Assessing the morphological trends of juvenile populations of two threatened carcharhinid shark species at St. Joseph Atoll, Seychelles

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Introduction

Sharks belong to the class Chondrichthyes and play crucial roles in marine ecosystems, acting as the primary ecological stabilizer (Dibattista et al., 2007; Dulvy et al., 2014; Heupel et al., 2014). Their slow growth, late maturation, low fecundity and long reproductive cycles render them highly vulnerable to anthropogenic threats with many species undergoing population declines (Field et al., 2009; Dulvy et al., 2014). The quantity of energy reserves accumulated and stored throughout the early stages of life is recognized to be strongly connected to the health, growth, and general development of sharks (Hodgkiss et al., 2017). Predation risk, parasitic infestation, and environmental conditions can all have an impact on an animal's health (Hussey et al., 2009). There is no parental care provided to juvenile sharks (Schlaff et al., 2020) and consequently, they rely heavily on prey availability and habitat suitability which, in turn, contribute to their foraging success, overall condition and fitness.

Condition indices are used to assess the health of animals and to evaluate how life-history strategies, ecological interactions and environmental threats affect them. Some of the most commonly used indices, such as condition factor (CF), are calculated from an animal's morphological measurements and have been used extensively to assess condition in fish (e.g. Lizama and Ambrosio, 2002; Mozsar et al., 2015; Contreras-Reyes, 2016; de Vries et al., 2020). Relatively few studies, however, have utilized body condition metrics to assess condition in sharks. Hussey et al. (2009) used multiple condition indices to assess condition in dusky sharks. Elias et al. (2004), Hodgkiss et al. (2017) and Rapi et al. (2020) all used CF to assess tope sharks, juvenile lemon sharks and grey reef sharks respectively.

Investigating the factors that influence condition and health in sharks, especially at key life stages, can help to better understand changes in their population dynamics, fitness and rates of survival, for example.

Various factors could play a role in driving variations in body condition. In some species of shark, gender-specific variation in growth rates have been reported (Klimley, 1987; Parsons, 1993) which may correlate with variations in condition. Further, as juvenile sharks must commit energy resources to their growth and development, potential differences in the commitment of energy reserves to gonad development could influence sex-based differences in condition. Sharks are ectothermic, and temperature affects metabolic efficiency, foraging patterns and movements in sharks (Morrisey and Gruber, 1993; Speed et al., 2010). Therefore, seasonal temperature shifts may impact condition in nursery-bound sharks. Sharks occupying different areas within the broader nursery habitat may be affected by variable conditions or resource availability and this too may influence condition. Additionally, most sharks make changes in their movements, habitat use and diets as they grow (Grubbs, 2010) which may affect their relative condition.

The blacktip reef shark *Carcharhinus melanopterus* and the sicklefin lemon shark *Negaprion acutidens* are two carcharhinid species that are listed as 'Vulnerable' (Simpfendorfer et al., 2020) and 'Endangered' (Simpfendorfer et al., 2021), respectively, on the IUCN Red List of Threatened Species, with populations of both declining globally. The St. Joseph Atoll in the Amirantes, Seychelles, is home to juvenile populations of these species. Previous research has investigated how they share this space, grow and survive (Weideli et al., 2019a). The aim of this research was to use data collected from mark-recapture studies of juvenile *C. melanopterus* and *N. acutidens* populations to assess variations in CF across sex, season, habitat zones and size class. Juvenile populations represent the most important life stages contributing to the recovery of species populations as a whole (Field et al., 2009; Kinney and Simpfendorfer, 2009; Speed et al., 2010) and this study hoped to provide useful information on the status of critical juvenile populations of these two shark species. Further, baseline information about the condition and health of shark populations is important for gauging the impacts of potential future environmental disturbances and threats (Hussey et al. 2009).

