420 × 300 mm, Plexiglas) with five tuna pieces (*Thunnus spp*.) and GoPro Hero3. Feeding place was randomly placed in the tank (dashed squares). C) GoPro Hero3 equipped with a polarized lens mounted 2.5 m above the centre of the pool. D) Example picture taken of two blacktip reef sharks *Carcharhinus melanopterus* with ceiling camera GoPro Hero3. All pictures by Ornella Céline Weideli | SOSF.



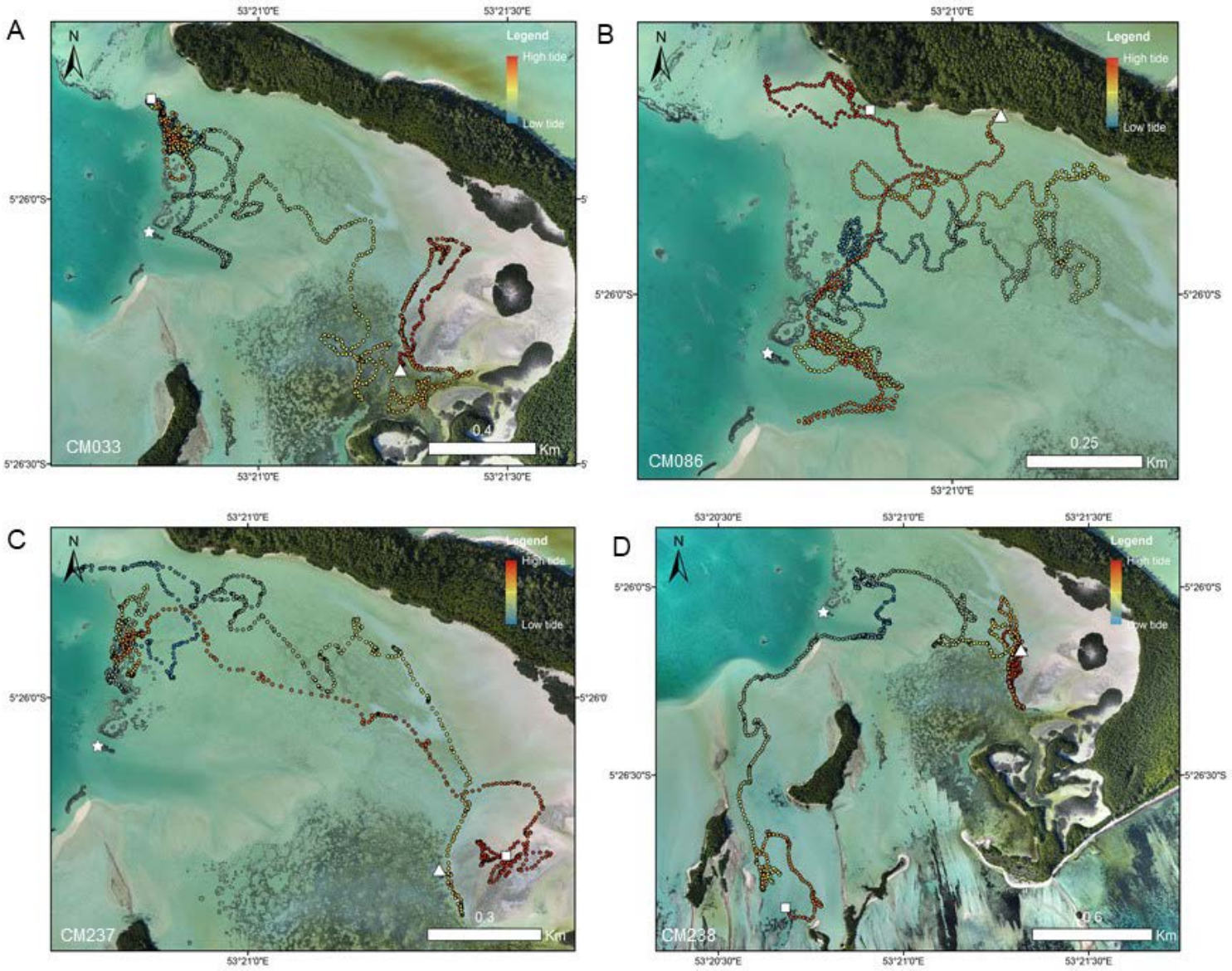
Supplementary information S2. Manual active tracking of juvenile sharks at St. Joseph Atoll, Seychelles. A) Galvanic timed release (GTR) attached to the first dorsal fin by a padded wire. B) Commercially available fishing foam floats used for tracking. C) Manual active tracking in very shallow waters. Note that the kayaks are being towed by the two-person team. Pictures: A) Ornella Céline Weideli | SOSF; B) www.decatholon.ch; C) Rainer von Brandis | SOSF.

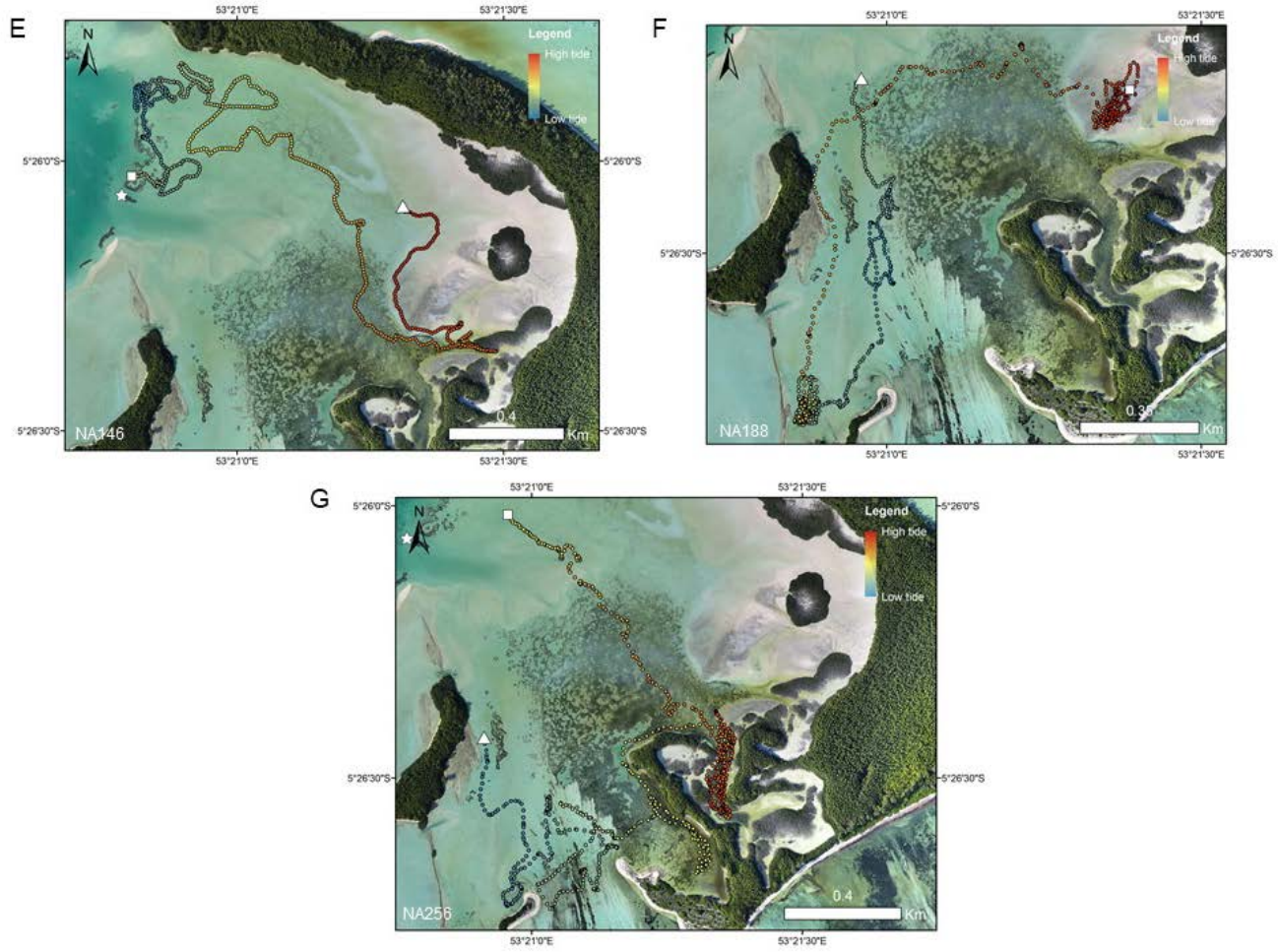


Supplementary information S3. Pictures from GoPro Hero3 camera placed on the feeding plate. A) At 42 seconds into the experiment, the sicklefin lemon shark *Negaprion acutidens* starts eating. B) While *Negaprion acutidens* eats, the blacktip reef shark *Carcharhinus melanopterus* uses the opposite of the pool. C) *Carcharhinus melanopterus* approaches the feeding plate only after all prey items are eaten by *Negaprion acutidens*. All pictures by Ornella Céline Weideli | SOSF.

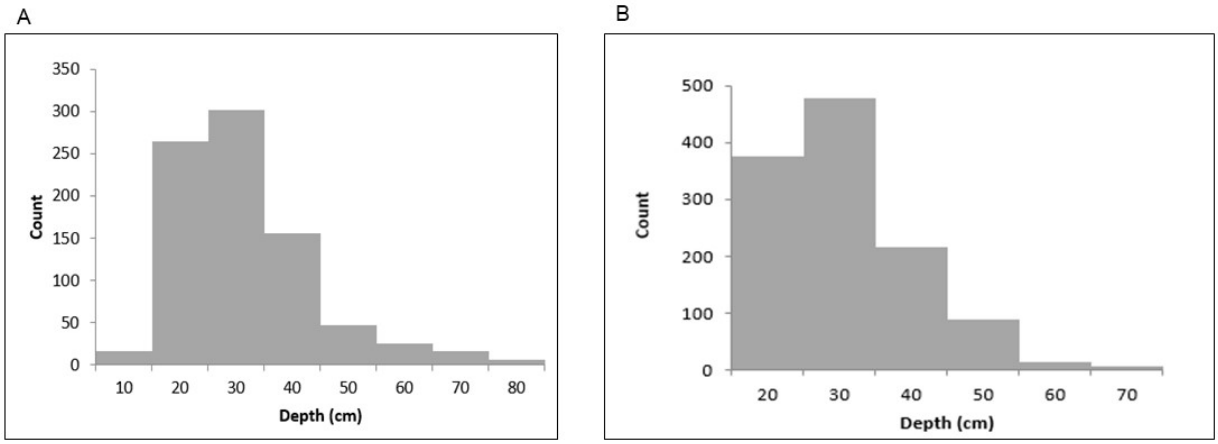
Supplementary information S4 Details of eleven manually tracked juvenile blacktip reef sharks *Carcharhinus melanopterus* and sicklefin lemon sharks *Negaprion acutidens* at St. Joseph Atoll, Seychelles. Individual sharks listed in order of when they were tracked. PCL: Precaudal length

Species	Shark ID	Sex	PCL (cm)	Date captured	Season N°	Time captured (h)	Duration (h:m)
Carcharhinus melanopterus	086	M	39.3	23 Nov. 2014	1	08:15	9:24
Negaprion acutidens	117	F	54.0	01 Dec. 2014	1	11:04	6:39
Negaprion acutidens	128	M	47.5	05. Dec. 2014	1	08:38	8:46
Negaprion acutidens	146	F	50.4	11. Dec. 2014	1	08:48	8:18
Carcharhinus melanopterus	237	F	45.0	06 April 2015	2	08:30	8:43
Carcharhinus melanopterus	238	F	49.5	07 April 2015	2	08:00	7:10
Negaprion acutidens	188	F	53.0	16 April 2015	2	08:05	7:20
Carcharhinus melanopterus	260	F	51.7	17 April 2015	2	09:55	5:32
Carcharhinus melanopterus	033	F	42.3	24 April 2015	2	08:10	9:32
Negaprion acutidens	256	F	58.9	28 April 2015	2	09:40	5:50
Negaprion acutidens	411	M	50.2	21 Oct. 2015	3	08:05	9:45

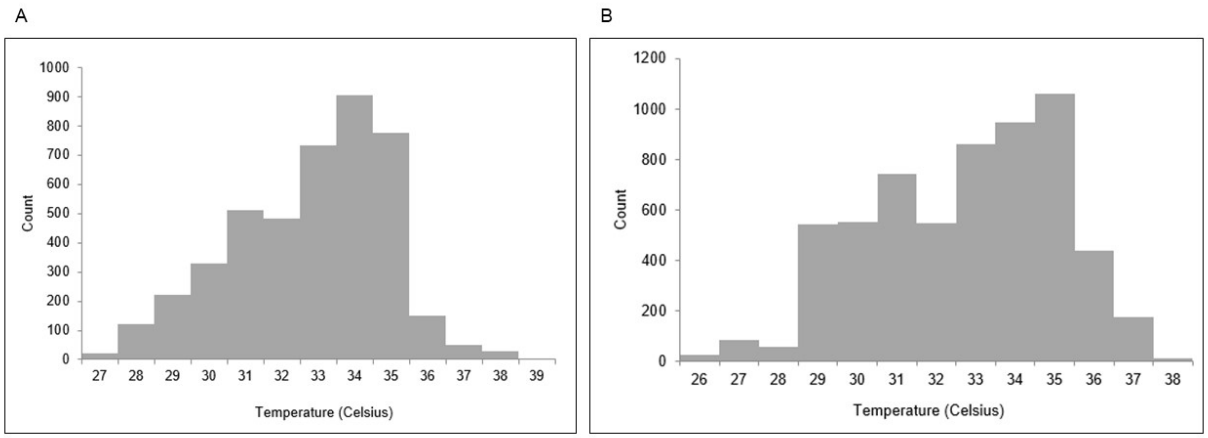

A
B
C
D
N
Legend
High tide
Low tide
CM033
CM086
CM237
CM238
0.4
0.25
0.3
0.6
Km
53°21'0"E
53°21'30"E
53°20'30"E
5°26'0"S
5°26'30"S



Supplementary information S5. Fine scale movement paths of manually tracked juvenile blacktip reef sharks *Carcharhinus melanopterus* (A - D) and juvenile sicklefin lemon sharks *Negaprion acutidens* (E - G) at St. Joseph Atoll, Seychelles. White triangle indicate the beginning of the track, white square represent the end of the track, and different coloured dots represent GPS coordinates at corresponding tidal stages. All individuals were tracked on a different day. The location of the water pressure logger is indicated by a white star. Drone image © Save Our Seas Foundation.



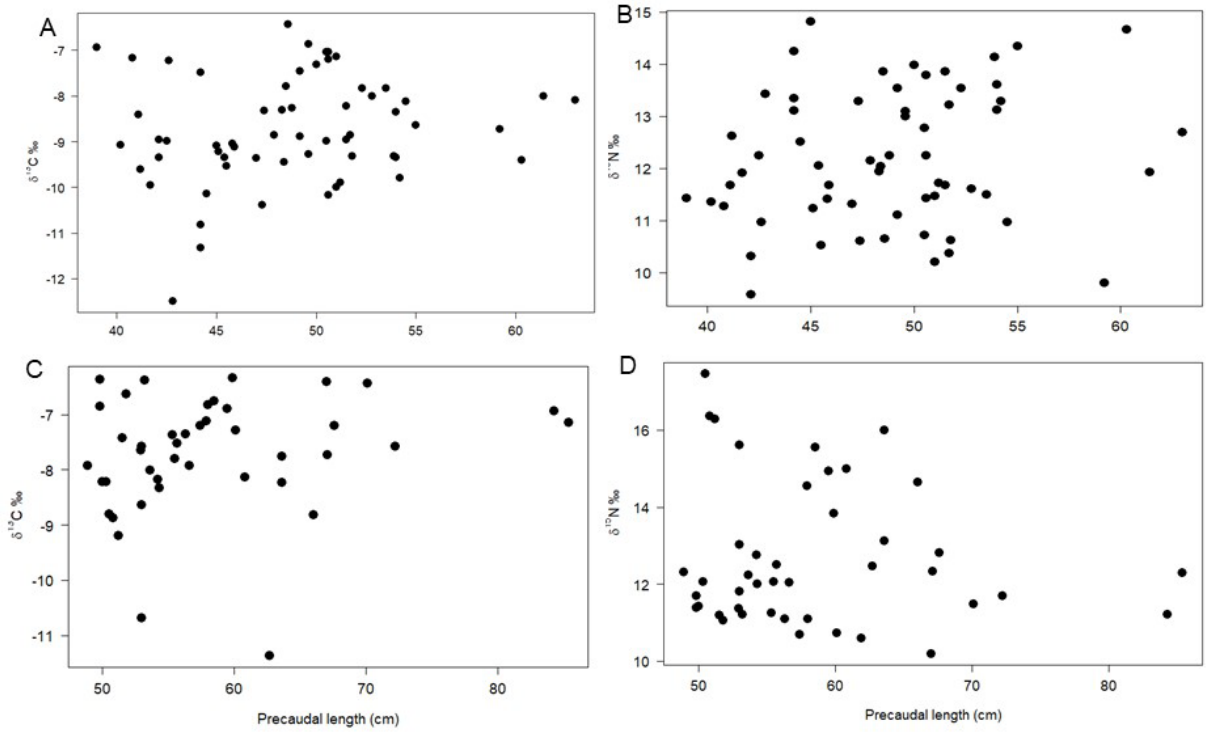
Supplementary information S6. Depth histogram for A) 12 *Carcharhinus melanopterus* and B) 14 *Negaprion acutidens*. Depth data were collected during manual tracking in a five-minute interval.



Supplementary information S7. Temperature histogram for 12 *Carcharhinus melanopterus* (A) and 14 *Negaprion acutidens*. Temperature data were collected during manual tracking in a one-minute interval.

			C. melanopterus	*N. acutidens*
Teleosts		**Family**	**84.62**	**94.19**
Acanthurus triostegus	Convict surgeonfish	Acanthuridae	1.10	0.00
Acentrogobius nebulosus	Shadow goby	Gobiidae	1.10	0.00
Amblygobius nocturnus	Nocturn goby	Gobiidae	0.00	0.00
Amblygobius semicinctus	Whitebarred goby	Gobiidae	1.10	0.00
Bothus patherinus	Leopard flounder	Bothidae	0.00	1.16
Caranx ignobilis	Giant Trevally	Carangidae	1.10	0.00
Caranx sexfasciatus	Bigeye trevally	Carangidae	1.10	1.16
Chanos chanos	Milk fish	Chanidae	0.00	1.16
Cheilinus chlorourus	White-dotted Maori Wrasse	Labridae	2.20	0.00
Chrysiptera biocellata	White-saddled damsel	Pomacentridae	1.10	0.00
Echnida nebulosa	Snowflake Moray Eel	Muraenidae	2.20	0.00
Fistularia commersonii	Bluespotted cornetfish	Fistulariidae	2.20	0.00
Fowleria variegata	Variegated cardinalfish	Apogonidae	1.10	0.00
Gerres longirostris	Strong-spine pursemouth	Gerreidae	7.69	6.98
Gnatholepis cauerensis	Eyebar sand goby	Gobiidae	0.00	1.16
Gymnothorax pictus	Peppered Moreay Eel	Muraenidae	1.10	0.00
Gymnothorax richardsonii	Richardson's moreay Eel	Muraenidae	1.10	1.16
Halichoeres scapularis	Zigzag wrasse	Labridae	1.10	0.00
Hypoatherina barnesi	Barnes' silverside	Atherinidae	1.10	0.00
Lethrinus lentjan	Red-ear emperor	Lethrinidae	0.00	1.16
Lethrinus nebulosus	Spangled emperor	Lethrinidae	1.10	0.00
Lethrinus obsoletus	Orange-striped emperor	Lethrinidae	0.00	1.16
Lethrinus xanthochilus	Yellowlip emperor	Lethrinidae	1.10	0.00
Lutjanus fulviflamma	Long-spot/dory snapper	Lutjanidae	1.10	2.33
Lutjanus monostigma	One-spot snapper	Lutjanidae	1.10	0.00
Mulloidichthys flavolineatus	Square-spot goatfish	Mullidae	1.10	0.00
Myrichthys colubrinus	Harlekin snake eel	Ophichthidae	1.10	1.16
Myripristis pralinia	Scarlet soldierfish	Holocentridae	0.00	1.16
Oplopomus oplopomus	Spinecheek goby	Gobiidae	0.00	1.16
Parupeneus macronemus	Long-barbel goatfish	Mullidae	2.20	0.00
Planiliza macrolepis	Largescale mullet	Mugilidae	8.79	30.23
Plotosus lineatus	Striped eel/ marine catfish	Plotosidae	2.20	0.00
Rhinecanthus aculeatus	Picasso triggerfish	Balistidae	1.10	0.00
Salarias fasciatus	Jewelled blenny	Blenniidae	2.20	0.00
Saurida gracilis	Gracile lizardfish	Synodontidae	1.10	0.00
Sebastapistes strongia	Bar-chin scorpionfish	Scorpaenidae	1.10	0.00
Selar crumenphthalmus	Big-eye scad	Carangidae	0.00	1.16
Spratelloides delicatulus	Delicate round herring	Clupeidae	1.10	1.16
Stethojulis strigiventer	Three-ribbon wrasse	Labridae	3.30	0.00
Aulostomus chinensis	Trumpetfish	Alustomidae	1.10	0.00
Terapon jarbua	Crescent grunter	Terapontidae	0.00	2.33
Zanclus cornutus	Moorish idol	Zanclidae	1.10	0.00
Unidentified teleost			35.16	38.37
Crustacean			**6.59**	**3.49**
Unidentified crab			6.59	3.49
Cephalopoda			**8.79**	**1.16**
Unidentified cephalopod			8.79	1.16
Bivalvia			0.00	**1.16**
Unidentified bivalvia			0.00	1.16
Miscellaneous			0.00	**1.16**
Unidentified seagrass			0.00	1.16

Supplementary information S8. Summary of the percentage of occurrence (%O) of prey species from the diet of blacktip reef sharks *Carcharhinus melanopterus* and sicklefin lemon sharks *Negaprion acutidens* at St. Joseph Atoll, Seychelles.



Supplementary information S9. Isotopic values in plasma for blacktip reef sharks *Carcharhinus melanopterus* (a, b) and sicklefin lemon sharks *Negaprion acutidens* (c, d) to examine ontogenic shifts in diet and foraging location. No significant relationship between $L_{PC} - \delta^{13}C$ and $L_{PC} - \delta^{15}N$ for either of the two study species was found. Stable isotope values are in ‰.

Chapter 4 | Size frequency, dispersal distances and variable growth rates of young sharks in a multi-species aggregation

This chapter is published as:

Weideli, O.C., Papastamatiou Y.P., Planes S. (2019) Size frequency, dispersal distances and variable growth rates of young sharks in a multi-species aggregation. *Journal of Fish Biology*. 94(5):789–797. https://doi.org/10.1111/jfb.13968.

Size frequency, dispersal distances and variable growth rates of young sharks in a multi-species aggregation

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Received: 31 October 2018 | **Accepted**: 15 March 2019 | **Published online**: 18 March 2019

Keywords: Annual growth, dispersal distances, Indian Ocean, juvenile sharks, neonatal size, resource limitation

4.1 Abstract

During a mark-recapture survey from November 2014 until April 2017, 333 neonatal and juvenile blacktip reef sharks *Carcharhinus melanopterus* and 302 neonatal and juvenile sicklefin lemon sharks *Negaprion acutidens* were tagged and measured at the uninhabited and isolated St. Joseph Atoll (Republic of Seychelles). Both species demonstrated seasonal reproductive synchronicity and relatively large sizes at birth. Despite the extended times at liberty (> 2.5 years), the majority of recaptures were found in close proximity to the initial tagging location (< 500 m). Annual growth rates of *C. melanopterus* (n = 24) and *N. acutidens* (n = 62) ranged from 6.6 to 31.7 cm year^{-1} (mean ± SE; 16.2 ± 1.2 cm year^{-1}) and 0.2 to 32.2 cm year^{-1} (11.8 ± 1 cm year^{-1}), respectively and are to date the most variable ever recorded in wild juvenile sharks. High abundances of both species coupled with long-term and repeated recaptures are indicative of a habitat where juveniles can reside for their first years of life. However, large variability in annual growth rates in both species may suggest high intra and interspecific competition induced by a possibly resource limited, isolated habitat.

4.2 Introduction

Several species of reef-associated viviparous sharks use shallow nearshore areas for parturition (Springer, 1967). Frequent observations of young sharks in such sheltered habitats suggest that these areas are not only used as pupping grounds, but also as nurseries, potentially providing the required resources needed by juvenile sharks (Springer, 1967; Branstetter, 1990; Simpfendorfer & Milward, 1993). High abundances of neonatal and young sharks does not alone classify a habitat as a shark nursery; to meet the criteria of a shark nursery, long-term site attachment as well as a repetitive use across years need to be prevalent (Heupel et al., 2007). Although this approach helps define shark nurseries for management and conservation decisions (Heupel et al., 2007, 2018a), the functional importance of these coastal habitats for young sharks remains contentious. Recent work suggests that shallow, nearshore nurseries may not be as protective and resource abundant as previously thought (Branstetter, 1990), because high pup mortality, weight loss and slow growth rates are found in monospecific shark nurseries (Heupel & Simpfendorfer, 2002; Lowe, 2002; Duncan & Holland, 2006). In addition, multi-species nurseries report significant trophic and spatial niche segregation, potentially due to interspecific competition for limited prey resources (Bethea et al., 2004; Kinney, 2011; Kinney et al., 2011; Matich et al., 2017c).

Shallow areas that are used by coastal sharks as potential shark nursery habitats have been studied in remote islands and atolls, coastlines, embayment, lagoons, brackish estuaries and rivers (Heupel & Hueter, 2002; DeAngelis et al., 2008; Reyier et al., 2008; Chapman et al., 2009; Heithaus et al., 2009; Papastamatiou et al., 2009a; Chin et al., 2013b; Mourier et al., 2013b; Cardeosa et al., 2017). Despite being ecologically diverse, these habitats must all provide a certain abundance of prey to meet young shark requirements as well as refuge from predators, yet the relative importance of these two aspects is in debate (Heupel & Hueter, 2002; Duncan & Holland, 2006; Jennings et al., 2008). Nonetheless, prey abundance and refuge possibilities might be of even greater importance to philopatric species, as well as in remote and isolated habitats, where alternative nurseries are not available (Heupel et al., 2007; Chapman et al., 2015). Philopatric female lemon sharks *Negaprion brevirostris* (Poey 1868), for example, depict geographically exact natal philopatry to one specific nursery site, even though the closest, alternative nursery is less than 4 km away (Feldheim et al., 2013). Given the susceptibility of nursery bound sharks to habitat development,

stress through recreational fishing and climate change (Jennings et al., 2008; Danylchuk et al., 2014; Bouyoucos et al., 2018), long-term population assessments are required.

The blacktip reef shark *Carcharhinus melanopterus* (Quoy & Gaimard 1824) is to date the only species from the genus Carcharhinus (Blainville 1816), where natal philopatry has been identified (Mourier & Planes, 2013; Chapman et al., 2015). *Carcharhinus melanopterus* is broadly distributed throughout the Indo-Pacific Ocean, ranging from higher latitudinal coastal environments in clear (Speed et al., 2011), turbid and eutrophic waters (Chin et al., 2013a, 2013b) to remote Pacific islands and atolls (Bonham, 1960; Papastamatiou et al., 2009a; Mourier et al., 2013b). The sicklefin lemon shark *Negaprion acutidens* (Rüppell 1837), as well as *C. melanopterus*, demonstrate nursery site-fidelity (Mourier et al., 2013a; Chapman et al., 2015), but occupy a narrower range of nearshore habitats throughout the Indo-Pacific Ocean (Speed et al., 2012; Mourier et al., 2013a; Papastamatiou et al., 2014; Oh et al., 2017). *Carcharhinus melanopterus* and *N. acutidens* are also found in the western Indian Ocean, but data is limited to four studies (Stevens, 1984; Filmalter et al., 2013; Lea et al., 2016; Hodgkiss et al., 2017), with only Stevens (1984) including the juvenile life-stage of both species. At St. Joseph Atoll, Seychelles, another remote and relatively pristine reef system, both juvenile shark species are found to coexist. We conducted a 3 year mark-recapture study to assess the population characteristics (size frequency, time at liberty, dispersal distances) and annual growth rates of coexisting juvenile *C. melanopterus* and *N. acutidens* and to estimate if these characteristics conform with previous studies that suggest nearshore areas to be resource limited, which may have detrimental impacts on residential sharks from isolated areas.

4.3 Material and methods

All research on sharks at the St. Joseph Atoll, Seychelles was approved by and conducted with the knowledge of Ministry of Environment, Energy and Climate Change, Seychelles. Animal handling and tagging methods were conducted in accordance with the approved guidelines of S. Planes by the Autorisation de pratiquer des expériences sur les animaux n° 006725 (1995) from the ministry of Agriculture.

4.3.1 Study site

The St. Joseph Atoll (05°26' S 53°20' E), together with neighbouring D'Arros island, is part of a chain that make up the Amirantes group in the outer islands in the Republic of Seychelles in the western Indian Ocean (Figure 4.1). St. Joseph Atoll and D'Arros are separated by a 1 km wide and 70 m deep channel and the nearest islands are 40 km away. Recently, St. Joseph Atoll and D'Arros island were declared a special reserve with a no-take zone extending 1 km from the low-tide mark (Lea et al., 2016). The relatively pristine St. Joseph Atoll consists of seven uninhabited and undeveloped islands that surround a central oval shaped lagoon. The atoll's total area measures 21.8 km^2 with extensive reef flats that account for 70% of the atoll's total area. The lagoon dynamic is highly influenced by the tidal cycle that can flood the reef flats to depths of 2 m during spring high tides and completely expose them to air at spring low tides. Large parts of the islands are fringed by the mangrove *Pemphis acidula*, for which the dense shrub and root systems serve as habitats for small reef-associated animals.

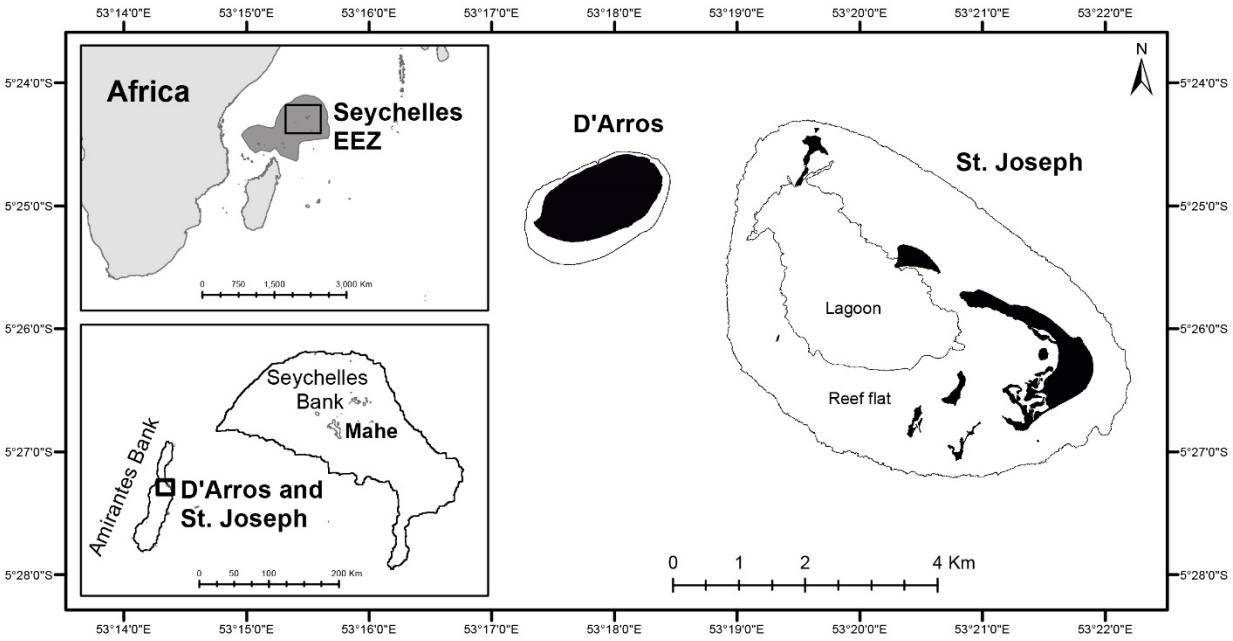

Figure 4.1 Location of the Republic of Seychelles and St. Joseph Atoll and map of the study location with the neighbouring D'Arros island. Permanent landmasses are shown in black. Map created by Ryan Daly | SOSF.

4.3.2 Sampling

Young sharks were caught in six sampling trips from November 2014 to April 2017. Shark sampling trips lasted 2 – 8 weeks (see Supplementary Information S1). Sampling took place during the transition phases between the north-west monsoon and the onset of the south-east trade winds (March - April), and when the north-west monsoon regime takes over (mid-September until November). While the season of the south-east trade winds is dominated by cooler and drier air, the north-west monsoon predominately brings hot and wet weather to Seychelles. Sharks were captured on the reef flats with a 20 m wide, 1.5 m high, gillnet with 5 cm mesh size during high, low, flooding and ebbing tides, but no fishing was conducted during extreme spring high tides or during the night due to handling complexity. Upon capture, young sharks were sexed and total length (L_T, mm) was measured. Passive integrated transponder (PIT) tags (Biomark; www.biomark.com) were inserted below the first dorsal fin and umbilical scar stage (USS), the most reliable indicator of the neonate life-stage, was recorded and photographed. A quantitative three-point USS system was applied, where USS1 was used if scar was fully open, USS2, if scar was partially open and USS3 for fully closed scars. Individuals with USS1 and USS2 were considered as neonate sharks with maximum age of up to 4 weeks (Barker et al., 2005; Duncan & Holland, 2006; Chin et al., 2015). Sharks with closed scars (USS3) were identified as juveniles and no differentiation was made between visible and completely healed scars. The length of the pupping season was determined by the time period when individuals with USS1 or USS2 were captured, since assigning age to a specific size-class cannot be applied due to systematic size overlap between age-classes (Barker et al., 2005). Temperature data was recorded every 15 min with eight stationary Hobo temperature loggers (www.hobo.com) distributed across the reef flats surveyed.

4.3.3 Time at liberty and recapture distances

Data on recaptured sharks was used to explore time at liberty, recapture frequency and recapture distances. Time at liberty was calculated by counting the days between the initial and recapture dates. For individuals that were at liberty longer than 90 days, distance and direction of movements between capture locations were estimated and subsequently displayed in QGIS (www.qgis.org). To evaluate whether size and longer times at liberty resulted in recapture locations further away from the initial capture location, shark total length (L_T) and time at liberty were correlated with

distance and tested using Pearson's correlation coefficient. For individuals that were captured on multiple occasions, maximum distances were retained for calculations.

4.3.4 Growth rates

Growth increments were estimated by using the period at liberty and the change in growth over that time using the formula: change in L_T 365/days at liberty. Because size measurements from all size classes are needed to construct a growth curve (Fabens, 1965), annual growth rates were calculated instead. Individuals that were recaptured in the same sampling season (hereafter referred as over-recaptures) were excluded from all analyses because measurement errors over short periods (< 90 days) can potentially be larger than the growth rate itself (Casey et al., 1985). To preclude the influence of age (USS as an indicator of age-class) on growth, annual growth rates were calculated separately for neonates and juveniles. Similarly, to preclude the influence of temperature on growth, individuals captured and recaptured during the higher temperature season were compared with individuals captured and recaptured during the lower temperature season by Wilcoxon rank-sum test. Furthermore, Pearson's correlation coefficient was used to elucidate if faster growing individuals depict higher recapture probabilities (longer times at liberty) and further recapture distances. For individuals that were captured on multiple occasions in different parturition seasons, length increments and time at liberty were summed, while maximum distances were retained for calculations. Significance was set at $p < 0.05$, results are reported as means ± SE, and all analyses were conducted in R Studio 3.5.1 (www.r-project. org) (RStudio Team, 2016).

4.4 Results

During six sampling trips between November 2014 and April 2017, a total number of 647 juvenile sharks were caught at depths of 10 to 85 cm (33.4 ± 0.4 cm) on the reef flats of St. Joseph Atoll. *Carcharhinus melanopterus* with open and not yet healed umbilical scars (USS 1 or 2), indicating recent birth, were caught each year from mid-September until mid-April. *Negaprion acutidens* demonstrated shorter pupping seasons with neonates only found from mid-September until early December, except for three neonates that were captured in March and April. Eighty percent of both shark species were captured during both flood and ebb tides, leading to a significant tidal bias (*C. melanopterus*: $\chi^2 = 72.99$, $p < 0.001$; *N. acutidens*: $\chi^2 = 81.92$, $p < 0.001$). Temperature during the

three consecutive parturition seasons (mid-September until end of April) were higher (30.0 ± 0.0°C) than during the rest of the year (27.6 ± 0.0°C, May until mid-September). Fishing occurred at various times of the day at different locations on the sand and reef flats, depending on the tides, weather and access to sites.

4.4.1 Size frequencies and sex ratio

Length measurements were obtained from 635 individuals; 333 *C. melanopterus* and 302 *N. acutidens*, respectively. *Carcharhinus melanopterus* ranged in L_T from 39.5 to 104.5 cm (57.5 ± 0.5 cm) and *N. acutidens* from 56.6 to 110.6 cm (66.5 ± 0.45 cm; Figure 4.2a). Size at birth (neonate sharks with USS 1 or 2) varied substantially in *C. melanopterus* from 39.5 to 63 cm L_T (50.5 ± 0.32 cm, n = 142; Figure 4.2b) with significantly smaller pups at the beginning of the pupping season (t = -3.14, df = 140, $p < 0.05$). *Negaprion acutidens* also varied in neonatal sizes from 56.6 to 69.6 cm L_T (63.2 ± 0.24 cm, n = 118; Figure 4.2b), but the time of parturition had no statistically significant influence on shark size (t = 0.68, df = 116, $p > 0.05$). A total of 163 male and 170 female *C. melanopterus* were caught, which did not differ significantly from unity (χ^2 test, $p > 0.05$). Similarly, the sex ratio did not differ from unity in *N. acutidens*, where 136 males and 166 females were caught (χ^2 test, $p > 0.05$).

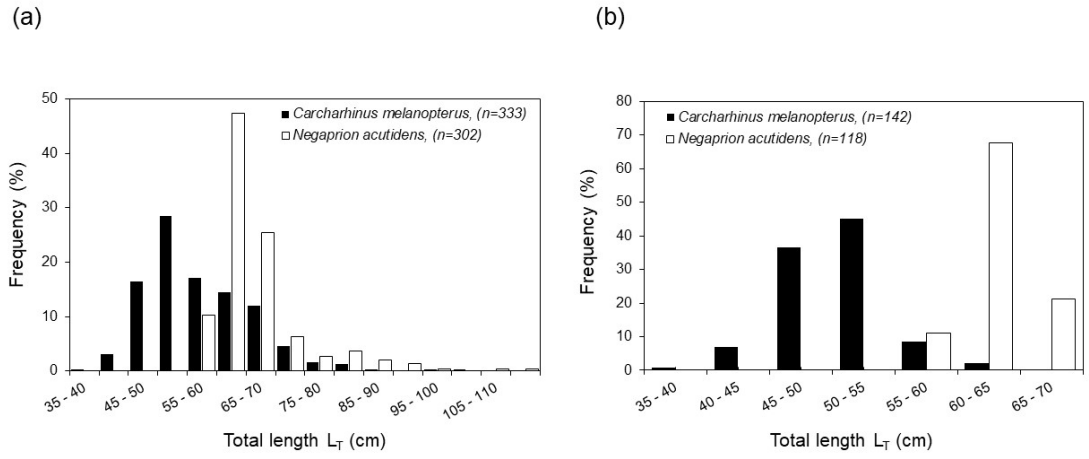

Figure 4.2 Total length (L_T) - frequency histograms for (a) juveniles, including neonatal *Carcharhinus melanopterus* (n = 333; black bar), and *Negaprion acutidens* (n = 302; white bar), and (b) solely neonatal *Carcharhinus melanopterus* (n = 142; black bar), and *Negaprion acutidens* (n = 118; white bar) captured at St. Joseph Atoll between November 2014 and April 2017.

4.4.2 Time at liberty and recapture distances

Over the scope of the study, we recaptured 24 out of 333 (8.4%) individual *C. melanopterus*, of which three (12.5%) were caught on multiple occasions in different seasons (two were captured twice and one individual three times). Time at liberty ranged from 96 to 925 days with an average of 287 ± 44 days. From 302 *N. acutidens* that were tagged, 62 individuals (26.8%) were recaptures. Fifteen individuals (24.2%) were recaptured on multiple occasions, of which 12 were recaptured twice, two individuals three times and one individual was recaptured four times over the period of 2 years. Time at liberty for *N. acutidens* ranged from 90 to 842 days with an average of 321 ± 26 days. Tagging procedures did not seem to negatively affect or harm animals, as high numbers of over-recaptures (*C. melanopterus*, $n = 24$; *N. acutidens*, $n = 48$) were recorded in all seasons. Note that sharks that are recaptured on the same day are not included in over-recaptures. Instead they were released immediately in order to avoid post-release mortality. Distances between initial and recapture locations ranged from 49 to 4417 m (730 ± 197 m) in *C. melanopterus* (Figure 4.3a) and 13 to 1541.7 m (579 ± 65 m) in *N. acutidens* (Figure 4.3b). While approximately half the recaptures of both species (50% in *C. melanopterus* and 56% in *N. acutidens*) were captured within 0 to 500 m from their initial capture location, nearly all sharks (92% of *C. melanopterus* and 95% of *N. acutidens*, respectively) were recaptured between 0 to 1500 m (Figure 4.4). The longest recapture distances in *C. melanopterus* (4417 and 2515 m, respectively) were measured in two individuals that were both recaptured in deeper channels through hand-line fishing by another research project (Figure 4.3a). All *N. acutidens* were recaptured on the reef flats. Recaptures of both species reported significantly further distances with longer times at liberty (*C. melanopterus*: $F_{1,22} = 15.4$, $p < 0.001$; $r^2 = 0.42$; *N. acutidens*: $F_{1,60} = 15.18$, $p < 0.001$; $r^2 = 0.2$; Figure 4.5), while recapture distances were not correlated with shark total lengths (*C. melanopterus*: $F_{1,22} = 0.102$, $p > 0.05$; *N. acutidens*: $F_{1,60} = 0.003$, $p > 0.05$).