Methodology

Study location and sampling

This study was conducted at St. Joseph Atoll (05°26′S, 53°20′E), Amirantes, Seychelles. St. Joseph is a near-pristine site, consisting of uninhabited and undeveloped islands. The atoll provides shallow, sheltered habitats for *C. melanopterus* and *N. acutidens*. The lagoon is dynamic and highly tidal with large areas exposed at low tides. The shoreline has two mangrove species, the dominant *Pemphis acidula* with limited patches of *Rhizophora mucronata* (Stoddart et al., 1979; Lea et al., 2016).

Sampling was conducted from May 2021 to March 2022. Across all tides (high, low, flooding and ebbing), sharks were caught with a 20 m wide, 1.5 m high gillnet with a 5 cm mesh size. All sampling was conducted at one of six habitat zones (Figure 1) encompassing a variety of different areas juvenile sharks are known to inhabit (Weideli et al., 2019b). At zones 1-5, nets were placed over the sand flats adjacent to the shoreline. At zone 6, nets were set over the seagrass beds on the reef flat. Specific sampling locations within a zone were selected at random and, following the deployment of the nets, GPS coordinates were recorded at each location (Garmin 73 handheld unit). Sampling took place across all of the zones and the selection of which zone to survey was based on the prevailing weather conditions and tidally-related access restrictions. During all capture sessions environmental data were recorded at the immediate site. Temperature (°c), salinity (ppt) and dissolved oxygen (mg. l^{-1}) were all recorded using a YSI Pro 2030 water meter (YSI Inc.).

Captured juvenile sharks were sexed (determined by the presence of claspers [male reproductive organ]) and morphometric measurements were taken. This included total length (TL – tip of the snout to the tip of the upper caudal lobe), fork length (FL – tip of the snout to the anterior-most point in the fork of the caudal fin) and precaudal length (PCL – tip of the snout to the pre-caudal pit) (see Weideli et al., 2019a for more information on morphometric measurements). All sharks were weighed using a net sling and a newton meter, to the nearest 50 g. Below the first dorsal fin, a passive integrated transponder (PIT) tag (Biomark) was inserted under the skin of each shark and served as its unique identifier. This data collection process took between two and five minutes and all sharks swam away well upon release.

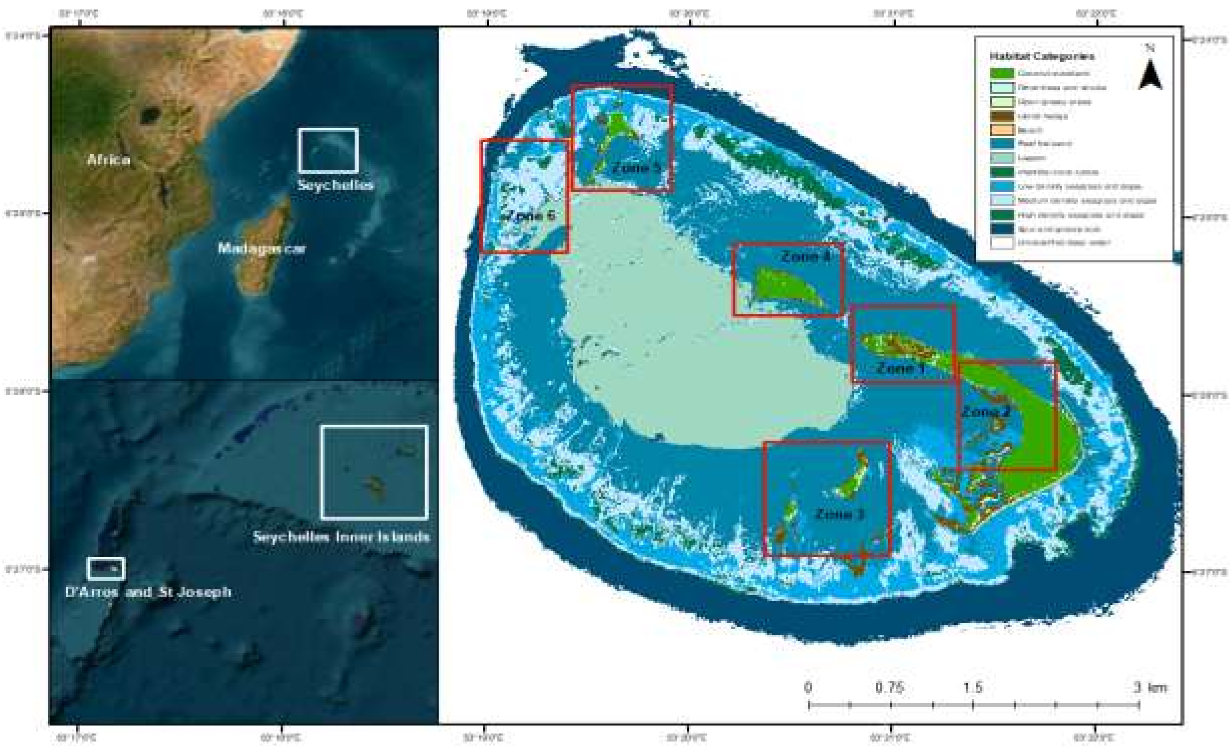

Figure 1: The study site of St. Joseph Atoll, Republic of Seychelles. Red boxes indicate the various habitat zones within which mark-recapture experiments were held. Upper left inset shows geographical position of Seychelles (highlighted by white box) and lower left inset shows position of D'Arros Island and St. Joseph Atoll relative to the Seychelles inner islands (highlighted with white boxes)

Map base layer source: Esri®

Data analysis

All recaptures of previously caught sharks were excluded from this analysis to eliminate same-individual replicates and ensure independence of all data analysed. Seasons were defined by the known monsoon seasons at St. Joseph (Northwest: NW – December to March, Southeast: SE – April to November) (Filmalter et al., 2013). Size-classes were generated to group different sized sharks. Size class 1 included sharks up to 49 cm PCL. Size-class 2 included sharks from 50-69 cm PCL and size-class 3 included all sharks ≥ 70 cm PCL.

All analyses were conducted in R (vers. 3.5.3) within the RStudio Interface (R Foundation for Statistical Computing). The relative condition of each of the captured sharks of each species was calculated using Fulton's condition factor (CF) (as in Hussey et al., 2009 and Hodgkiss et al., 2017):

$$CF = [\text{total body mass (kg)} / \text{PCL (cm)}^3] \times 10^5$$

Data were separated by species and then tested for normality using Shapiro Wilk tests. Variations in CF across different factors were then statistically tested for each species' dataset. The Wilcoxon rank-sum test was employed to analyse the variation in CF across sex (male vs female) and season (NW vs SE). Kruskal-Wallis