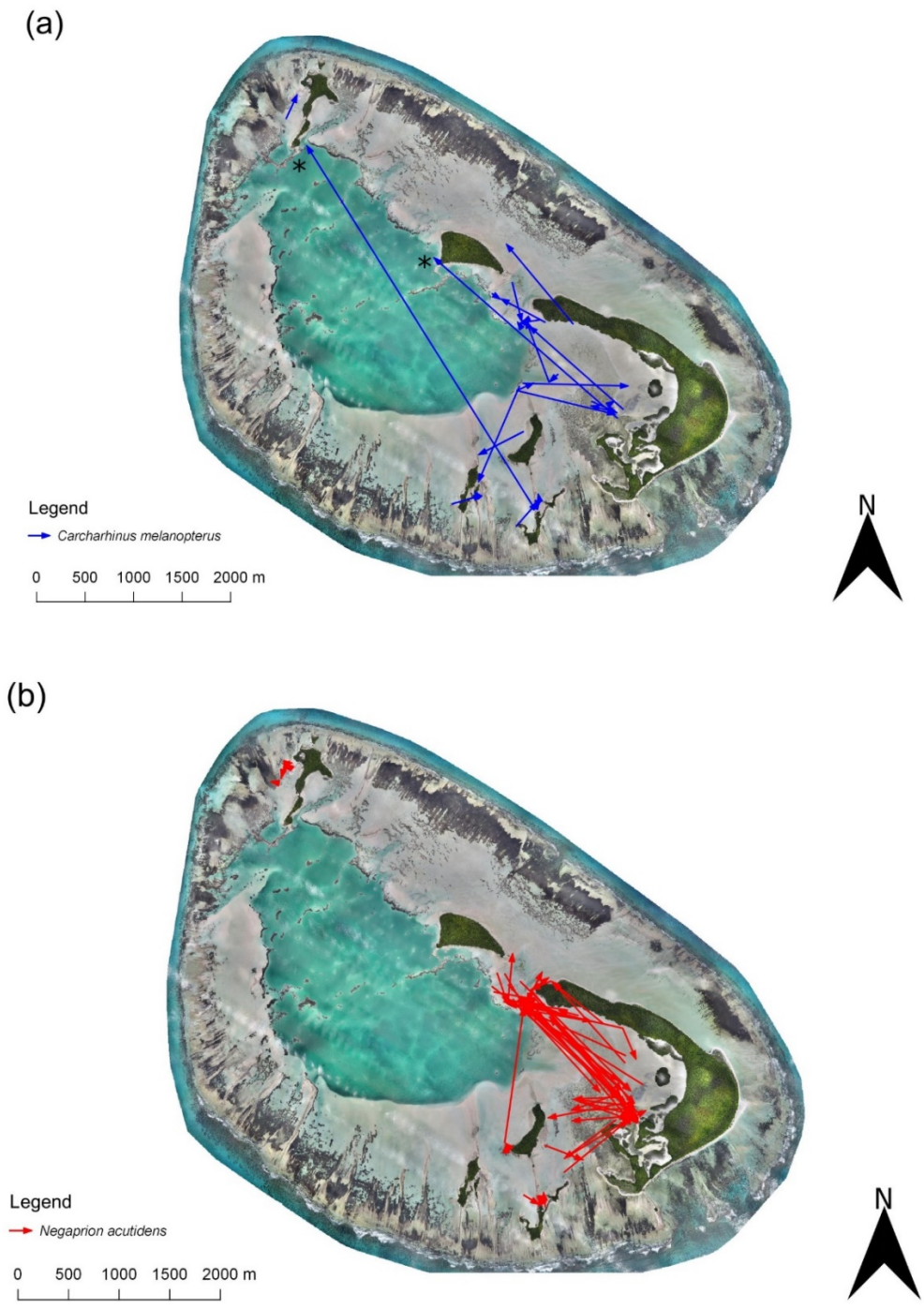

Figure 4.3 Direction between initial and recapture locations in (a) *Carcharhinus melanopterus* (blue arrow; $n = 24$) and (b) *Negaprion acutidens* (red arrow; $n = 62$) at St. Joseph Atoll, Seychelles. Arrowheads indicate direction of dispersal. Due to the high number of arrows and for simplicity, only a sub-sample of movements are displayed. (*) Positions where two *Carcharhinus melanopterus* were captured by handlines in deeper channels rather than on the adjacent reef flats; all *Negaprion acutidens* were captured on the reef flats.

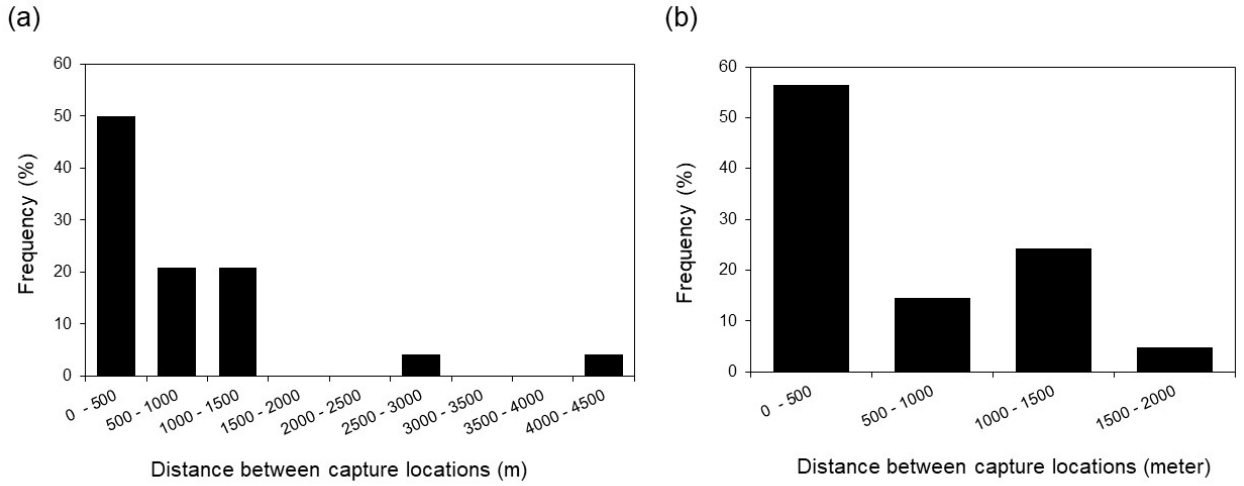

Figure 4.4 Frequency histogram of the dispersal distance between initial and recapture locations for (a) *Carcharhinus melanopterus* (*n* = 24), and (b) *Negaprion acutidens* (*n* = 62). Maximum distance was used for sharks with multiple recaptures.

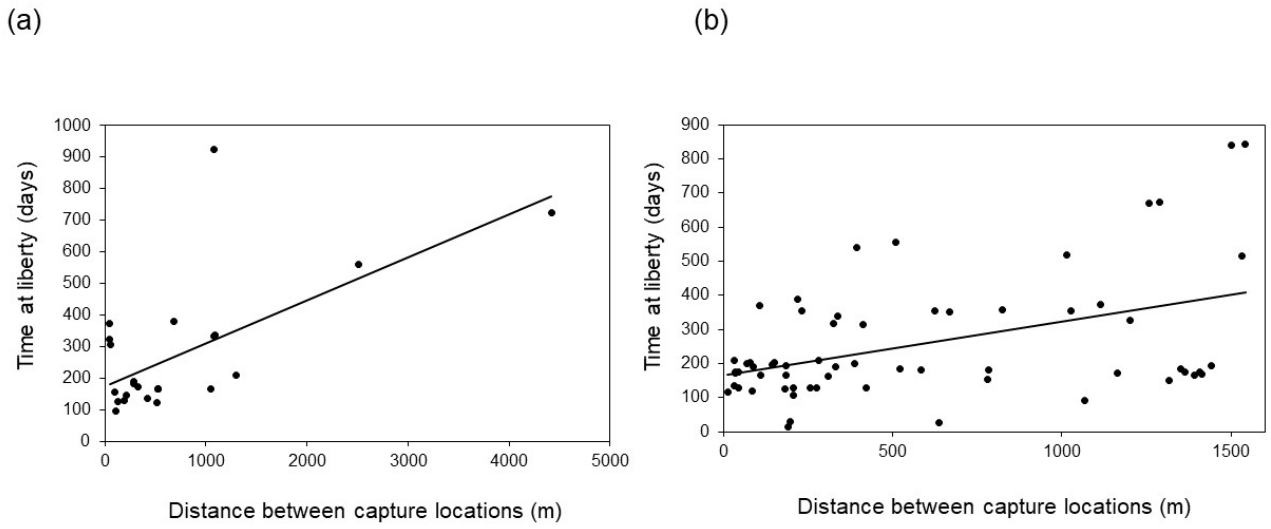

Figure 4.5 Time at liberty with recapture distance in (a) juvenile *Carcharhinus melanopterus* (y = 0.136x + 175.173, $r^2 = 0.42$, *n* = 24, *p* < 0.05), and (b) juvenile *Negaprion acutidens* (y = 0.158x + 163.89, $r^2 = 0.2$, *n* = 62, *p* < 0.05). Maximum distance was used for sharks with multiple recaptures.

4.4.3 Growth rates

Annual growth rates calculated from recaptured individuals varied substantially from 6.6 to 31.6 cm year^{-1} (16.2 ± 1.2 cm year^{-1}) in *C. melanopterus* (n = 24) and 0.2 to 32.2 cm year^{-1} (11.8 ± 1 cm year^{-1}) in *N. acutidens* (n = 62). When divided into *C. melanopterus* sub-classes, growth varied from 6.6 to 31.6 cm year^{-1} (15.5 ± 1.9 cm year^{-1}; n = 14) in individuals that were initially captured as neonates and from 13 to 22.4 cm year^{-1} (17.2 ± 1.2 cm year^{-1}; n = 10) in individuals first captured as juveniles (Figure 4.6a). Similarly, growth rates in *N. acutidens* varied from 0.2 to 28.2 cm year^{-1} (8.2 ± 1.2 cm year^{-1}; n = 27) in individuals captured as neonates and from 0.8 to 32.2 cm year^{-1} (14.9 ± 1.5 cm year^{-1}; n = 31) in individuals captured as juveniles (Figure 4.6b). To ensure that the broad variation is not related to different temperature seasons, growth rates from the higher temperature period (October – April) were compared with the lower temperature period (April – October), but no statistically significant difference was found (Wilcoxon rank-sum test; *C. melanopterus*: $p > 0.05$; *N. acutidens*: $p > 0.05$). Growth rates in *C. melanopterus* were related neither to times at liberty ($F_{1,22} = 2.92$, $p > 0.05$) nor distances ($F_{1,22} = 0.48$; $p > 0.05$). Similarly, no relationships were found in *N. acutidens* for growth rates with time at liberty ($F_{1,60} = 1.23$, $p > 0.05$), or with dispersal distances ($F_{1,60} = 2.54$, $p > 0.05$).

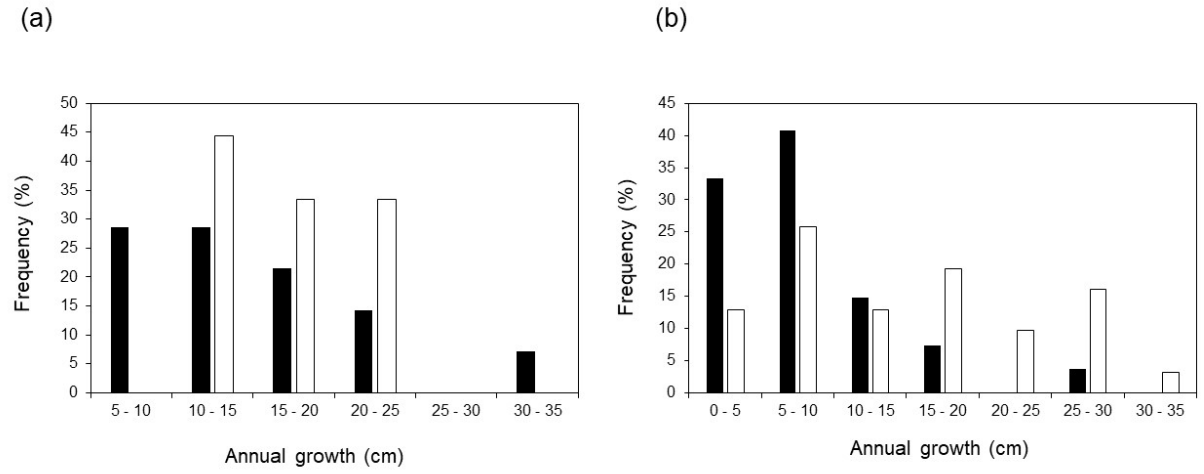

Figure 4.6 Annual growth rate-frequency histogram for (a) *Carcharhinus melanopterus* (black bar, neonates at initial capture, n = 14; white bar, juveniles at initial capture, n = 10), and (b) *Negaprion acutidens* (black bar, neonates at initial capture, n = 27; white bar, juveniles at initial capture, n = 31).

4.5 Discussion

Several coastal viviparous shark species have been reported to use nearshore areas for parturition and during early life-stages. Despite the benefits that nearshore nursery areas potentially provide, recent studies on nursery-bound sharks found evidence of high pup mortality, slow growth, weight loss and niche segregation (Heupel & Simpfendorfer, 2002; Lowe, 2002; Bethea et al., 2004; Duncan & Holland, 2006; Kinney et al., 2011; Matich et al., 2017c). We found distinctive population characteristics that provide compelling evidence of a multi-species shark aggregation composed of a significant number of two residential populations. Long-term and repeated recaptures with short recapture distances are indicative of a habitat where juveniles can remain for the first years of life. However, large variability in annual growth rates provide new insights into remote and communally used habitats and suggest high intra and interspecific competition induced by a resource limited habitat.

Several shallow nearshore areas have been reported to be inhabited by multiple juvenile shark populations (Stevens, 1984; Kinney et al., 2011; Speed et al., 2011; Matich et al., 2017c) and St. Joseph Atoll provides such a habitat. The seasonal and consecutive occurrence of neonatal *C. melanopterus* suggests a parturition cycle from mid-September to mid-March, which is broadly consistent with the cycle in Aldabra situated 900 km south-east of St. Joseph Atoll (Stevens, 1984). The slightly shorter pupping season of *N. acutidens* is in accordance with the cycle of sharks at Curieuse Marine National Park situated 250 km north-west of St. Joseph Atoll, where parturition lasts for 2.5 months (Hodgkiss et al., 2017). Also, parturition times coincide with the time of the highest atoll water temperatures, similar to parturition periods of *C. melanopterus* throughout its range (Mourier et al., 2013b). The pupping seasons for both species could potentially start earlier or last longer, but due to lack of sampling effort between April and September, no further conclusions can be drawn. Capture rates were higher during changing tides (flood and ebb tides) compared with slack tides (high and low water). These results are, however, most probably explained by our sampling effort biased towards regimes of changing tides. Catch data further reveals both shark species with sex ratios that were not significantly different from unity, which is similar to other populations of juvenile *C. melanopterus* (Papastamatiou et al., 2009a; Chin et al., 2013b) and *N. acutidens* (Stevens, 1984). Neonatal *C. melanopterus* from St. Joseph Atoll are bigger compared with pups from other remote, reef-associated habitats in the Pacific Ocean

(Bonham, 1960; Papastamatiou et al., 2009a) and smaller than pups from eutrophic and turbid nurseries in western Australia and the Pacific island of Moorea (Chin et al., 2013b; Mourier et al., 2013b; Oh et al., 2017). Neonatal *N. acutidens* at St. Joseph Atoll are comparable with other locations in the Indian Ocean, such as Aldabra (60 cm L_T; Stevens, 1984) and Curieuse (62.5 cm L_T; Hodgkiss et al., 2017), but are significantly smaller than individuals from western Australia (75.2 cm L_T; Oh et al., 2017) and Moorea (66.9 cm L_T; J. L. Rummer, unpublished data, August 2018). Possible explanations as to why neonatal size may spatially vary could include: (a) response to latitudes, with larger bodied pups found at higher latitudes (Mayr, 1942; Lombardi-Carlson et al., 2003); (b) differences in maternal sizes; (c) genetic differences; (d) variable levels of interspecific competition in adults due to resource availability, (e) litter sizes; (f) parturition months, or a mixture may also influence neonatal sizes (Barker et al., 2005; Hussey et al., 2010). We also noted the wide range of new-born sizes in both species. If maternal size has a substantial influence on offspring sizes, large variation in pup size may be a function of mature females of different size and age classes that uses diverse parturition strategies to optimise the survival of their offspring (Lombardi-Carlson et al., 2003; Hussey et al., 2010). Additionally, the broad neonatal size range may be related to parturition months, but only *C. melanopterus* demonstrated significantly smaller pups earlier in the pupping season. Nevertheless, future assessments of young nearshore shark populations will be needed to explain why neonatal sizes vary between locations, as well as parental analyses, to better understand if smaller and younger females are giving birth to smaller pups.

Extended times at liberty in numerous recaptured individuals, of which several were recaptured as many as five times, provide compelling evidence that juvenile sharks reside in the shallow areas of St. Joseph Atoll during their first years of life. Mark-recapture studies in other nearshore habitats depict juvenile recapture numbers to be either low or dominated by short times at liberties of a few weeks. Indeed, of 24 recaptured *C. melanopterus*, at Cleveland Bay, QLD, Australia, only five were juveniles (Chin et al., 2013b), while at Curieuse National Park, despite the intensive sampling effort, only one recaptured *N. acutidens* originated from the previous pupping season, while the rest consisted entirely of over-recaptures (Hodgkiss et al., 2017). Similarly, at Marquesas Keys, Florida, USA, only four *N. brevirostris* were recaptured after 1 year from a total number of 186 tagged juveniles (Barker et al., 2005). While only Chin et al. (2013a) conducted acoustic tracking

to demonstrate dispersal into adjacent habitats, early juvenile emigration may be the major cause of small recapture numbers and short times at liberty, especially in areas where alternative nursery areas are in close proximity. Alternatively, in remote areas with no adjacent nursery habitats, such as Bimini, Bahamas, and Atols das Rocas, Brazil, recapture rates of *N. brevirostris* were comparable with *N. acutidens* of this study (Gruber et al., 1988; Freitas et al., 2006). It's also noteworthy that despite demonstrating similar times at liberties and recapture distances to *N. acutidens*, the recapture rate of *C. melanopterus* at St. Joseph Atoll was less than a quarter of that for *N. acutidens*. Since no alternative nursery is near, from which dispersal could have occurred, these lower values are potentially attributable to a larger local population or higher mortality rates of *C. melanopterus* compared with *N. acutidens* (J. S. E. Lea, personal observation, September 2017). Despite having small litter sizes of 1- 6 pups (Mourier et al., 2013b), *C. melanopterus* is a medium-bodied and relatively fast-growing reef shark, while *N. acutidens* is a slower growing large-sized shark, known for being able to sustain a population with less than 50 breeding animals (Mourier et al., 2013a). Furthermore, species-specific reproduce cycles may also contribute to the difference in recapture numbers. *Negaprion acutidens* are known for biennial reproductive cycles (Mourier et al., 2013a), while female *C. melanopterus* are giving birth annually (Mourier et al., 2013b). However, reproductive cycles may vary within species inhabiting different areas (Driggers III & Hoffmayer, 2009). Another explanation for the difference in recapture numbers between species might be attributable to some bias in sampling gear. We observed that the gillnet used in this study with 5 cm mesh sizes failed on several occasions to capture particularly small and slender *C. melanopterus* while they were around the net. Lastly, juvenile *C. melanopterus* are less residential compared with *N. acutidens* (Oh et al., 2017) and may utilize more distant areas on the atoll's reef flats, which were not part of our sampling effort. Nonetheless, the high numbers of long-term recaptures caught in close proximities to the initial capture locations suggests that St. Joseph Atoll provides environmental conditions that allows long-term site attachment by multiple juvenile shark species.

Despite that 50% of recaptures were found in very close proximity to their initial tagging site (< 500 m), times at liberty, as opposed to shark size, were significantly related to recapture distances in both species. The increase of dispersal distance with longer times at liberty was especially evident in two *C. melanopterus* that were recaptured by hand-line fishing by another research

project. Both individuals were captured in deeper parts of the lagoon far from the initial capture locations after 560 and 724 days, respectively. Ontogenic increases in activity space have been convincingly demonstrated in *N. brevirostris* from Bimini, where young sharks start expanding their movements over larger areas and deeper water (Morrissey & Gruber, 1993b; Franks, 2007). While our observation may also indicate an increase in dispersal distance with ontogeny, our sample size is small and additional methodology such as long-term passive acoustic tracking would be needed to draw further conclusions.