tests with Dunn's pairwise post-hoc testing was used to test across size-class (size class 1, 2 and 3) and habitat zones (Zones 1-6). Environmental data (temperature, salinity and dissolved oxygen) were tested for differences between season and habitat zone using Wilcoxon rank-sum and Kruskal-Wallis tests respectively.

Results

A total of 195 juvenile sharks (142 *Carcharhinus melanopterus* and 53 *Negaprion acutidens,* Table 1) were captured and measured across habitat zones at St. Joseph Atoll across all tides and at depths ranging from 10 to 85 cm. Captured *C. melanopterus* sharks ranged in size from 35 to 95 cm PCL (45.51 cm ± 8.25 cm mean ± SD). Captured *N. acutidens* sharks ranged in size from 41 to 94 cm PCL (54.1 cm ± 7.95 cm mean ± SD).

Table 1: Numbers of sharks caught across different habitat zones with total effort (Effort: time spent fishing for sharks rounded to the nearest hour) and catch per unit effort (CPUE: mean number of sharks caught per hour at different habitat zones)

Zone	C. mel	N. acu	Total sharks	Effort (h)	CPUE (sharks h^{-1})
1	80	30	110	18	6.1
2	14	13	27	8	3.4
3	5	2	7	4	1.8
4	14	0	14	5	2.8
5	11	8	19	6	3.2
6	18	0	18	14	1.3
All	**142**	**53**	**195**	**55**	**3.1**

Shapiro-Wilk results demonstrated high significance for *Carcharhinus melanopterus* (w = 0.93, p-value = 7.942e-06; not normally distributed) and non-significance for *Negaprion acutidens* (w = 0.97, p-value = 0.316; normally distributed). Results did demonstrate heteroscedasticity (deviations of a predicted variable monitored over different values of an independent variable), thus non-parametric, one-way and multiple comparison tests were applied to fit both datasets.