Fast annual growth rates suggest that individuals are capable of meeting far in excess of their prey requirements since rate of consumption directly influences growth rate and survival (Cortes & Gruber, 1993; Lowe, 2002). Indeed, in a captive study, Randall (1977) observed two new-born *C. melanopterus* growing up to 22 cm year^{-1} during their first 2 years of life. Similarly, Gruber and Stout (1983) found precaudal length (L_{PC}) growth rates of captive juvenile *N. brevirostris* to be 23.4 cm year^{-1} L_{PC}, demonstrating the capacity for fast growth, if food is not scarce. While growth in captivity cannot be taken as indicative of growth in the wild, several *C. melanopterus* and *N. acutidens* from this study demonstrated annual growth rates as high as 20 cm year^{-1} L_T or higher. Comparable fast growth rates are available for wild *N. brevirostris* from Atols das Rocas with 19.5 cm year^{-1} L_T (Freitas et al., 2006). Our study not only reported similarly fast growth rates, but concurrently also found neonates and juveniles of both species with growth rates less than 5 cm year^{-1} L_T. This results in a wide range of growth rates that are to date the broadest reported in young wild sharks. In a recent study, variable growth rates of *N. brevirostris* were attributed to different foraging strategies, with slow growing individuals (1.4 cm year^{-1} L_{PC}) foraging predominantly in mangrove habitats (predator safe) and fast-growing individuals (9.5 cm year^{-1} L_{PC}) more likely to forage over predator exposed seagrass beds (Hussey et al., 2017). Unlike Bimini, where a resource abundant (Newman & Gruber, 2002) and mono-species habitat implies a lack of competitive patterns, St. Joseph Atoll is an oligotrophic and potentially resource-limited habitat. Therefore, variable growth rates are more likely to have resulted from intra and inter-specific competition for limited prey. While evidences of prey limitations in nearshore areas are increasing (Heupel & Hueter, 2002; Duncan & Holland, 2006; Kinney et al., 2011), they are also likely to be the leading cause for resource partitioning in response to competition (Kinney et al., 2011; Matich et al., 2017c). To gain further clarification if limited prey resources may have led to

competition and subsequent fast (successful competitors) and slow growing (poor competitors) individuals, future investigations on resource and habitat use will be important for estimating the level of competition at St. Joseph Atoll.

In conclusion, our data suggest that St. Joseph Atoll is a critical habitat for parturition as well as an essential location ensuring the life cycle of two coexisting and residential juvenile shark populations. Despite large neonatal sizes, high capture and recapture numbers, short dispersal distances and long times at liberty that suggest favourable environmental conditions, large variability in annual growth rates suggests prey resources to be limited, a condition suggested from several near-shore locations (Duncan & Holland, 2006; Kinney et al., 2011; Matich et al., 2017c). While early dispersal to nearby shallow areas with more abundant prey resources may be possible in certain locations (Chin et al., 2013a), young sharks from atoll habitats are unlikely to perform such behaviour. Even if they do, the surrounding open water is going to contain less refuges and higher abundances of predatory elasmobranchs (Lea et al., 2015b, 2016, 2018), therefore the protection given by shallow atoll habitats may compensate, over the long term, for its resource limitation. Thus, we support the progress of the special reserve developed for D'Arros island and St. Joseph Atoll (Lea et al., 2016) that allows the conservation of this critical habitat and the species that depend on it.

Acknowledgements

The authors wish to thank R. and C. Daly, C. Boyes, R. von Brandis and K. Bullock and numerous SOSF - D'Arros Research Centre assistants for their invaluable support in the field. Authors are thankful to A. Chin for helpful and critical discussions during the process of writing, to J. S. E. Lea for discussing data from Lea et al. 2016, to P. Megert for support with ArcGIS/QGIS and to Drone Adventures for creating high resolution atoll maps. The manuscript benefited greatly from the input of two anonymous reviewers.

Author contribution

O.C.W. designed and coordinated the study; O.C.W. collected field data; O.C.W. analysed the data and wrote the manuscript with support and inputs from Y.P.P. and S.P; all authors gave final approval for publication. O.C.W. secured the funding to support this work.

Supplementary Information

Field season	Dates	Duration	Weather conditions
1	November - December 2014	6 weeks	Start of northeast monsoon
2	March - April 2015	8 weeks	End of northeast monsoon
3	October - November 2015	8 weeks	Start of northeast monsoon
4	April 2016	2 weeks	End of northeast monsoon
5	October 2016	2 weeks	Start of northeast monsoon
6	March - April 2017	3 weeks	End of northeast monsoon

Supplementary information S1. Information about the timing, duration and predominant weather condition during field seasons at St. Joseph Atoll, Seychelles.

Chapter 5 | Same species, different prerequisites: investigating body condition and foraging success in young reef sharks between an atoll and an island system

This chapter is published as:

Weideli, O.C., Bouyoucos, I.A., Papastamatiou, Y.P., Mescam, G., Rummer, J.L, Planes, S. (2019) Same species, different prerequisites: investigating body condition and foraging success in young reef sharks between an atoll and an island system. *Scientific Reports*. 9, 13447. http://doi: 10.1038/s41598-019-49761-2.

Same species, different prerequisites: investigating body condition and foraging success in young reef sharks between an atoll and an island system

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Received: 17 May 2019 | **Accepted**: 27 August 2019 **| Published online**: 17 September 2019

5.1 Abstract

Acquiring and storing energy is vital to sharks of all age-classes. Viviparous shark embryos receive endogenous maternal energy reserves to sustain the first weeks after birth. Then, in order to maintain body condition, sharks must start foraging. Our goal was to understand whether maternal energy investments vary between blacktip reef sharks (*Carcharhinus melanopterus*) from two populations and to what extent body condition and the initiation of foraging might be affected by presumably variable maternal investments. A total of 546 young sharks were captured at St. Joseph Atoll (Seychelles) and Moorea (French Polynesia) between 2014 and 2018, and indices of body condition and percentage of stomachs containing prey were measured. Maternal investment was found to be site-specific, with significantly larger, heavier, and better conditioned individuals in Moorea. Despite these advantages, as time progressed, Moorea sharks exhibited significant decreases in body condition and were slower to initiate foraging. We suggest that the young sharks'

foraging success is independent of the quality of maternal energy resources, and that other factors, such as prey availability, prey quality, and/or anthropogenic stressors are likely responsible for the observed differences across sites. Insights into intraspecific variations in early life-stages may further support site-specific management strategies for young sharks from nearshore habitats.

5.2 Introduction

Acquiring and storing energy reserves to maintain body functions and survival is vital to animals of all age-classes (Stearns, 1992). To estimate energy reserves during various life-stages, body condition, as a proxy of animal health, is commonly used (Jakob et al., 1996), with animals in good body condition presumably associated with relatively larger energy reserves (Jakob et al., 1996; Green, 2001). At birth, an animal's body condition is determined by the parents, notably by the mother (Bernardo, 1996). Depending on maternal size and age at parturition, the diet, as well as the environmental conditions to which the mother was exposed during gestation, the offspring's size, body mass, and body condition can vary among and within species. Indeed, coral reef fishes from high quality habitats pass on larger yolk reserves to their offspring than parents living in low quality habitats (Donelson et al., 2008). In the first weeks after birth, young animals with no parental care are required to gradually incorporate autonomous foraging activities to their daily routine to sustain their energy reserves. Hence, young animals depend on prey resources and habitat quality, in addition to remaining maternal energy resources. While strong positive relationships between parental energy reserves and factors such as offspring condition and time to exogenous feeding have been noted for teleost fishes and marine reptiles (Berkeley et al., 2004; Gagliano & McCormick, 2007; Wallace et al., 2007; Donelson et al., 2008), little work has been done on maternal energy investment in elasmobranchs. As maternal investment may vary with life-history traits (e.g., size, body condition) and habitat, it is also important to understand if and to what extent the level of maternal energy investment affects the offspring's condition and foraging development during the first weeks of life.

Elasmobranchs occur across a range of heterogenous habitats and experience variable environmental conditions and levels of anthropogenic threats that differentially affect life-history traits (Lombardi-Carlson et al., 2003). While intraspecific differences in life-history traits may be

less distinctive in sharks with broad movement patterns, genetically and geographically isolated sharks with restricted movements and site-fidelity are known to exhibit pronounced intraspecific differences in size at birth, growth rates, and litter sizes (Lombardi-Carlson et al., 2003; Bradley et al., 2017a). Adult reef-sharks from the family Carcharhinidae have been the focus of a number of studies investigating such differences (Bradley et al., 2017a), but fewer studies have characterized intra-specific variability among populations of young animals. Barker et al. (2005), for example, reported that larger sized female lemon sharks (*Negaprion brevirostris*) from Florida's Marquesas Keys (USA), give birth to larger offspring than the smaller sized females from a nearby nursery. Likewise, Hussey et al. (2010) revealed an increase in maternal reproductive output (larger neonatal mass) with increasing maternal size in two carcharhinid sharks. While such size and body mass measurements can help assess characteristics of young shark populations (**Chapter 4**), their relationship (e.g., body mass per unit size) provides invaluable information on energy reserves, overall body condition and fitness (Jakob et al., 1996; Green, 2001; DiBattista et al., 2007; Hussey et al., 2017). Despite the importance of energy reserves, it is unclear as to whether maternal energy investment varies across shark populations adopting different life-history traits.

At birth, viviparous sharks receive endogenous energy reserves, primarily stored as lipids in livers, from their mothers (Duncan & Holland, 2006; Hoffmayer et al., 2006; Hussey et al., 2010; Corsso et al., 2018). Although this maternal energy allocation can lead to significantly enlarged livers in neonatal sharks (up to 20% of total body mass; Hussey et al., 2010), this endogenous energy transfer is finite. As opposed to marine mammals, where exogenous maternal energy provisioning (e.g., lactation) can last months to years, depending on species (Oftedal, 1997), sharks receive no maternal aftercare. This results in energy resources being utilized within the first weeks, as demonstrated by decreasing condition indices (Hussey et al., 2010, Corsso et al., 2018). Similarly, Duncan and Holland (2006) reported mass loss in young sharks following parturition, most likely a sign of depleted energy reserves. To counteract such declines, young sharks are required to incorporate autonomous foraging to their daily routine. As it is difficult to directly observe young sharks foraging in the wild, biomarkers that indirectly estimate when young sharks shift from relying on maternal energy resources to feeding autonomously have recently been established (Belicka et al., 2012; Matich et al., 2015). Although biomarkers, such as bulk stable isotopes, alone

(Olin et al., 2011; Matich et al., 2015) or in combination with fatty acids (Belicka et al., 2012), provide insights into autonomous foraging developments and broad estimates of body condition (Papastamatiou et al., 2010; Matich et al., 2019), isotopic turnover rates impede timely and precise estimates of body condition as well as foraging development. To date, Hussey et al. (2010) executed the only study that simultaneously and precisely assessed changes in body condition and estimated foraging development in early life-stages of sharks via a combination of lethal and non-lethal measurements to calculate such developments across different umbilical scar healing stages. Given that the study only focused on a single location that precludes investigations on variabilities among populations, further work is needed to understand if and how body condition and the development of autonomous foraging may vary across species inhabiting different habitats.

To examine intraspecific variability in body condition and foraging development during the first weeks of life, we collected life-history data from neonatal and juvenile blacktip reef sharks (*Carcharhinus melanopterus*), a species with high levels of genetic population structure (Vignaud et al., 2014; Delser et al., 2016), from two remote habitats in the Indo-Pacific Ocean. While Moorea (French Polynesia) is a remote island with human-impacted shorelines in the South Pacific, St. Joseph Atoll (Seychelles), located in the western Indian Ocean, consists of a small and uninhabited ring of islands with adjacent shallow reef flats. Our objectives were to use non-lethal methods to determine (1) whether maternal energy investment varies between *C. melanopterus* populations potentially adopting different life-history strategies, and (2) if and to what extent body condition and foraging development might be affected by presumably variable maternal investments. We hypothesized that better conditioned neonates (e.g., neonates with higher energy stores) would show a slower decrease in body condition and a faster acquisition of foraging skills during the first weeks of life. Considering the steady increase in human activities in nearshore areas that is resulting in declining prey abundance and habitats (Duncan & Holland, 2006; Jennings et al., 2008; Thiault et al., 2017) as well as predicted higher water temperatures due to climate change (Levitus et al., 2000; Cheng et al., 2019), a better understanding of maternal energy investments, body condition, and autonomous foraging development during early life-stages of sharks has important implications for conservation (Rosa et al., 2017). Insights into potential intraspecific differences in such characteristics may further support site-specific management strategies for sharks from remote and potentially prey-limited habitats (Kinney & Simpfendorfer, 2009; Knip et al., 2012).

5.3 Material and methods

5.3.1 Ethical approval

Sharks for this study were captured as part of long-term fisheries-independent surveys in Moorea, French Polynesia and on St. Joseph Atoll, Republic of Seychelles. Ethical approval for Moorea was given by James Cook University Animal Ethics Committee protocol A2089 and permission to work with sharks in French Polynesia was obtained from the Ministère de l'Environnement (Arrete N° 9524). Research on sharks at St. Joseph Atoll was approved by, and conducted with the knowledge of Ministry of Environment, Energy, and Climate Change, Seychelles. Animal handling and tagging methods were conducted in accordance with the approved guidelines of S. Planes by the Autorisation de pratiquer des expériences sur les animaux n° 006725 (1995) from the ministry of Agriculture.

5.3.2 Study location and sampling

Some of the sharks for this study were captured as part of long-term fisheries-independent surveys in Moorea, French Polynesia (17°30′S, 149°51′W). Moorea is surrounded by fringing reefs and lagoons that are adjacent to shallow nearshore areas serving as putative nursery grounds for young *C. melanopterus* (Mourier et al., 2013b; Matich et al., 2017c). Juvenile *C. melanopterus* were captured using gillnets (50.0 m × 1.5 m, 5.0 cm mesh) during the parturition months (September - February) in 2016/2017 and 2017/2018. Captured individuals were immediately removed from the net, and handling time was kept to a minimum (< 7 min.) to avoid excessive capture-related stress (Glaus et al., 2018). Sharks captured in 2016/2017 were externally tagged using coloured T-bar anchor tags (Hallprint®, Hindmarsh Valley, SA, Australia) and internally with passive integrated transponder (PIT) tags (Biolog-ID) in 2017/2018 to allow recaptured animals to be identified. During these sampling events, precaudal length (L_{PC}, the length from the tip of the snout to the precaudal notch) and three girth measurements were measured to the nearest 0.1 cm with a tape measure for each shark: 1) pectoral girth (G_{PEC}), the circumference of the shark measured at the base of the pectoral fin insertion, anterior to the dorsal fin, 2) dorsal girth (G_{DOR}), the circumference measured at the base of the first dorsal fin insertion, and 3) caudal girth (G_{CAU}), measured anterior to the caudal fin in the precaudal notch (see Supplementary Information S1). Umbilical scar stage (USS), a reliable indicator of neonatal life-stages (Hussey et al., 2010; Duncan & Holland, 2006;

Chin et al., 2015), was quantified into three categories. USS1 was applied if scar was fully open, USS2, if scar was semi-healed, and USS3 for fully healed scars (see Supplementary Information S2). Individuals with USS1 and USS2 were considered as neonate sharks with an estimated maximal age of four weeks (Hussey et al., 2010; Duncan & Holland, 2006; Chin et al., 2015). Sharks with closed scars (USS3) were identified as young-of-the-year (> four weeks old) and no differentiation was made between visible and well-healed scars. We were unable to differentiate between young-of-the-year and older sharks, due to systematic size overlap between different age-classes (Barker et al., 2005). The USS of each shark was photographed alongside a ruler, and total body mass (M_{TB}) was measured with a hand-held scale to the nearest 10 g. After completing basic measurements, a subset of *C. melanopterus* individuals also had their stomachs flushed, similar to Bangley et al. (2013). Different diameters of transparent acrylic tubes (2.5, 3.2, and 3.8 cm outer tube diameter) were used according to the shark sizes (< 60 cm, between 60-70 cm and >70 cm L_T, respectively). The beveled and lubricated tubes were inserted through the mouth, esophagus, and into the stomach while the sharks were kept in the water. As soon as the stomach and the tube were filled with water, the shark was turned upside down to flush the stomach. The stomach items were captured in a sieve, and the percentage of stomachs containing prey was recorded. This procedure was solely conducted on sharks in good condition (e.g., no open wounds) and was kept to a maximum of three consecutive procedures per individual. Environmental temperatures were recorded every ten minutes during parturition season with stationary Hobo ® temperature loggers (UA-002-64, Onset Computer Corporation, Bourne, MA, USA) deployed in capture locations.

Fieldwork was further conducted in the western Indian Ocean at St. Joseph Atoll (05°26′S, 53°20′E) in the Republic of Seychelles. St. Joseph Atoll is a near-pristine and non-inhabited atoll that offers shallow, protected areas for at least two species of young sharks (**Chapter 4**). Juvenile *C. melanopterus* were captured with gillnets (20.0 m × 1.5 m, 5.0 cm mesh) during the parturition months (October - December and March - April) in 2014/2015, 2015/2016 and 2016/2017. Captured *C. melanopterus* were immediately removed from the net, and handling time was kept to a minimum (< 7 min.) to avoid excessive capture-related stress (Bouyoucos et al., 2018). Sharks were internally tagged using PIT tags (Biomark®) to allow recaptured sharks to be identified. The L_{PC}, girth, USS, and M_{TB} were measured for each shark, and gastric lavage was subsequently conducted using a sub-sample of sharks following Moorea's protocol. All sharks were released at

site within minutes of capture. Temperatures were recorded every fifteen minutes during parturition season with stationary Hobo ® temperature loggers (U22-001, Onset Computer Corporation Bourne, MA, USA) distributed across the area surveyed.

5.3.3 Data analyses

Where applicable, data were checked for normality using Shapiro-Wilk tests prior to analyses in R version 3.5.3 (R Core Team, 2016) within the RStudio interface ver. 1.0.153 (RStudio Team 2016). For all tests, the level of statistical significance α was set at 0.05, and results are reported as means ± SD. To investigate potential intraspecific life-history variabilities in neonates and temperature differences across habitats, mean L_{PC}, M_{TB} and water temperatues were compared with two sample t-tests, and frequency histograms were subsequently constructed. Total body mass for a given L_{PC} was used to estimate body condition, assuming that individuals in a good condition would be heavier than those in poorer condition of the same length. Thus, we determined allometric length-mass relationships by using the formula $\log y = \log a + b \log x$. These coefficients were used in $M_{TB} = a\, L_{PC}\, b$, where M_{TB} is total body mass (g) and L_{PC} is length (cm).

Two independent indices of individual body condition were also calculated. The Fulton's body condition index, also known as Fulton's K (Fulton, 1904), calculates a morphometric index of a fish's body condition with the following equation:

$$K = 10^5\, M_{TB}\, (L_{PC}^3)^{-1}$$

We also constructed a non-lethal and morphometric condition index, based on the assumption that individuals with larger livers for a given body length are in better condition (Hussey et al., 2010). Similar to Irschick & Hammerschlag (2014), three measurements along the shark's body were chosen to incorporate the size and anatomical location of the liver, as well as the shark's shape, which is wider along the anterior part of the body (Gallagher et al., 2014). While massive body sizes prevent measuring the circumference in previous studies (Gallagher et al., 2014; Irschick & Hammerschlag, 2014), we were able to take three circumference measurements to calculate the girth factor (GF) as a proxy for body condition using the following equation:

$$GF = [G_{PEC} + G_{DOR} + G_{CAU}] L_{PC}^{-1}$$

Resulting condition indices (K and GF) were compared across locations using a two sample t-tests. To demonstrate and validate the absence of inadvertent co-linearity between the two body condition indices and L_{PC} (Jakob et al., 1996; Green, 2001), linear least-squares regressions were performed for L_{PC} with K and GF, respectively.

In order to follow the transition of body condition with the closure of the umbilicus (increasing USS), analysis of variance (ANOVA) and Tukey's honest significant difference (HSD) tests were used for post-hoc multiple comparisons for Fulton's K at Moorea. As the other comparisons did not conform to a normal distribution, Kruskal-Wallis tests were applied. Post-hoc multiple comparisons were subsequently evaluated using Dunn tests, while *p*-values were adjusted using the Holm method to reduce type I error. In addition, individual sharks from Moorea that were captured on multiple occasions were used to further validate body condition changes during early life stages. Recaptured individuals allowed us to calculate the change in body condition between two capture events by subtracting body condition indices (K and GF) of the initial capture from the recapture event. Similarly, recaptured individuals allowed us to estimate if changes in body condition depend on the body condition at initial capture. For both calculations, values were plotted for each individual in a linear least-square regression. Furthermore, Fulton's K was linearly regressed against girth factor GF of recaptured *C. melanopterus* to demonstrate that changes in K could be predicted by changes in GF. Finally, in order to estimate level of autonomous foraging success during increasing USS, a sub-sample of *C. melanopterus* from Moorea and St. Joseph Atoll had gastric lavages performed. The obtained stomach status (% of stomachs containing prey) were compared with χ^2 test.

5.4 Results

5.4.1 Intraspecific variation in maternal energy investments

In Moorea, during the parturition seasons in 2016/2017 and 2017/2018, a total of 313 neonatal and juvenile *C. melanopterus* were captured and measured. Of those, 163 individuals (52%) were categorized as neonates (based on the presence of open or semi-healed umbilical scars) ranging

from 368 to 466 mm L_{PC} (418.42 ± 18.90 mm, Figure 5.1a) and weighting 670 to 1500 g (1025.22 ± 148.75 g, Figure 5.1b). At St. Joseph Atoll, during the parturition seasons in 2014/2015, 2015/2016 and 2016/2017, a total of 233 neonatal and juvenile *C. melanopterus* were collected. Of those, 173 individuals (74%) were categorized as neonates ranging from 287 to 459 mm L_{PC} (372.22 ± 27.66 mm, Figure 5.1a) and weighting 300 to 1375 g (694.99 ± 182.71 g, Figure 5.1b). Neonatal *C. melanopterus* from Moorea were significantly larger (two sample t-test: t = 17.769, df = 334, $p < 0.0001$) and heavier at birth (two sample t-test: t = 17.917, df = 325, $p < 0.0001$) than individuals from St. Joseph Atoll. Mean water temperatures during the pupping seasons were significantly lower in Moorea (29.5 °C ± 0.003) compared to St. Joseph Atoll (30.0 °C ± 0.003; two sample t-test: t = −101.87, df = 1040400, $p < 0.0001$; see Supplementary Information S3).

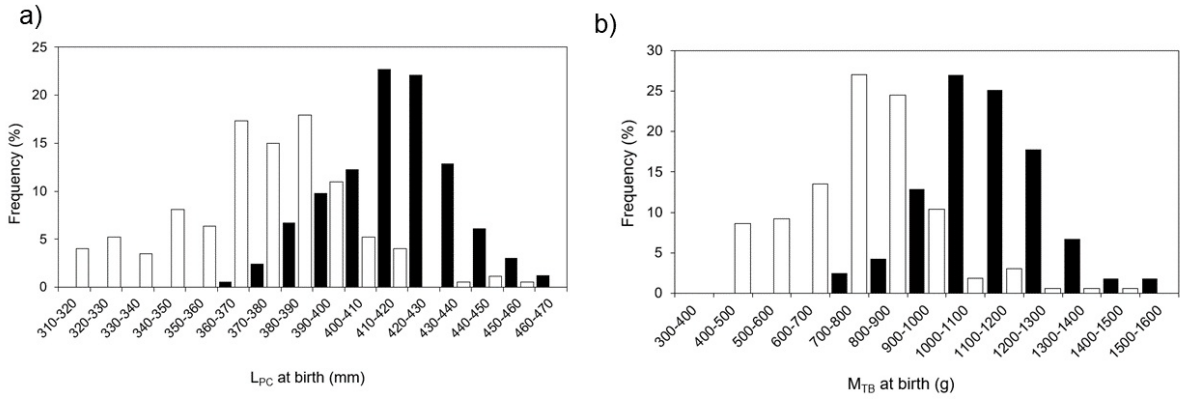


Figure 5.1 Percentage frequency histogram of (a) precaudal length (L_{PC}) and (b) total body mass (M_{TB}) in neonatal *Carcharhinus melanopterus* (USS1 and USS2) from Moorea (black, $n = 163$) and St. Joseph Atoll (white, $n = 173$ and 164, respectively).

Body condition, as calculated via three methods, differed significantly across locations. Neonatal sharks from Moorea were heavier for any given size than those at St. Joseph Atoll ($F_{1,234} = 20.89$, $p < 0.001$), and length-body mass results suggest positive allometric growth (Figure 5.2). Independent indices of body condition were significantly higher in Moorea sharks compared to St. Joseph sharks, as calculated by Fulton's K (two sample t-test: $t = 6.083$, df = 535, $p < 0.0001$; Figure 5.3a) and GF (two sample t-test: t = 7.036, df = 402, $p < 0.0001$; Figure 5.3b). Linear regressions revealed no relationships between L_{PC} and Fulton's K (St. Joseph: $F_{1,222} = 2.247$, $p =$

0.135), and L_{PC} and GF (Moorea: $F_{1,311} = 1.035$, $p = 0.310$; St. Joseph Atoll: $F_{1,89} = 3.397$, $p = 0.069$; see Supplementary Information S4). However, linear regressions revealed significant negative relationships between L_{PC} and Fulton's K in Moorea sharks ($F_{1,311} = 11.280$, $r^2 = 0.04$, $p = 0.0009$; see Supplementary Information S4).

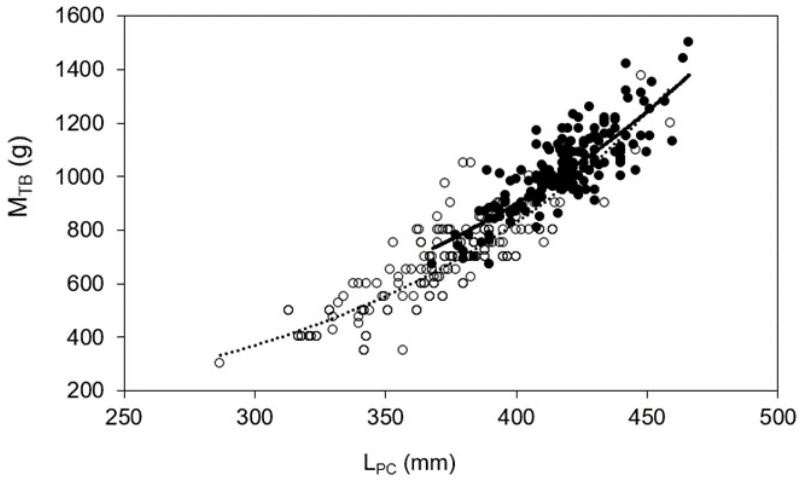


Figure 5.2 Relationship between total body mass (M_{TB}) and precaudal length (L_{PC}) of neonatal *Carcharhinus melanopterus* (USS1 and USS2) from Moorea (black, $y = 0.042472x^{2.70}$, $r^2 = 0.69$, $n = 163$) and St. Joseph Atoll ($y = 0.013947x^{2.98}$, $r^2 = 0.73$, $n = 164$).

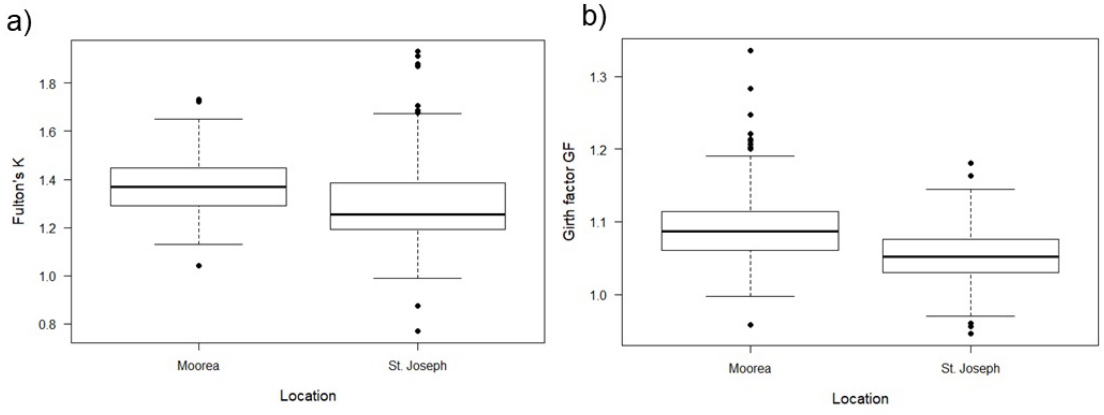


Figure 5.3 Comparison of body condition indices across locations. (a) Fulton's K for neonatal and juvenile *Carcharhinus melanopterus* from Moorea ($n = 313$) and St. Joseph Atoll ($n = 224$). (b) Girth factor GF for neonatal and juvenile *Carcharhinus melanopterus* from Moorea ($n = 313$) and St. Joseph Atoll ($n = 91$). Boxes indicate the interquartile range with the median shown by horizontal lines, minimum and maximum values shown by whiskers, and points representing outliers.

5.4.2 Intraspecific variation in change of body condition

In cases where body condition indices did not conform to a normal distribution (Shapiro-Wilks test, $p < 0.05$), non-parametric one-way and multiple comparison tests were applied. Body condition, as estimated by Fulton's K in young sharks from Moorea, differed significantly among increasing umbilical scars stages (ANOVA, $F_{2,310} = 6.907$, $p = 0.001$). Specifically, pair-wise comparisons showed statistical differences between USS1 and USS3 (Tukey's HSD, t = −0.06, $p = 0.001$) and decreasing, albeit non-significant, body condition between USS1 and USS2 (Tukey's HSD, t = −0.03, $p = 0.179$) and between USS2 and USS3 (Tukey's HSD, t = −0.03, $p = 0.097$, Figure 5.4a). Body condition, as estimated by girth factor GF in young sharks from Moorea, decreased significantly as umbilical scar stages increased (Kruskal-Wallis test, $\chi^2 = 48.513$, df = 2, $p < 0.001$). Pair-wise comparisons reported significant differences between all three umbilical scar stage classes (Dunn test, USS1/USS2: $p = 0.0006$; USS1/USS3: $p < 0.0001$; USS2/USS3: $p = 0.0003$, Figure 5.4c). On the contrary, no significant differences were found between Fulton's K

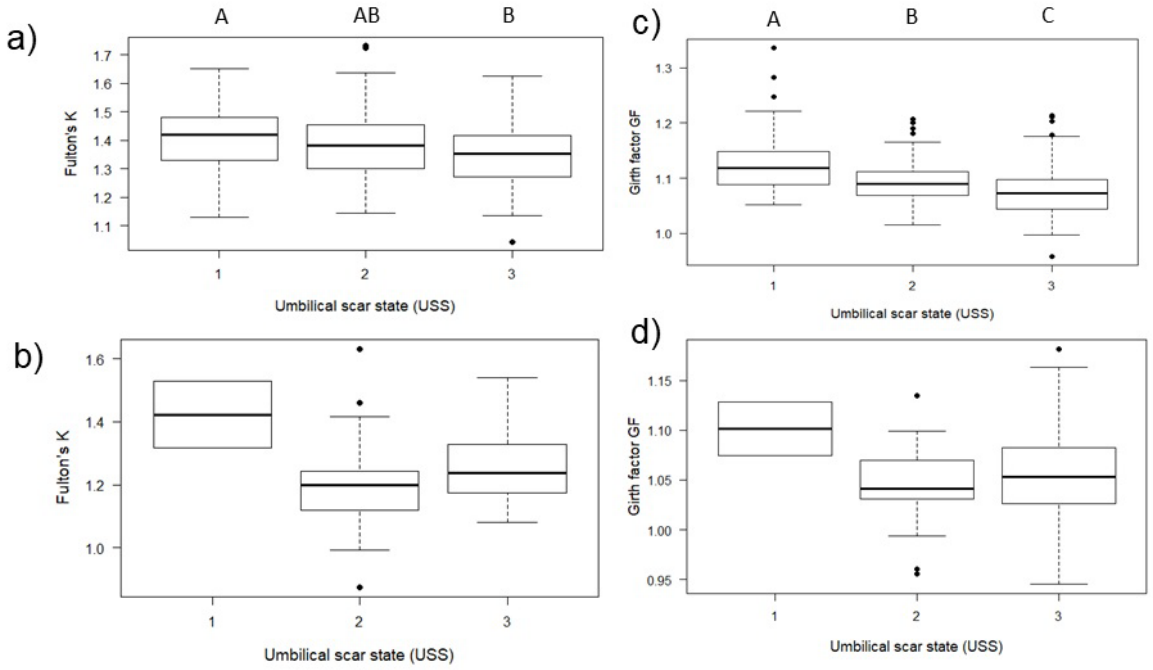


Figure 5.4 Transition of body condition indices with increasing umbilical scar stages (USS) in *Carcharhinus melanopterus*. Fulton's K at (a) Moorea and (b) St. Joseph Atoll, and girth factor GF at (c) Moorea, and (d) St. Joseph. Boxes indicate the interquartile range with the median shown by horizontal lines, minimum and maximum values shown by whiskers, and black dots represent outliers. Letters above plots in (a) and (c) indicate statistically significant differences between groups. Sample size in Moorea: USS1 $n = 59$, USS2 $n = 104$, USS 3 $n = 150$. Sample size at St. Joseph Atoll: USS1 $n = 2$, USS2 $n = 29$, USS3 $n = 60$.

and GF with increasing umbilical scar healing stages in young sharks from St. Joseph Atoll (Kruskal-Wallis test, $\chi^2 = 8.6627$, df = 2, $p = 0.056$ and $\chi^2 = 2.8051$, df = 2, $p = 0.246$, respectively; Figure 5.4b & d).

The 45 individuals that were recaptured in Moorea during one parturition season were at liberty from 4 to 72 days (see Supplementary Information S5), and linear regressions showed significant negative relationships between changes in Fulton's K and time at liberty ($F_{1,43} = 5.41$, $p = 0.025$, $r^2 = 0.11$, Figure 5.5a). Linear regression further revealed decreasing, albeit non-significant, relationships between GF and time at liberty ($F_{1,45} = 2.75$, $p = 0.104$; Figure 5.5b).

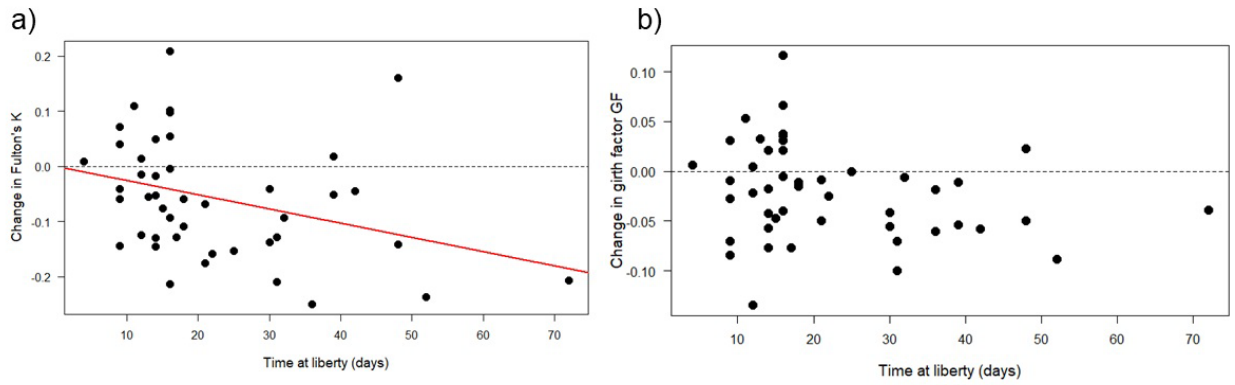


Figure 5.5 Changes in body condition indices with time at liberty in *Carcharhinus melanopterus* from Moorea. (a) Change in Fulton's K over time at liberty, and (b) change in girth factor GF over time at liberty. Data were obtained from neonatal sharks that were measured twice within the same parturition season (min. 4 days, max. 72 days, $n = 45$). The regression line for predicting changes in Fulton's K from time at liberty is shown in red ($y = 0.001 – 0.003x$, $r^2 = 0.11$). Note that each dot represents the change of body condition in one individual and that negative values (below the dashed line) depict a decrease of body condition in an individual shark.

Similarly, linear regression indicated significant negative relationships between change in body condition with body condition at initial capture (Fulton's K; $F_{1,43} = 28.46$, $r^2 = 0.40$, $p < 0.0001$; Figure 5.6a; GF: $F_{1,43} = 31.71$, $r^2 = 0.42$, $p < 0.0001$, Figure 5.6b). When differences in body condition indices were regressed against one another, data showed that changes in Fulton's K could be predicted by changes in GF ($F_{1,43} = 16.83$, $r^2 = 0.28$, $p = 0.0002$; see Supplementary Information S6), suggesting that estimates of either condition index were consistent within individuals.

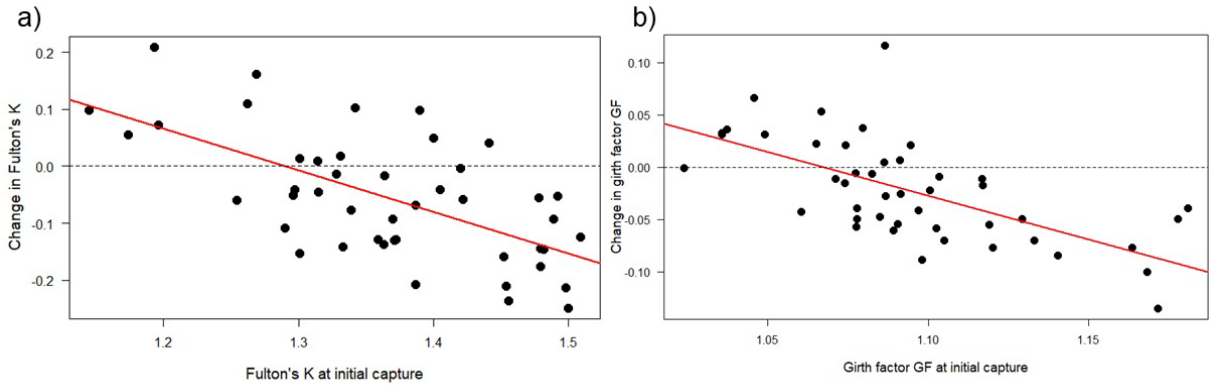


Figure 5.6 Changes in body condition indices versus body condition indices at initial capture in neonatal *Carcharhinus melanopterus* from Moorea. (a) Change in Fulton's K versus Fulton's K at initial capture, and (b) change in girth factor GF versus girth factor GF at initial capture. Data were obtained from sharks that were measured twice within the same parturition season (min. 4 days, max. 72 days, n = 45). The regression lines are shown in red (K: y = 0.94 - 0.73x, r^2 = 0.40 and GF: y = 0.90 - 0.84x, r^2 = 0.42, respectively). Note that each dot represents the change of body condition in one individual and that negative values (below the dashed line) depict a decrease of body condition in an individual shark.

5.4.3 Intraspecific variation in foraging success

In Moorea, over the scope of one parturition season (year 2016/2017), 165 gastric lavages in *C. melanopterus* resulted in 78 full (47%), and 87 empty (53%) stomachs. At St. Joseph Atoll, over the scope of two parturition seasons (years 2015/2016 and 2016/2017), 109 gastric lavages in young *C. melanopterus* provided 93 full (85%) and 16 empty (15%) stomachs, leading to a significant bias of stomach fullness with locations (χ^2 = 36.60, p < 0.0001). When separated by USS, the frequency of stomachs containing prey items (increased foraging success) increased from 30% (USS1; n = 17) and 47% (USS2; n = 51) to 51% by USS3 in Moorea (n = 97; Figure 5.7). At St. Joseph Atoll at USS2, 100% of sampled stomachs had prey items in them (n = 8), and 84% of 101 individuals at USS3 had stomachs containing prey (Figure 5.7). The smallest acrylic tubes (2.5 cm outer diameter) were still too large to be used with the smallest individuals from St. Joseph Atoll, resulting in a lack of sampled USS1 individuals.

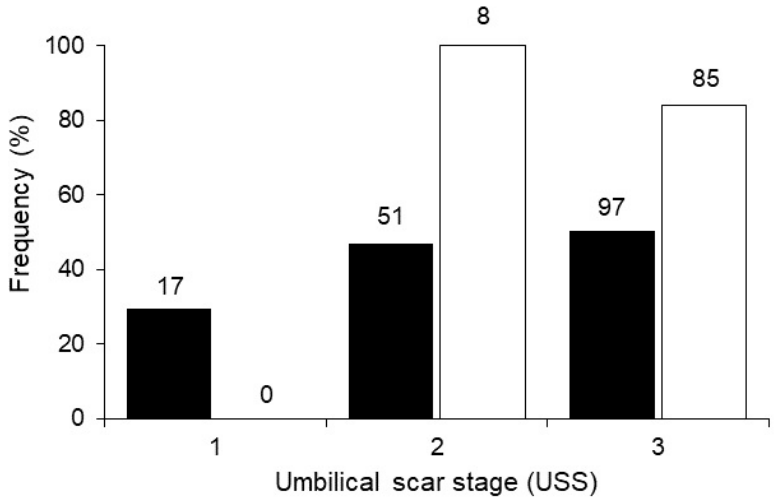


Figure 5.7 Frequency histogram of percentage stomachs containing prey with increasing umbilical scar stage (USS) in *Carcharhinus melanopterus* from Moorea (black, *n* = 165) and St. Joseph Atoll (white, *n* = 109). Numbers above each column represent the total sample size of sharks for a given umbilical scar stage (USS) on which gastric lavages were performed.