Sex

For *C. melanopterus,* 62 females and 59 males were caught, equating to a sex ratio of 1.05:1 (female:male). For *N. acutidens* 23 females and 29 males were caught, equating to a sex ratio of 1:1.18 (female:male). Data for both species evidenced no significant differences in CF between sex (Table 2), as shown below in figures 2a and 2b.

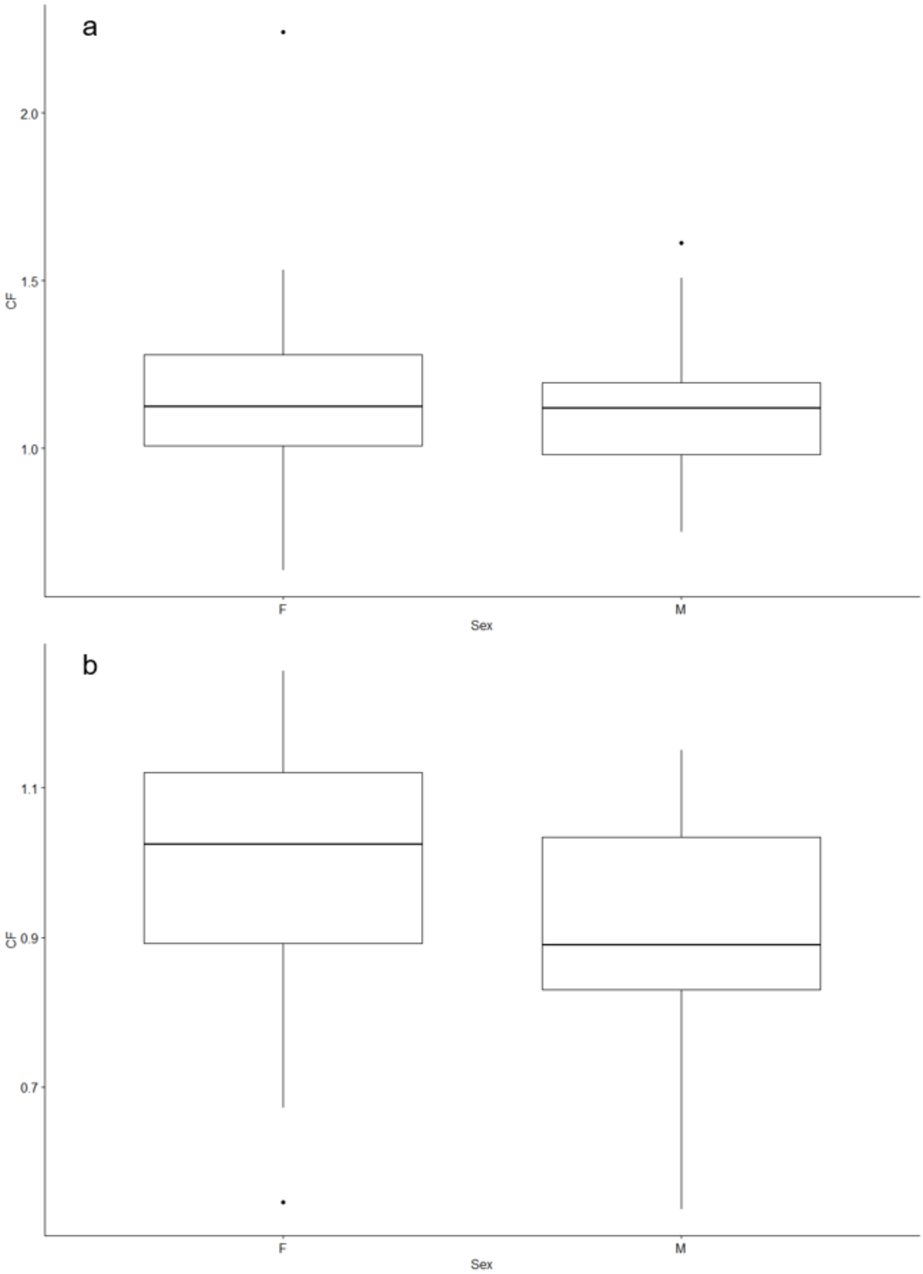

Figure 2: Box plots displaying CF differences between males and females for a) Carcharhinus melanopterus and b) Negaprion acutidens

Seasons

Significant seasonal differences in CF were detected for *C. melanopterus*, indicating that this population is impacted by seasonal monsoon changes (Table 2, Fig 3a). Sharks had a mean CF of 1.23 (±0.03 SE) in the NW season and 1.06 (± 0.02 SE) in the SE season. Meanwhile, CF data for *N.acutidens* showed no seasonal variations in condition (Table 2, Fig 3b).