5.5 Discussion

This study represents the first non-lethal investigation of body condition and concurrent estimates of autonomous foraging development in young *C. melanopterus* from two isolated shark populations. Our data provide compelling evidence that maternal investment is site-specific, with significantly larger sizes, greater body masses, and larger body condition measurements in Moorea sharks when compared to St. Joseph sharks. Furthermore, our data suggest that, despite this better head start, young sharks in Moorea exhibited significant decreases in body condition and developed foraging habits slower than sharks from St. Joseph Atoll (e.g., fewer than half of the stomachs lavaged from sharks in Moorea had contents during later development stages). These differences in foraging success likely explain the significant decrease in body condition in Moorea sharks, while sharks at St. Joseph Atoll maintained their body condition. Likewise, data from recaptured individuals from Moorea confirm the significant decrease in body condition with increasing time at liberty (up to 72 days). Recaptured individuals that initially had higher body condition indices were most likely to exhibit declines in body condition during the first weeks/months of life.

The fact that we observed larger and heavier neonates with greater mass per unit length and higher condition indices in Moorea versus St. Joseph Atoll suggests that neonates in Moorea are being well provisioned by larger, better conditioned mothers with potentially lower fecundity. Indeed, adult *C. melanopterus* from Moorea tend to be larger (Mourier et al., 2013b) than adult *C. melanopterus* from St. Joseph Atoll (Lea et al., 2016) and are therefore likely to produce larger, heavier and better conditioned young (Hussey et al., 2012). Body size is strongly heritable, and it's also common for geographically separated shark populations of the same species to be genetically and morphologically different (Lombardi-Carlson et al., 2003; Mourier et al., 2013b; Bradley et al., 2017a). Body size, or at least body condition, can further be influenced by a species' diet. Data on natural prey abundance were not collected in either of the two study locations, but provisioning sites in Moorea are numerous, and adult *C. melanopterus* frequent such sites (Kiszka et al., 2016). While direct impacts of provisioning on body condition is either sparsely documented (Maljković & Côté, 2011; Brena et al., 2015) or show minimal impacts on the sharks' diet (Abrantes et al., 2018), provisioned female *C. melanopterus* may benefit from high-trophic level food, which, in turn, is likely to augment maternal investments (e.g., more endogenous energy resources) for their offspring. Further, the exclusive economic zone (EEZ) of French Polynesia banned fishing for *C. melanopterus* in 2006 (Ward-Paige & Worm, 2017). This fishing ban may have helped protect larger and better conditioned females, which in turn give birth to larger, heavier, and better conditioned offspring (Hussey et al., 2010). Lastly, it could be argued that differences in fecundity influence variable pup sizes in Moorea and St. Joseph Atoll. While the estimated average litter size of sharks from Moorea and Aldabra (~ 900 km southwest of St. Joseph) is three pups, Moorea's sharks demonstrate annual reproductive cycles as opposed to biennial cycles in Seychelles (Stevens, 1984; Porcher, 2005; Mourier et al., 2013b). This suggests that, considering the very limited data, fecundity does not explain our findings, because more frequent cycles in Moorea would likely infer smaller pups. While any of these mechanisms alone or in combination could explain the intraspecific variation in the level of maternal investment of female *C. melanopterus*, identifying the specific factors that result in female sharks in Moorea being larger and giving birth to larger, heavier, and better conditioned offspring was beyond the scope of this study.

The rate at which body condition and autonomous foraging success changed as umbilical scars began disappearing varied between Moorea and St. Joseph sharks, suggesting early development may be site-specific for young *C. melanopterus*. Although the rapid decrease of body condition in *C. melanopterus* from Moorea is not surprising, considering documented declines in body conditions in other young sharks (Hussey et al., 2010; Duncan & Holland, 2006; Corsso et al., 2018; Olin et al., 2011), the relatively high maternal investment in Moorea was expected to lead to slower declines in body condition (e.g., due to more energy reserves at birth) and faster foraging development and success compared to sharks from St. Joseph Atoll. Our study, however, demonstrates significant declines in body condition and slower foraging development in sharks from Moorea, therefore suggesting that the quality of the maternal energy investment is not correlated with the foraging success of the young. Other factors, such as environmental conditions, prey resources, variable foraging strategies, and/or anthropogenic stressors are all likely, in some part, to be responsible for the observed differences across sites.

Environmental conditions, such as seawater temperatures, were measured in Moorea and St. Joseph Atoll. Despite significantly lower mean temperatures during pupping seasons in Moorea (29.5 °C ± 0.003) compared to St. Joseph Atoll (30.0 °C ± 0.003), temperature ranges were highly comparable (see Supplementary Information S3). These small differences in mean temperatures lead to standard metabolic rates (SMR; the cost of maintenance metabolism) of 160.5 and 162.7 mg O_2 kg^{-1} h^{-1}, respectively (Bouyoucos, IA, unpublished data). A difference in SMR of 1.4% is, however, negligible in maintenance costs and is therefore likely not responsible for the observed site-specific differences in changes of body condition. However, if ocean temperatures continue to increase, a decrease in body condition during early life-stages may be more pronounced, because higher water temperatures can have decelerating effects on growth (Rosa et al., 2014).

Variable rates of decreasing body condition and foraging development in young sharks may have also been shaped by different levels of inter- and intraspecific competition in young sharks for limited prey resources. Recent studies categorize nearshore areas as resource-limited, a condition that may especially be distinctive in remote areas, where multiple juvenile shark species co-occur and compete for similar prey (Bethea et al., 2004; Kinney et al., 2011; Matich et al., 2017c). Both Moorea and St. Joseph Atoll are inhabited by multiple populations of young sharks (**Chapter 4**;

Matich et al., 2015), therefore, competition is likely to occur at both locations. Indeed, co-occurrence and potential competition in Moorea lead to isotopic niche partitioning between juvenile *C. melanopterus* and sicklefin lemon sharks (*Negaprion acutidens*); yet, body condition as well as growth rates were not affected by the coexisting species (Matich et al., 2017). Even if prey abundances were not quantitatively assessed in any of the two study sites, small reef-associated teleosts [e.g., the predominant prey of young sharks (Stevens, 1984; Newman et al., 2010)] are often observed in St. Joseph Atoll at site of collection (Weideli, OC, personal observation) and in 85% of stomachs investigated. These observations suggest that prey availability at St. Joseph Atoll is sufficient, resulting in potentially weak competitive interactions between young sharks. At Moorea, during gillnet deployments (n = 175) for this study, potential prey species were rarely observed; although this does not prove their absence. Future studies assessing competitive patterns among coexisting shark species and prey availability are, however, needed to draw further conclusions as to why body condition and foraging development during the first weeks of life change at different rates in Moorea and St. Joseph Atoll.

In addition to prey availability, the caloric value of ingested prey as well as foraging strategies may differ between sites. Juvenile scalloped hammerheads (*Sphyrna lewini*) have been reported consuming energetically poor prey (Duncan & Holland, 2006; Lowe, 2002), which may explain the observed decreases in body mass after parturition (Duncan & Holland, 2006). The liver lipids that young sharks in Moorea receive as a maternal headstart are potentially higher in energy compared to their ingested prey. This caloric difference may help explain the body condition decrease as their umbilical scars begin to disappear, similar to the loss of maternal isotopic signals observed in young bull sharks (*Carcharhinus leucas*) and Atlantic sharpnose sharks (*Rhizoprionodon terraenovae*; Olin et al., 2011). On the contrary, small or negligible differences in energetic value of maternal energy resources compared to young sharks' prey may explain the maintained body condition observed in St. Joseph sharks. Low caloric prey may also help to explain how an increase in foraging success from 30% and 47% to 51% of stomachs containing prey (Figure 5.7) can result in decreasing body condition in Moorea sharks. Similar findings have been reported by Hussey et al. (2010), where body condition of neonatal dusky sharks (*Carcharhinus obscurus*) decreased despite increasing stomach content mass (increasing feeding activities). Nonetheless, this is highly speculative, and more stomach items, especially those from

extremely young sharks (e.g., USS1 fresh umbilical scars), as well as the actual caloric value of the stomach contents are needed to better understand the relationship between decreasing body condition despite increasing foraging success.

Prey resources and their caloric value may deteriorate in nearshore areas with substantial anthropogenic impacts (Thiault et al., 2017, 2019). Indeed, the abundance of small reef-associated teleosts is declining through large-scale habitat degradation (Lotze et al., 2006; Jennings et al., 2008), and artisanal fishing (Blaber et al., 2000). Likewise, anthropogenic habitat degradation underpins the declines in the abundance of energetically high-value prey species (e.g., small scarids) with a concurrent increase of low caloric gobies and shrimps in the shallow areas of Kāne'ohe Bay, Hawai'i USA; (USA; Bush, 2003). This transition to lower caloric-value prey is thought to be partially responsible for the declining body mass in *S. lewini* during their first weeks of life (Duncan & Holland, 2006). Anthropogenic stressors, however, can also have direct impacts on young sharks. Increasing temperatures and salinity, for example, allowed young *C. leucas* to expand into formerly uninhabited bays (Bangley et al., 2018) with potentially different prey resources and also into areas where artisanal nearshore fisheries frequently capture young sharks (Glaus et al., 2015, 2018). Even if young sharks are not the target species in artisanal fisheries and are subsequently released, accidental capture events cause stress (Bouyoucos et al., 2018). Young *C. melanopterus*, for example, require at least 8 h recovery after a single accidental gillnet capture event; during this time, about 15% of the energy used for daily swimming is lost (Bouyoucos et al., 2018). Despite enforcement of partially protected areas (no-take zones) around Moorea (de Loma et al., 2008), artisanal fishing is far more likely to occur within the coastal areas of Moorea when compared to near-pristine and uninhabited St. Joseph Atoll, with its uninterrupted reserve boundary (Lea et al., 2016; Ward-Paige & Worm, 2017). Similarly, human activities at Moorea (e.g., boat traffic, boat channel dredging, and shoreline activities) may constrain young shark habitats, with sharks potentially avoiding deeper channels or areas with boat traffic.

The observed relationship between decreasing body condition with increasing USS in Moorea sharks is further supported by data from individual sharks that were captured on multiple occasions. This is, to our best knowledge, the first evidence of a significant decrease of body condition with time at liberty in individual wild sharks (Figure 5.5). Results from such recaptures

also depict that individuals with higher body condition indices (K as well as GF) at initial capture had more pronounced decreases in body condition during the first weeks of life (Figure 5.6). This is analogous to the findings across habitats, in which sharks from Moorea with higher maternal investments were subject to significant decreases in body condition (Figure 5.4 a & c) compared to sharks from St. Joseph Atoll, where such a decline was absent (Figure 5.4 b & d). Since all recaptured individuals at Moorea were exposed to similar environmental conditions (e.g., prey availability, prey quality, and anthropogenic stressors), other factors must have contributed to the within-population differences around Moorea. One plausible answer could be that sharks with higher initial body condition are less driven to start foraging because they can rely on ample endogenous energy resources for an extended period of time. On the contrary, individuals with lower initial body condition are forced to develop foraging skills at an earlier age, hence demonstrating a positive change in body condition between capture events. This is speculative, because unexperienced young sharks are generally considered as asynchronous opportunistic foragers (Newman et al., 2011), and dietary information were not collected from recaptured sharks. Also, body condition is only a proxy that may mask other behavioural or physiological traits that may have influenced our findings. Future work should therefore aim to collect dietary information (e.g., stomach contents or isotopic information) from recaptured sharks to validate changes in body condition between multiple capture events. Finally, prospective studies are recommended to include long-term recaptures to elucidate whether the body condition changes that are observed during early-life stages influence later development stages or if these early body condition changes are negligible for older age-classes.

In conclusion, our findings suggest and support that decreases in body condition within the first weeks of life are common for young viviparous sharks and not only result from natural depletions of maternal energy resources, but will also in some part be affected by prey availability, prey quality, foraging strategies, and/or anthropogenic stressors (Hussey et al., 2010; Duncan & Holland, 2006). Our approach, using two populations of *C. melanopterus*, further enabled us to discriminate between different maternal investments in which young sharks from Moorea with higher maternal energy resources were found to demonstrate significant decreases in body condition and slower foraging development compared to sharks from St. Joseph Atoll. A comparable observation was provided within the Moorea population in which better-conditioned

individuals were subject to a higher loss of body condition. It is therefore expected that young sharks with relatively lower body condition are forced to develop foraging skills at an earlier life-stage, resulting in higher proportions of stomachs containing prey and a positive change in body condition between recaptures. This finding suggests that the habitat quality (e.g., prey abundance and quality) might be especially important for sharks with limited maternal energy resources, and generally for sharks that occur in isolated, nearshore habitats, where deeper surrounding waters or anthropogenically-induced channels impede or prevent dispersal to nearby, potentially prey-rich habitats.

The continued global expansion of human activities (e.g., overfishing, climate change, coastal development, and pollution) poses the greatest risk to reef-associated, shallow water shark species (Dulvy et al., 2014). Therefore, generating site-specific information on early development of reef sharks is critical (Heupel et al., 2019). During these early life-stages, young sharks not only depend on the maternal energy resources, but also rely on these nearshore areas for shelter and/ or to access adequate prey resources. Therefore, to achieve sound conservation measures for *C. melanopterus* and other viviparous reef sharks, management strategies need to come together to effectively protect breeding populations as well as young sharks and their shallow nearshore habitats.

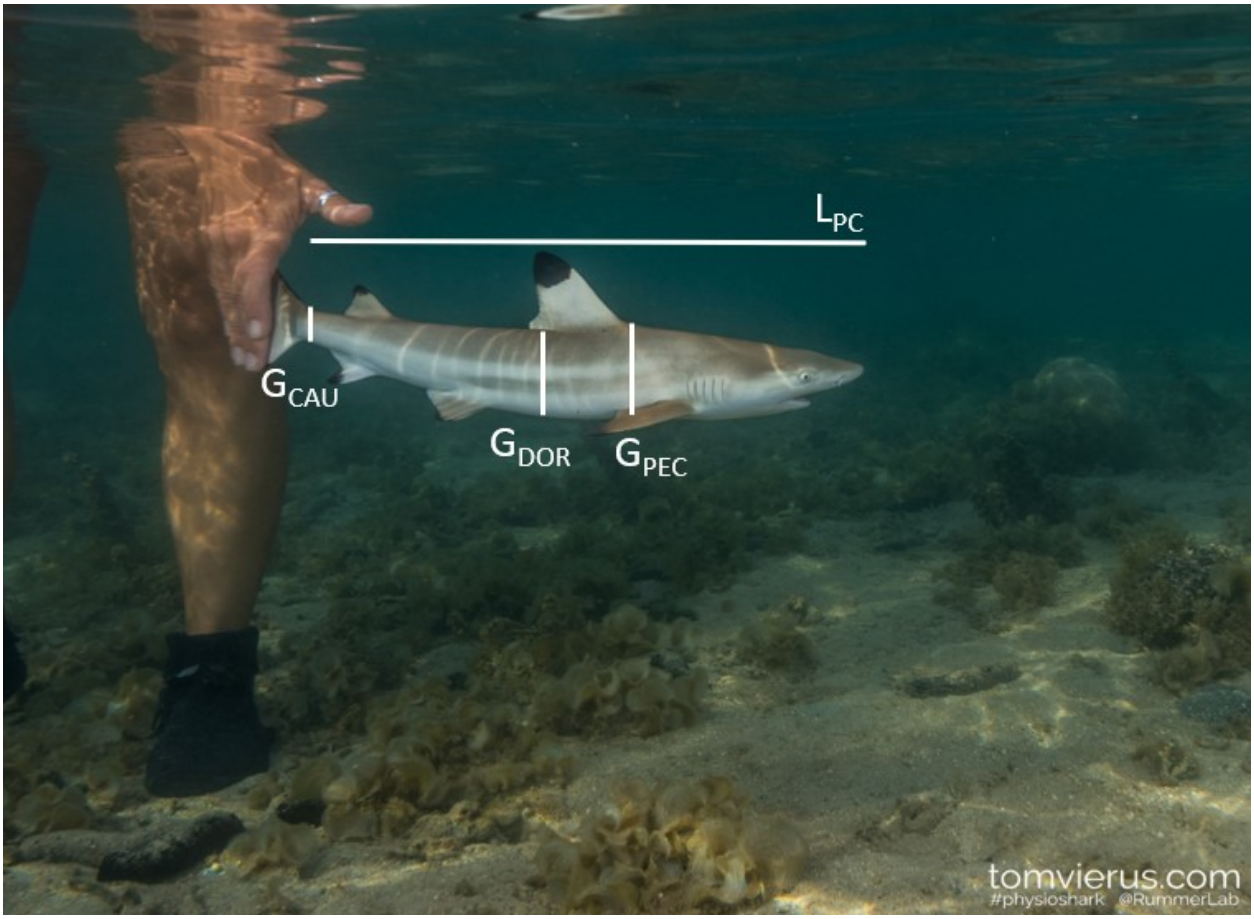
Acknowledgements

This research was gratefully funded by the Save Our Seas Foundation (SOSF Keystone Grant 290) and the Basler Stiftung für biologische Forschung awarded to OCW. IAB was supported by a James Cook University Postgraduate Research Scholarship. JLR was supported by an Australian Research Council (ARC) Early Career Discovery Fellowship [PDE150101266] (2015 - 2017) and the L'Oréal-UNESCO Women in Science Foundation Fellowship (2015 - 2016) concomitantly with an infrastructure and research allocation from the ARC Centre of Excellence for Coral Reef Studies at James Cook University. The authors wish to thank the staff and volunteers involved in this study, especially E. Jacquesson and E. Duncan at the CRIOBE, and R. Daly, C. Daly, C. Boyes, R. von Brandis, and K. Bullock at the SOSF - D'Arros Research Centre.

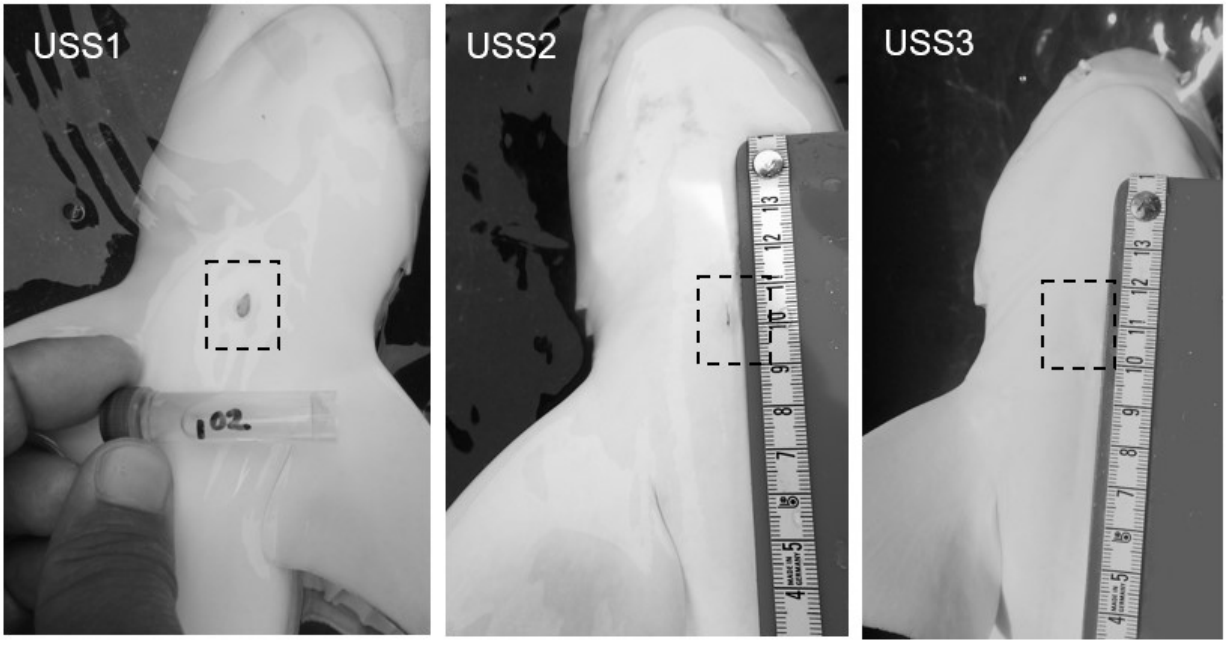
Author contributions

O.C.W. designed and coordinated the study; O.C.W., I.A.B. and J.L.R. collected field data; O.C.W. analysed the data and interpreted the data with Y.P.P. and I.A.B. O.C.W. wrote the manuscript with support and inputs from I.A.B, Y.P.P., G.M., J.L.R. and S.P.; all authors gave final approval for publication. O.C.W., I.A.B., S.P. and J.L.R. secured the funding to support this study.

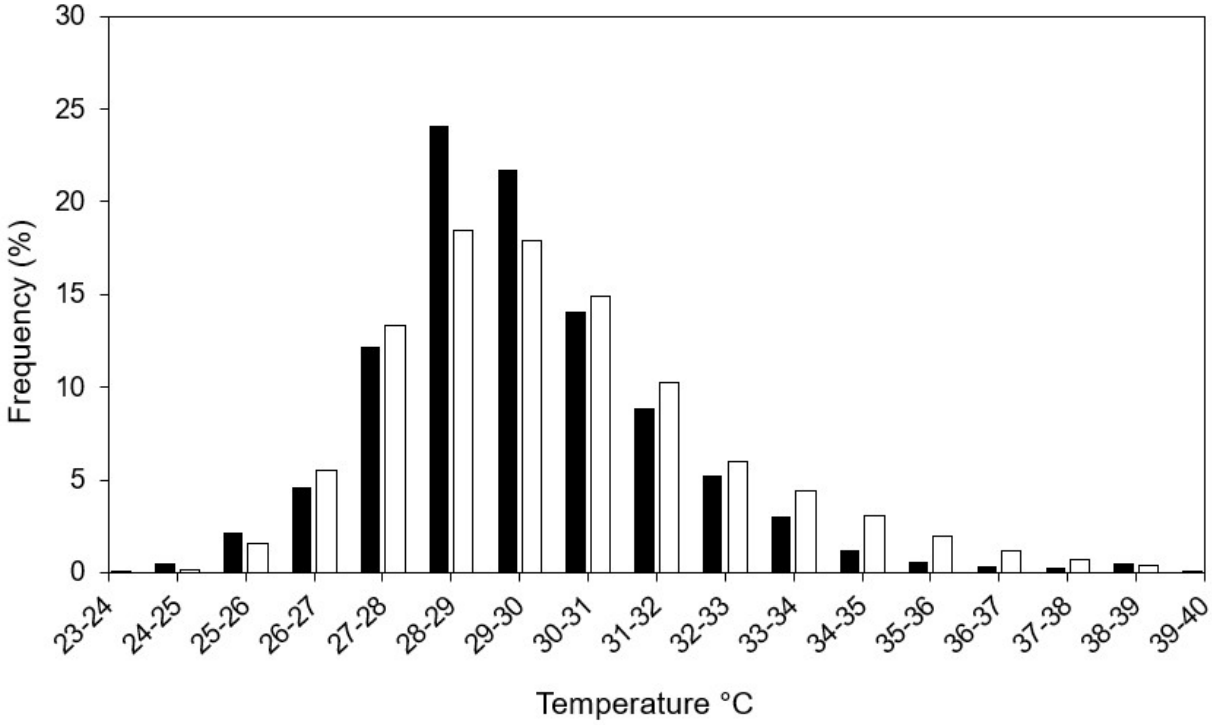
Supplementary information



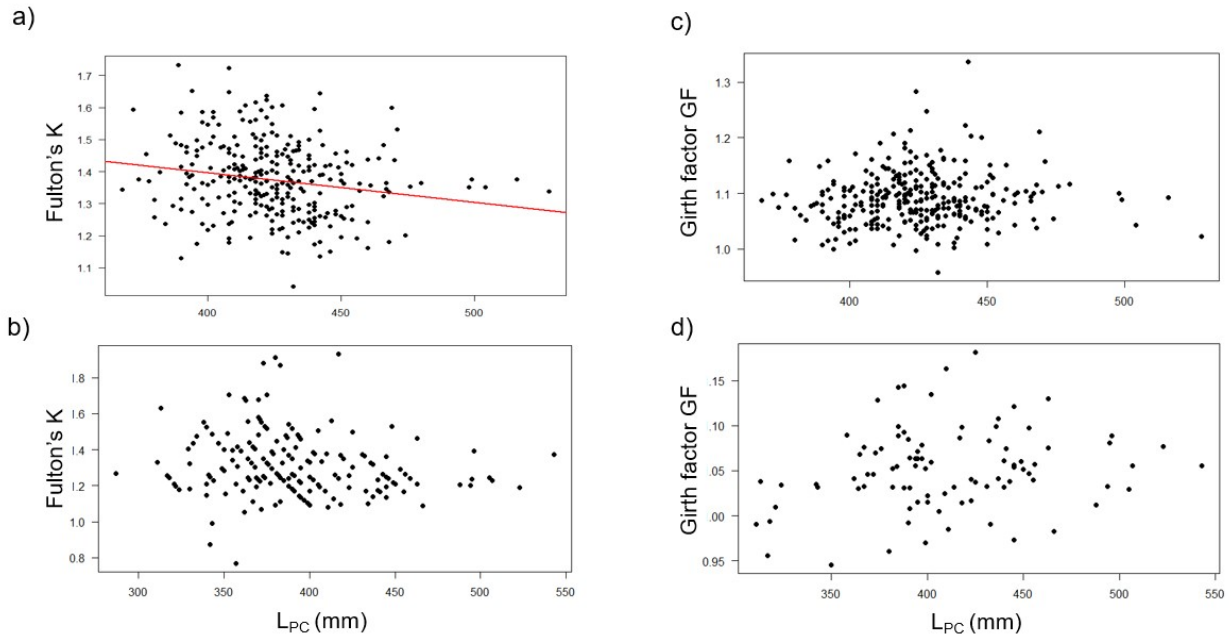
Supplementary Information S1. Location of measurements on juvenile *Carcharhinus melanopterus*: pectoral girth (G_{PEC}), dorsal girth (G_{DOR}), caudal girth (G_{CAU}), and precaudal length (L_{PC}). Picture: Tom Vierus.



Supplementary Information S2. Umbilical scar stages (USS) in young *Carcharhinus melanopterus*. USS1 = open umbilical scar, USS2 = semi-healed scar, USS3 = fully healed scar. Note that no differentiation is made between visible and well-healed scars in USS3. Picture: Ornella Céline Weideli | SOSF.



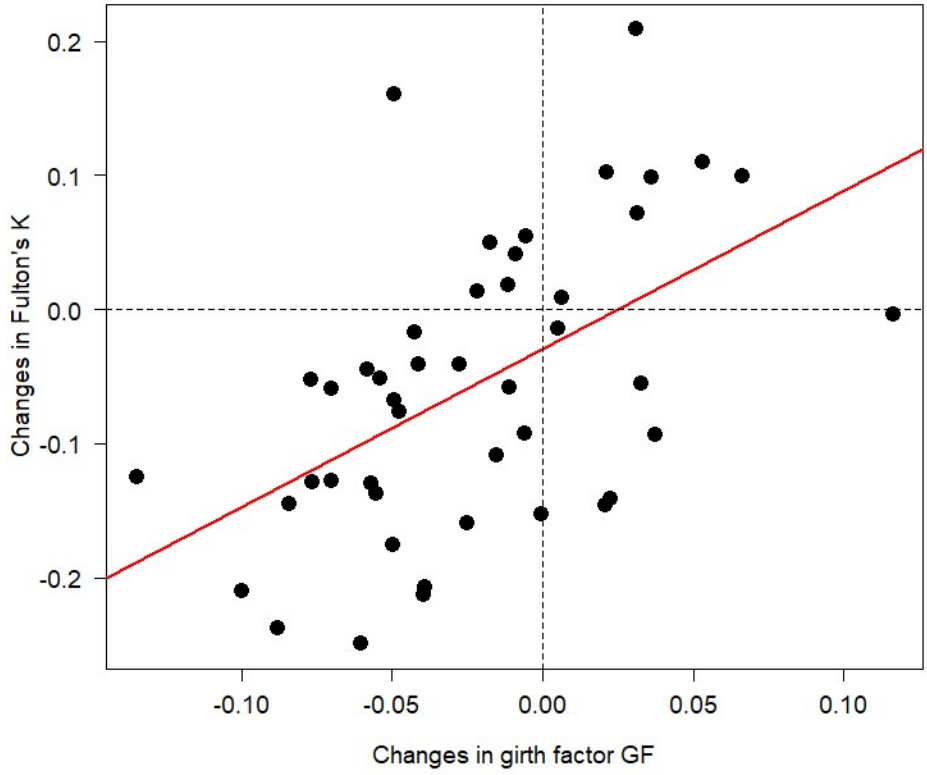
Supplementary Information S3. Percentage frequency histogram of temperature measured during pupping seasons in Moorea (2016/2017 and 2017/2018, black) and St. Joseph Atoll (2015/2016, 2016/2017, and 2017/2018, white). Temperature was measured every fifteen and ten minutes in Moorea and St. Joseph Atoll, respectively.



Supplementary Information S4. Scatterplots of pre-caudal length (L_{PC}) and Fulton's K for young *Carcharhinus melanopterus* from a) Moorea (*n* = 313), and b) St. Joseph Atoll (*n* = 91) and girth factor GF for *Carcharhinus melanopterus* from c) Moorea (*n* = 313), and d) St. Joseph Atoll (*n* = 224). The regression line for predicting changes in Fulton's K with pre-caudal length (L_{PC}) in Moorea is shown in red (y = 1.77 – 0.0009x, r^2 = 0.04).

ID	Sex	Initial capture	Recapture	Time (days)	Δ GF	Δ K
cm66	Male	15-Nov-16	28-Nov-16	13	0.033	-0.056
cm68	Female	16-Nov-16	01-Dec-16	15	-0.047	-0.077
cm79	Male	18-Nov-16	02-Dec-16	14	-0.077	-0.052
cm77	Male	18-Nov-16	02-Dec-16	14	-0.043	-0.017
cm85	Male	20-Nov-16	04-Dec-16	14	-0.017	0.050
cm45	Female	02-Nov-16	04-Dec-16	32	-0.006	-0.093
cm71	Male	17-Nov-16	09-Dec-16	22	-0.025	-0.159
cm111	Male	01-Dec-16	10-Dec-16	9	-0.084	-0.144
cm110	Female	01-Dec-16	10-Dec-16	9	-0.070	-0.059
cm112	Female	01-Dec-16	10-Dec-16	9	-0.009	0.041
cm72	Female	17-Nov-16	23-Dec-16	36	-0.060	-0.249
cm112	Female	10-Dec-16	24-Dec-16	14	0.021	-0.146
cm145	Female	13-Dec-16	03-Jan-17	21	-0.050	-0.176
cm158	Female	22-Dec-16	07-Jan-17	16	0.067	0.099
cm155	Female	22-Dec-16	07-Jan-17	16	0.031	0.209
cm159	Male	22-Dec-16	07-Jan-17	16	0.021	0.102
cm173	Male	07-Jan-17	21-Jan-17	14	-0.057	-0.130
cm160	Female	22-Dec-16	21-Jan-17	30	-0.041	-0.041
cm152	Male	19-Dec-16	27-Jan-17	39	-0.011	0.018
cm192	Male	08-Feb-17	20-Feb-17	12	0.005	-0.014
cm192	Male	20-Feb-17	24-Feb-17	4	0.006	0.009
cm190	Male	08-Feb-17	28-Mar-17	48	-0.050	0.161
Cm2	Female	26-Sep-17	08-Oct-17	12	-0.135	-0.124
Cm31	Female	26-Oct-17	13-Nov-17	18	-0.011	-0.058
Cm26	Male	26-Oct-17	13-Nov-17	18	-0.015	-0.109
Cm53	Male	02-Nov-17	14-Nov-17	12	-0.022	0.014
Cm41	Male	30-Oct-17	20-Nov-17	21	-0.050	-0.068
Cm65	Female	07-Nov-17	23-Nov-17	16	-0.039	-0.213
Cm46	Male	02-Nov-17	27-Nov-17	25	-0.001	-0.153
Cm61	Male	06-Nov-17	06-Dec-17	30	-0.055	-0.137
Cm62	Male	06-Nov-17	07-Dec-17	31	-0.070	-0.128
Cm58	Female	06-Nov-17	07-Dec-17	31	-0.100	-0.210
Cm43	Male	31-Oct-17	18-Dec-17	48	0.023	-0.141
Cm102	Male	06-Dec-17	22-Dec-17	16	-0.006	0.055
Cm106	Male	06-Dec-17	22-Dec-17	16	0.036	0.098
Cm101	Female	06-Dec-17	22-Dec-17	16	0.116	-0.004
Cm107	Female	06-Dec-17	22-Dec-17	16	0.037	-0.093
Cm57	Female	06-Nov-17	28-Dec-17	52	-0.088	-0.237
Cm116	Male	18-Dec-17	29-Dec-17	11	0.053	0.110
Cm41	Male	30-Oct-17	10-Jan-18	72	-0.039	-0.207
Cm116	Male	29-Dec-17	15-Jan-18	17	-0.077	-0.129
Cm112	Male	08-Dec-17	16-Jan-18	39	-0.054	-0.051
Cm99	Female	06-Dec-17	17-Jan-18	42	-0.058	-0.045

Supplementary Information S5. Overview of 45 neonatal *Carcharhinus melanopterus* from Moorea that were recaptured within the same parturition season (minimum 4 and maximum 72 days). Values demonstrate the difference (Δ) in body condition indices between initial capture and recapture. GF: Girth factor; K: Fulton's K.



Supplementary Information S6. Changes in Fulton's K versus changes in girth factor GF in neonatal *Carcharhinus melanopterus* from Moorea. Data were obtained from 45 sharks that were measured twice within the same parturition season (minimum 4 days, maximum 72 days). The regression line for predicting changes in Fulton's K from changes in GF is shown in red ($y = - 0.03 + 1.18x$, $r^2 = 0.28$).

Chapter 6 | General Discussion

6.1 Summary and synthesis of main results

6.1.1 Overview

The overreaching aim of this thesis was to improve our understanding about the underlying mechanisms that enable and affect the coexistence of ecologically and morphologically similar shark species. Although sharks have been the subject of numerous studies that have investigated trophic, spatial, or temporal niche pattern across coexisting species, only a few have assessed more than one niche dimension (**Chapter 1**). To date, there have been no studies that linked niche pattern with competition effects, population characteristics, and fitness-related traits in sharks, consequently the origin and the potential effects of niche pattern (segregation and overlap) are unclear (**Chapter 1**). This thesis used multiple and innovative methods (mark-recapture sampling, active tracking, stable isotope analysis [SIA], stomach content analysis, and competition experiments) on sympatric juvenile *C. melanopterus* and juvenile *N. acutidens* to help fill these knowledge gaps, while also extending previous findings on preservation effects for blood stable isotopes (**Chapter 2**). This thesis primarily showed that competition effects (i.e., dominance hierarchies) contribute to niche pattern, but the extent will vary depending on which niche axes are considered (**Chapter 3**). Further, segregation patterns were expected to have varying consequences on sympatric shark species in the long-term (**Chapter 1**), but population characteristics and fitness-related traits were similar across species, and were indicative of healthy shark populations (**Chapter 4**). By using Moorea as an additional study location, I provided novel insights on sharks' early foraging developments, and demonstrated considerable intraspecific differences across study sites. Such intraspecific differences are likely caused by varying levels of prey availability, intraspecific competition, and anthropogenic impacts (**Chapter 5**). Although subsequent investigations will be needed to determine a complete picture of the underlying mechanisms of coexistence in sharks, this thesis extends and improves previous knowledge and has important ecological and management implications for the conservation of sympatric juvenile shark populations.

6.1.2 Effects on blood stable isotopes

The motivation to measure the effects of anticoagulants on stable isotope values of blood components was primarily based on the increased use of stable isotopes in elasmobranch research

(Vander-Zanden et al., 2015), as well as on my intentions to use SIA as part of the trophic niche investigation (**Chapter 3**). Despite the range of tissues that can be used for SIA (Hussey et al., 2012), blood components (red blood cells [RBC]) and blood plasma) provide the most recent insight into trophic interactions and are especially useful for studying the trophic ecology of young sharks (McMeans et al., 2009). Freshly drawn blood, however, coagulates quickly, thus blood samples are often treated with anticoagulants. As preservation effects of anticoagulants on stable isotope ratios vary among taxa and blood components (Bugoni et al., 2008; Kim & Koch, 2011; Lemons et al., 2012), further research was needed.

I've tested if commonly used anticoagulants (EDTA and SH) have the ability to bias blood stable isotopes to improve the accuracy and reliability of my data. My data suggest that RBC and plasma isotope values were highly similar in no-additive control samples and samples treated with the EDTA and SH (**Chapter 2**). The only significant differences between control and treated samples were found in RBC $\delta^{15}N$ values treated with either EDTA or SH, but differences were very small (0.0873‰ and 0.2018‰, respectively; **Chapter 2**). In line with previous studies (Kim & Koch, 2012), my study thus supports the use of anticoagulants for SIA, but the limited sample size (n = 10) may have resulted in smaller treatment effects and high variability in mean differences between control and treated plasma samples. Consequently, and as a precaution, only non-additive blood samples were used for isotopic analyses within the scope of this thesis (**Chapter 3**). I encourage future research on preservation effects because anticoagulants can considerably alleviate and simplify fieldwork procedures (i.e., no need for immediate centrifugation in the field).

6.1.3 Niche pattern and competition effects

In coexisting species, niche segregation is expected to alleviate the effects of interspecific competition (Gause, 1934; Pianka, 1974), and can potentially be accomplished through spatial and temporal avoidance, or variable prey selection (Schoener, 1968; Alatalo et al., 1987; Barlow et al., 2002; Wilson, 2010). Without knowledge on the competitive abilities (e.g., dominance hierarchies) of similar sympatric species, it is, however, unclear if segregation patterns result from competition or from other unknown factors (**Chapter 1**). Yet, it is difficult to study the complete range of possible factors that could influence sharks' niche pattern, due to their natural environment and elusive nature. Juvenile reef sharks that coexist in shallow nearshore areas, however, allowed me

to not only assess multiple niche dimensions, but their amenability to laboratory conditions (Chin et al., 2015; Bouyoucos et al., 2018) also enabled me to conduct dominance behaviour experiments in captivity.

This complementary study approach provided a rich source of information showing that competition effects contribute to niche segregations (**Chapter 3**), similar to a recent study on sympatric adult reef sharks (Papastamatiou et al., 2018). Daily space use, however, appeared to overlap across species, suggesting that risk effects (i.e.; predator-avoidance strategies) and tidal fluctuation can greatly influence the daily spatial use of juvenile sharks (Guttridge et al., 2012; George et al., 2019). Cumulatively, this study highlighted that segregation patterns can result from competition, but factors other than competition must also be considered. As such, my findings support a previous study that questioned competition as the sole driving force behind niche segregation (Wilson, 2010). Overall, using sympatric juvenile reef sharks to improve our understanding about the driving forces behind niche patterns was a successful approach, but further research will be needed to advance our understanding of these complex ecological interactions.

6.1.4 Population characteristics and fitness-related traits

Asymmetric niche pattern, if they persist over time, can have varying consequences for fitness-related traits (Martin & Martin, 2001; Munday, 2001; Eccard & Ylönen, 2003). For example, growth rates in coral-dwelling fish *Gobiodon brochus* are significantly reduced if *G. brochus* coexist with the dominant competitor *G. histrio* (Munday, 2001). Despite its importance for studying ecological interactions, such methodological approaches cannot be applied to large-bodied animals such as sharks (**Chapter 1**). Thus, there was an incentive to investigate this subject in juvenile sharks, as during early life-stages they are amenable to captivity (Chin et al., 2015; Bouyoucos et al., 2018).

The dominance behaviour experiments in captivity provided evidence to suggest that juvenile *N. acutidens* are dominant over juvenile *C. melanopterus* (**Chapter 3**). Consequently, negative fitness-related consequences were expected for the subordinate species, similar to other studies on fish and birds (Martin & Martin, 2001; Munday, 2001). My three-year mark-recapture approach, however, did not find negative fitness-related consequences in the subordinate species (**Chapter**

4). In contrast, data suggested that St. Joseph Atoll is an essential habitat contributing to the life cycle of both shark species. Furthermore, growth rates were found to be highly variable with some individuals growing over 20 cm per year, while others less than 5 cm per year. Surprisingly, these highly variable growth rates were found in both, the dominant and the subordinate species (**Chapter 4**). One possible explanation for the highly variable growth rates in both species is intraspecific competition together with high genetic variance. Atoll ecosystems are presumably oligotrophic habitats, thus intraspecific competition for limited prey resources is likely. Another likely explanation for highly variable growth rates are individual differences in personality traits (Finger et al., 2016). Risk-prone individuals with high-reward behaviour may use productive but dangerous areas resulting in fast growing individuals. In contrast, smaller and less bold sharks may predominantly occur in sheltered, but less productive areas, resulting in slower growing individuals (Hussey et al., 2017).

It is relevant to note that, although I was not able to find negative trends in fitness-related traits in *C. melanopterus*, this does not prove its absence. For this to be proven, growth rate estimates of *C. melanopterus* from areas with and without *N. acutidens* would have been needed. Although the habitat distribution was semi-truncated (*N. acutidens* used a smaller portion of available areas than *C. melanopterus*, Holmgren 1995, **Chapter 3**), growth rate data for *C. melanopterus* from areas without *N. acutidens* were too limited ($n = 5$) to further investigate this subject. Nevertheless, and despite our efforts to unveil fitness-related consequences of asymmetric niche pattern, my three-year mark-recapture approach is the longest and most comprehensive assessment of juvenile sharks from the western Indian Ocean and consequently provides new and suitable baseline data on which future studies can be built upon.