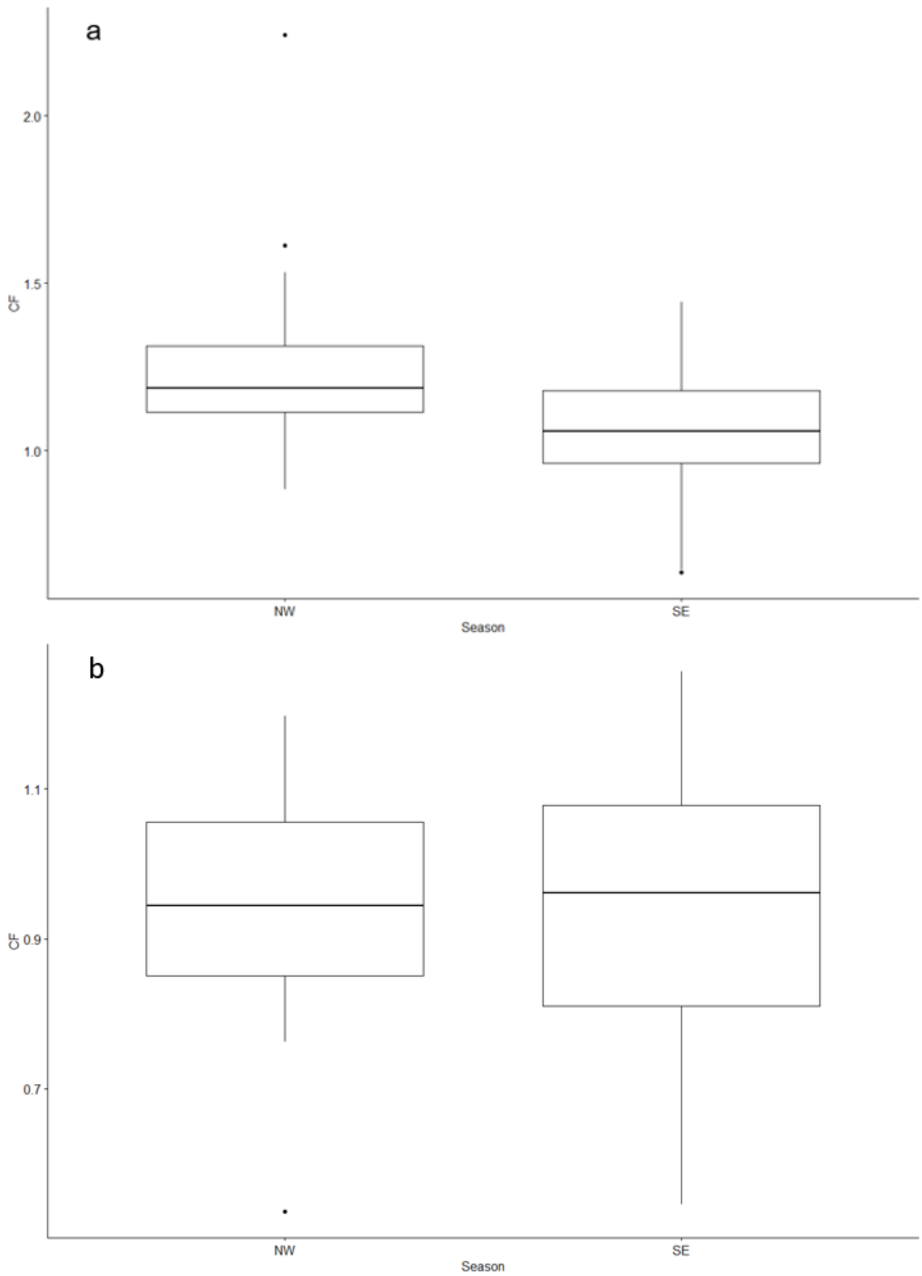

Figure 3: Box plots displaying CF differences between season for a) Carcharhinus melanopterus and b) Negaprion acutidens

Habitat zones

C. melanopterus were caught across all six habitat zones. Significant differences in CF between habitat zones were detected (Table 2, Fig 4a) and post-hoc pairwise testing identified zones 1-5 as significantly distinct from zone 6. Condition of sharks caught in zone 6 was markedly higher than in all other zones (zone 6 mean = 1.64 ± 0.15 SE, zones 1-5 mean = 1.11 ± 0.05 SE). *N. acutidens* were caught in zones 1, 2, 3 and 5. No significant differences in CF were detected between habitat zones for *N. acutidens* (Table 2, Fig 4b).