6.1.5 Foraging development

Intraspecific differences in population characteristics and fitness-related traits have been the focus of a number of studies in adult sharks of the family Carcharhinidae, but fewer studies have characterised intraspecific differences among geographically separated populations of juvenile sharks (**Chapter 1**). While the focus of such studies primarily lays on variable sizes at birth (e.g., Barker et al., 2005), it is unclear if early foraging developments may also vary within species from

different habitats. Therefore, there was an incentive to not only investigate foraging developments in juvenile sharks, but also to estimate whether they vary within species from different locations. This study compared *C. melanopterus* from St. Joseph Atoll with the multi-species aggregation at Moorea. By aiming at different early life-stages (USS 1 – 3) of *C. melanopterus*, I was able to compare early foraging developments and associated changes in body condition. Results provided evidence that *C. melanopterus* from St. Joseph Atoll, although smaller and lighter at birth, were faster to initiate foraging skills (85% of stomach contained prey) and demonstrated no significant decrease in body conditions as opposed to their conspecifics at Moorea (**Chapter 5**). These contrasting results were further supported by significant declines of body condition with time in 45 recaptured individuals at Moorea. Interestingly, the recaptured individuals that were initially better conditioned, demonstrated the highest declines in body condition (**Chapter 5**).

Whether contrasting early foraging developments between locations are due to differences in prey availability, prey quality, environmental factors and/or anthropogenic stressors is difficult to conclude because I did not specifically measure any of these factors. Thus, I can only speculate that the near-pristine conditions of St. Joseph Atoll enable natural conditions that positively affect juvenile sharks, similarly to what has been found in **Chapter 4**. Further, it is difficult to conclude why individuals with the highest initial body condition showed the highest body condition declines. One plausible answer could be that sharks with higher initial body condition are less driven to start foraging because they can rely on larger endogenous energy resources for an extended period of time. On the contrary, individuals with lower initial body condition are forced to develop foraging skills at an earlier age, hence demonstrating a positive change in body condition between capture events. To conclude, **Chapter 5** not only enabled novel insights on sharks' early foraging developments but allowed the characterization of intraspecific differences among geographically separated shark populations, highlighting species and habitat-specificity of juvenile shark populations.

6.2 Implications

6.2.1 Ecological implications

One of the main findings of this thesis indicated that sympatric juvenile *C. melanopterus* and *N. acutidens* differ in spatial and trophic niche dimensions as a likely consequence of competition (**Chapter 3**). Consequently, the ecological implications and roles within nearshore areas are likely different for each of the species (Bascompte et al., 2005). *Carcharhinus melanopterus* at St. Joseph Atoll were captured over a larger proportion of the sampling area than *N. acutidens*, confirming findings from Australia where *C. melanopterus* exhibit lower residency and broader movements compared to *N. acutidens* (Oh et al., 2017). Moreover, the dietary breadth of *C. melanopterus* was wider (B_A: 0.21, H': 0.75) and composed of a higher diversity of crustaceans, molluscs, and teleost species compared to *N. acutidens* (B_A: 0.12, H': 0.58, **Chapter 3**). According to these species-specific differences it is likely that *C. melanopterus* not only have a different role of structuring and shaping prey communities (Bascompte et al., 2005), but *C. melanopterus* might respond to environmental changes or anthropogenic disturbances in a different manner than *N. acutidens*.

One of the major anthropogenically related threats to juvenile sharks in nearshore areas, besides fishing, is the reduction of prey resources either through habitat developments or fishing practices (Jennings et al., 2008; Stump, 2013). Previous studies suggested species with wider resource niches are able to maintain a higher level of fitness in unstable environments and will not be as greatly affected by exposure to environmental changes and anthropogenic impacts as highly specialized species (Richmond et al., 2005; Munroe et al., 2014). Juvenile *C. melanopterus* may thus be more resilient to resource declines and anthropogenic disturbances compared to juvenile *N. acutidens*. A species' feeding strategy, however, can vary in response to environmental fluctuations and different theoretical predictions as to how species would respond to relative food scarcity exist (Figure 6.1; Perry & Pianka, 1997; Correa & Winemiller, 2014). Optimal foraging, for example, suggests that animals forage more opportunistically in relatively poor conditions, and are more specialized in areas of high resource abundance (Perry & Pianka, 1997). Consequently, if coexisting juvenile sharks, such as *C. melanopterus* and *N. acutidens* both start foraging more opportunistically and become more generalist with decreasing prey resources, dietary overlap may result in higher interspecific competition (Figure 6.1, Hypothesis 1: optimal foraging). Conversely,

competition theory predicts that in deteriorating environmental conditions, species will specialize and consume fewer alternative resources (i.e., reduction in trophic niche breadth), aiming to reduce interspecific dietary overlap and ultimately competition (Figure 6.1, Hypothesis 2; Pianka, 1974; Schoener, 1974; Barros et al., 2017).

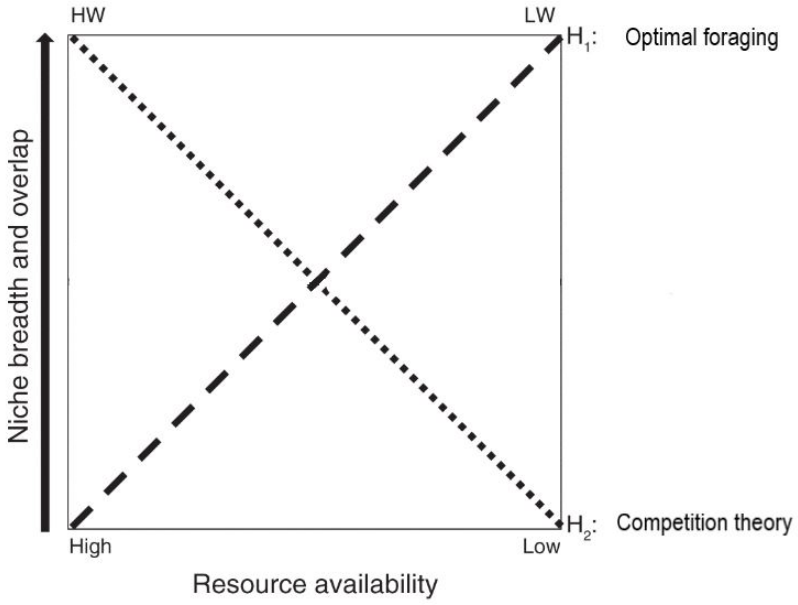


Figure 6.1 Divergent prediction for changes in dietary breadth and interspecific overlap in response to resource availability. Hypothesis 1: Optimal foraging theory, Hypothesis 2: Competition theory (Figure adapted and modified from Correa & Winemiller, 2014).

Predicting niche adaptations of sympatric juvenile sharks in response to changing environments is challenging, especially because foraging behavior is species-specific with some species expanding and others contracting dietary niches in relation to decreased food availability (Correa & Winemiller, 2014). Data from this thesis suggest that in highly restricted areas such as the St. Joseph Atoll, predator-avoidance strategies and tidal fluctuations may constrain movements of sympatric shark species within shallow habitats (**Chapter 3**), thus limiting the potential for spatial segregation for both species. Similarly, sharks investigated within the scope of this thesis were all young and inexperienced, thus it is questionable to what extend and at what rate sympatric juvenile sharks would be able to further reduce dietary overlap in response to declining prey resources. Overall, findings from St. Joseph Atoll provide evidence to suggest that sympatric and closely

related juvenile *C. melanopterus* and *N. acutidens* are likely limited in reducing niche overlap, thus patterns consistent with optimal foraging theory are to be expected if environmental conditions decrease (Figure 6.1, Hypothesis 1). Long-term investigations on sympatric juvenile *C. melanopterus* and *N. acutidens* from areas with variable levels of prey abundance could provide further insights about the potential consequences declining prey resources may have on species' niche overlap.

6.2.2 Conservation implications

The comprehensive data collected within this thesis provide important insight on species-specific resource use, population characteristics, and fitness-related traits that are key for planning thorough conservation management strategies (Heithaus, 2007). More precisely, this thesis highlighted that both juvenile shark populations occurred in high abundances, used the St. Joseph Atoll for an extend time of up to 2.5 years, showed limited dispersal distances, and were repetitive recaptured within the intertidal reef flats (**Chapter 4**). Furthermore, data from this thesis showed that juvenile sharks considerably increase foraging activities during the first weeks of life (**Chapter 5**). While such findings not only add important information to the limited understanding of sharks' early foraging developments (Hussey et al., 2010), they strongly support the importance of St. Joseph Atoll as an essential habitat providing ample prey resources for two coexisting juvenile shark populations.

To protect the near-pristine St. Joseph Atoll in the long-term, St. Joseph Atoll together with the neighbouring D'Arros island were recently declared as a designated Special Reserve (International Union for Conservation of Nature, IUCN Category 1a) with a no-take zone extending 1 km from the low-tide mark (Insert in Figure 6.2; Lea et al., 2016). This MPA will consequently be highly beneficial for juvenile *C. melanopterus* and *N. acutidens*, because the small-scale MPA (~53 km^2) completely overlaps with their distribution (**Chapter 3 & 4**). Moreover, this MPA will provide long-term protection for both juvenile populations, because both species are known to exhibit site fidelity to their nursery areas (Mourier & Planes, 2013; Chapman et al., 2015). However and despite of these benefits, a comprehensive protections is not guaranteed with this MPA, because adult sharks (especially adult *N. acutidens*) frequently occur outside the MPA's boundaries (Filmalter et al., 2013; Lea et al., 2016) where they may be targeted by fisheries (Temple et al.,

2017). To achieve an improved protection to all age-classes of sharks occurring around St. Joseph Atoll, MPA management strategies need to be couple with broader fisheries management strategies to reduce mortality of older and wider-ranging age-classes (Kinney & Simpfendorfer, 2009).

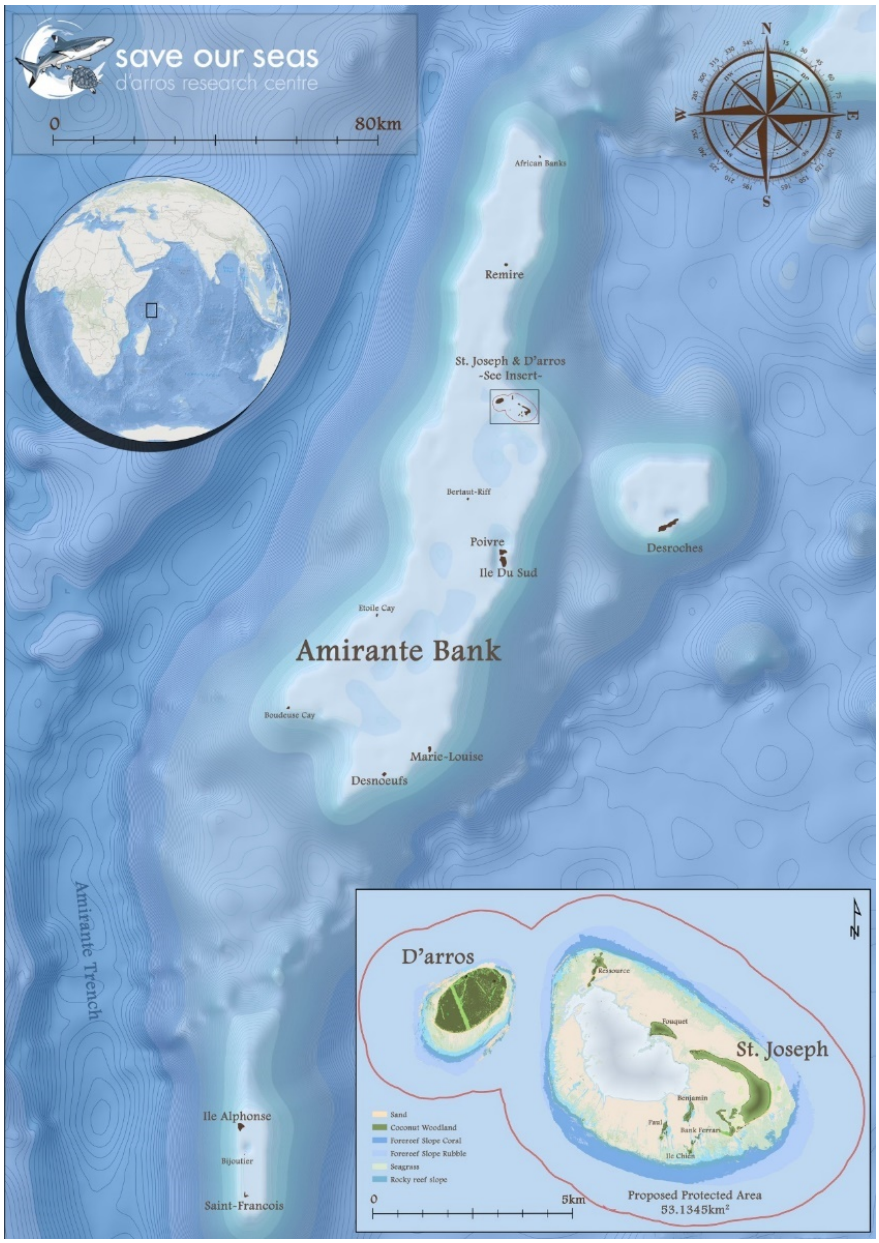


Figure 6.2 Map showing the St. Joseph Atoll located in the Amirantes Bank within the Outer Islands of the Republic of Seychelles in the West Indian Ocean. Insert indicates the designated marine protected area (MPA) with boundaries at 1 km from the high-tide mark (boundaries marked in red). Cartography by William Ruzek © Save Our Seas Foundation Copyright.

6.3 Future research directions

The research conducted in this thesis is the first to examine and link multiple niche dimensions, competitive abilities, population characteristics, and fitness-related traits in sympatric juvenile sharks. As such, there are numerous knowledge gaps on which future research could be built upon.

From a methodological standpoint, a key area for future work on sympatric sharks is long-term spatial investigations. Due to the very shallow water depths (< 50 cm), frequently used methods such as passive acoustic tracking, were impeded at St. Joseph Atoll. A passive acoustic receiver array placed in deeper areas of the atoll (where receivers are not exposed to air during neap low tide) could have potentially enabled a long-term assessment. It is, however, questionable if juvenile sharks would have been near such areas, as they tend to avoid areas of greater depths (**Chapter 3**; George et al., 2019). An ancillary approach to gain long-term data could be the use of GPS tracking, similar to what has been conducted in juvenile stingrays (Martins et al., 2019). While small GPS tracker provide highly accurate spatial data (Martins et al., 2019), there are multiple concerns related to the use of this method in juvenile sharks. The sharks' movements are fast and the towed floats with the GPS tracker could impede their movements, while fringing mangroves or shrubs could get entangled with the tracking device. Nevertheless, with regards to the specific environmental conditions at St. Joseph Atoll that exacerbate common methods to investigate spatial analysis, manual active tracking was possibly the most appropriate and least invasive method to study short-term movements of sympatric juvenile sharks (**Chapter 3**). Manual active tracking was further interesting because it allowed measurements of ARS behaviour, depths and temperature; data that could not have been be collected with other methods. Future studies investigating space use in sympatric juvenile sharks, could, however, benefit from a higher tracking effort, longer duration of tracks, and potentially extending tracking into night times.

The diet in this thesis was studied with two complimentary methods: stomach content analysis and stable isotope analysis (**Chapter 3**). Although validating dietary patterns by two dietary methods considerably extends the understanding of a species' diet (Dale et al., 2011; Kinney et al., 2011), both methods have advanced over recent years and have become more specific with regards to the level of identification (e.g., compound-specific amino acid stable isotope analysis and meta barcoding, respectively; Dale et al., 2011; Matley et al., 2018). Thus, it may be worth applying

those methods in future studies on the trophic ecology of sympatric sharks. Moreover, dietary calculations were solely based on % occurrence (%O) data, without additional information on number of prey and gravimetric data, which are common metrics in dietary investigations to calculate the index of relative importance (IRI; Pinkas et al., 1971; Dale et al., 2011). Future diet studies are thus strongly encouraged to aim at collecting all of these metrics, however, if gastric lavages is continued to be used, there is always the probability of inefficient stomach content removal that bias results (Bangley et al., 2013).

Assessing prey availability enables essential insights about sharks' foraging selectivity (Gutteridge et al., 2011; Munroe et al., 2014), however, due to time and logistical constrains, prey were not sampled within the scope of this thesis. Sampling methods to capture prey species include seine nets, trawls, gillnets, fish traps or baited remote underwater video surveys (BRUVS; Heupel & Hueter, 2002; Newman et al., 2010; Gutteridge et al., 2011) and are worth exploring in future studies. Moreover, predatory risk effects are essential factors because they have the ability to modify spatio-temporal pattern, feeding activity, and body condition of its prey (Heithaus & Dill, 2002; Heithaus et al., 2007; Barley et al., 2017). Risk effects in this study were solely based on previous publications (Filmalter et al., 2013; Lea et al., 2016) and field observations, but future research should quantify these effects. One possible approach could be to actively track adult sharks. An external attachment of an acoustic tag connected to a floating device (similar to Speed et al., 2013) would not only enable active acoustic tracking in the deeper lagoon, but the floating device would allow visual tracking in shallow areas where predator-prey interactions are to be expected.

Finally, logistical issues related to severe weather conditions in Moorea impeded my efforts in reaching more replicates for my dominance behaviour experiments (**Chapter 3**). While an increased numbers of competition experiments would have improved the significance and credibility of our results, thorough and more elaborated experimental protocols will also be needed to further our understanding of competitive abilities in juvenile sharks. Overall, these advances could be applied to address the remaining questions. For example, although this thesis was able to improve and extend our understanding of the underlying mechanisms of coexistence, I was unable to prove that segregation pattern were exclusively driven by competitive interactions across

species. Multi-dimensional niche investigations (gillnet sampling, active tracking, and dietary investigations) were executed within the scope of this thesis, but long-term data from different microhabitats (i.e.; from mixed and mono-species area) would be needed to appropriately investigate this approach. Analogously, despite the relatively large number of recaptures that provided growth rate estimates for both species, growth data when divided into mixed and mono-species areas were limited, thus long-term effects of competition on fitness-related traits could not be revealed within the scope of this thesis. Finally, although the thesis' findings may be locally beneficial (i.e., for MPA designation), future research should focus on different nearshore habitats to determine if juvenile sharks' behaviours are similar when they are not constrained by such isolated and restricted habitats. Nevertheless, this thesis provides the first ever multi-dimensional niche investigation that additionally demonstrated insight on competitive effects, population characteristics, and fitness-related traits. As such, my thesis demonstrates an important and crucial step towards an increased understanding on the underlying mechanisms of coexistence, specific to juvenile reef sharks from tropical nearshore areas.

Author contribution

O.C.W. wrote the original draft; O.C.W. edited subsequent drafts with support from, and under the supervision of Y.P.P. and S.P.

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RÉSUMÉ

Dans les zones de sympatrie, les espèces étroitement apparentées et morphologiquement similaires sont censées d'occuper des niches écologiques différentes afin d'éviter la concurrence. Cependant, sans connaissance des capacités de concurrence (e.g., les hiérarchies de dominance) et des caractéristiques des populations (e.g., les taux de croissance, caractéristiques d'adaptation), l'origine et les effets potentiels du modèle de niche (ségrégation et chevauchement) ne sont pas connus. J'ai trouvé des preuves convaincantes que les juvéniles des requins à pointes noires *Carcharhinus melanopterus* et les juvéniles des requins citron faucille *Negaprion acutidens* de l'atoll de Saint-Joseph, Seychelles, présentent des schémas de ségrégation à petite échelle pour éviter la concurrence. En revanche, de légères différences dans les caractéristiques de la population étaient plus susceptibles d'être causées par la disponibilité des proies, la concurrence intraspécifique et les impacts anthropiques. Finalement cette thèse met en évidence la pertinence d'études multidisciplinaires pour dévoiler les mécanismes sous-jacents de la coexistence.

MOTS CLÉS

Carcharhinus melanopterus, caractéristiques des populations, compétition, coexistence, *Negaprion acutidens*, niches écologiques.

ABSTRACT

In areas of sympatry, closely related and morphologically similar species are expected to occupy different ecological niches in order to avoid competition. However, without knowledge on competitive abilities (e.g. dominance hierarchies) and population characteristics (e.g. growth rates, fitness traits), the origin and the potential effects of niche pattern (segregation and overlap) are unknown. I found compelling evidence that juvenile blacktip reef sharks *Carcharhinus melanopterus* and juvenile sicklefin lemon sharks *Negaprion acutidens* from St. Joseph Atoll, Seychelles exhibit fine-scale segregation patterns as a means to avoid competition. In contrast, slight differences in population characteristics were more likely to be caused by prey availability, intraspecific competition, and anthropogenic impacts. This thesis highlights the need for multi-disciplinary investigations to unveil the underlaying mechanisms of coexistence.

KEYWORDS

Carcharhinus melanopterus, competition, coexistence, ecological niches*, Negaprion acutidens*, population characteristics.