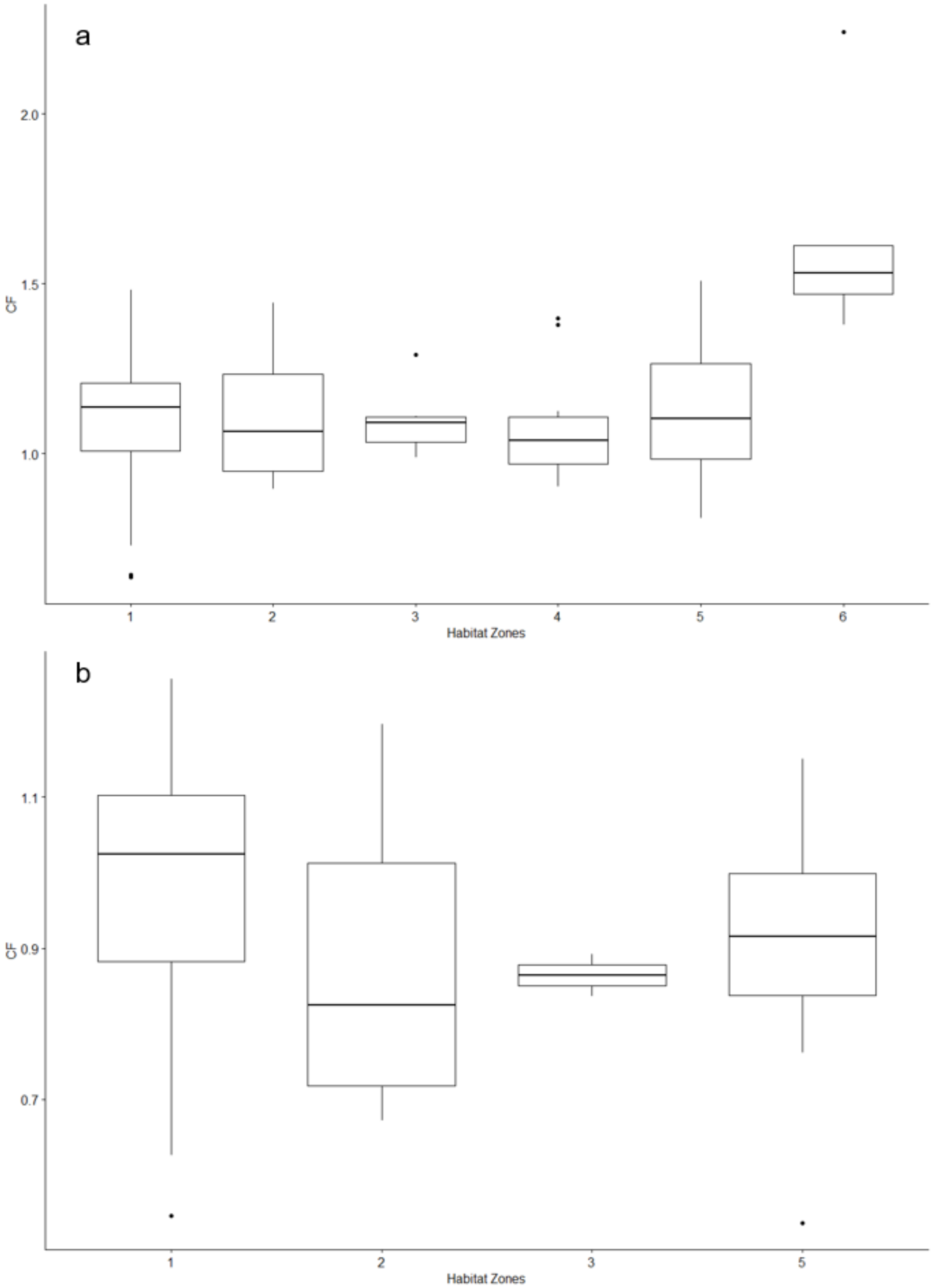

Figure 4: Box plots displaying CF differences between habitat zones for a) Carcharhinus melanopterus and b) Negaprion acutidens

Size class

Differences in CF between different size classes of *C. melanopterus* were detected with CF values of individuals in the largest size class significantly higher than those in the first two size classes (Table 2, Fig 5a). Mean CF of sharks in size classes 1 and 2 were 1.07 (± 0.02 SE) and 1.16 (± 0.03 SE) respectively, whereas mean CF of sharks in size class 3 was 1.42

(± 0.05 SE). No significant differences in CF between size classes were detected for *N. acutidens* (Table 2, Fig 5b).

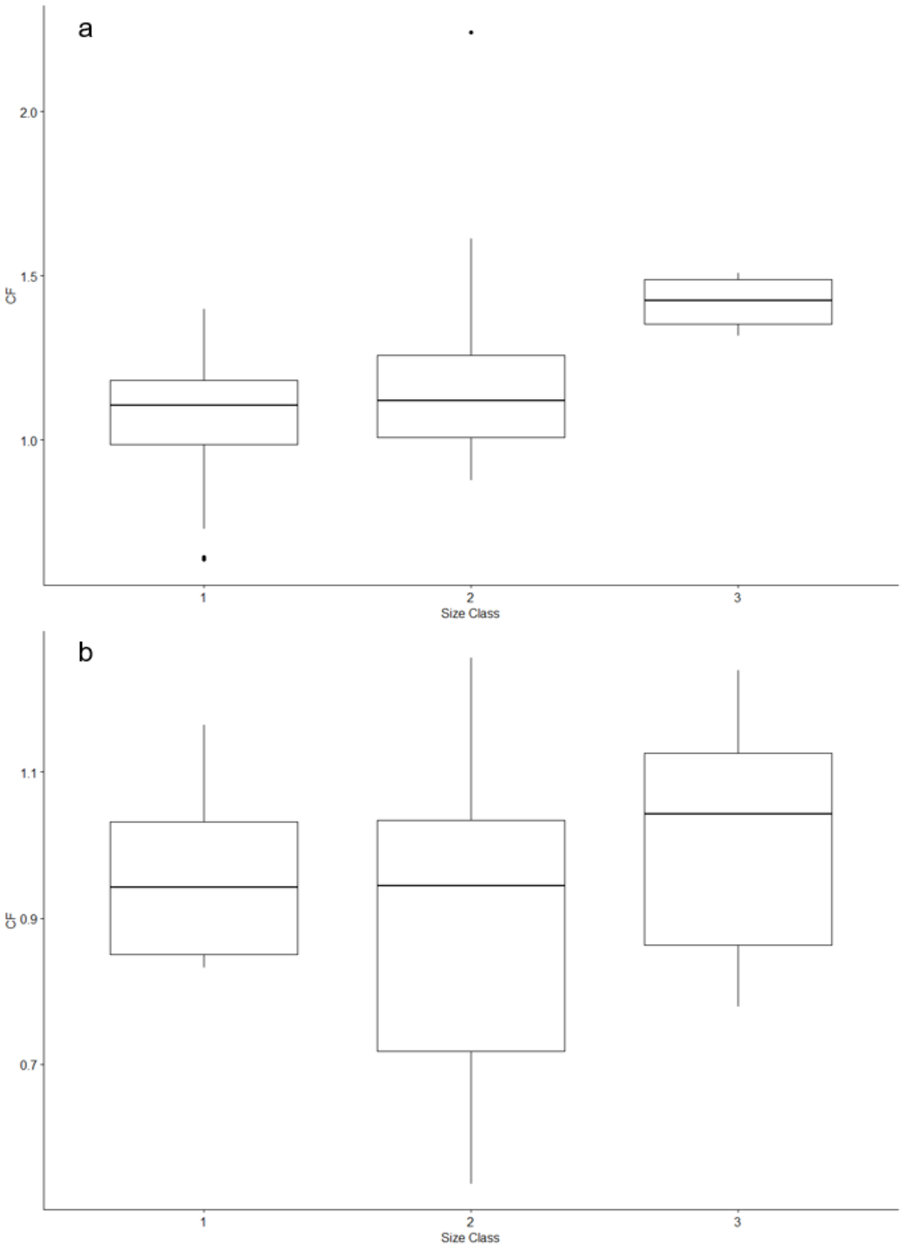

Figure 5: Box plots displaying CF differences between different size classes for a) Carcharhinus melanopterus and b) Negaprion acutidens

Table 2: Results of significance testing across different factors for the two shark species, Carcharhinus melanopterus and Negaprion acutidens

Species	Factor	Test Statistic	d.f.	Significance Value (p)
C. melanopterus	Sex	W = 2044	-	0.266
	Season	W = 2556.5	-	**1.394 e -05**
	Size Class	x^2 = 11.838	2	**0.003**
	Habitat Zones	x^2 = 14.537	5	**0.013**
N. acutidens	Sex	W = 416.5	-	0.075
	Season	W = 279	-	0.6331
	Size Class	x^2 = 3.5404	2	0.1703
	Habitat Zones	x^2 = 4.9505	5	0.1755

Significant p values (alpha = 0.05) highlighted in bold

Environmental data

No significant differences in temperature or salinity were detected between seasons, however dissolved oxygen levels were significantly higher during the NW season (Wilcoxon rank-sum: W = 1533, p = < 0.001, NW = 6.63 ± 0.88 mean ± SE, SE = 6.54 ± 0.35 mean ± SE). Statistical differences across habitat zones were detected for temperature (Kruskal-Wallis: x^2 = 46.75, d.f = 5, p = <0.001), salinity (Kruskal-Wallis: x^2 = 36.67, d.f = 5, p = <0.001) and dissolved oxygen (Kruskal-Wallis: x^2 = 14.87, d.f = 5, p = 0.01). Temperatures were highest in zone 1 (mean = 30.47ºC ± 0.17 SE) and lowest in zone 5 (mean = 27.66ºC ± 0.98 SE), salinity was highest in zone 4 (mean = 32.9 ppt ± 0.32 SE) and lowest in zone 2 (mean = 30.27 ppt ± 1.25 SE), dissolved oxygen was highest in zone 2 (mean = 6.90 mg l^{-1} ± 1.27 SE) and lowest in zone 4 (mean = 5.45 mg l^{-1} ± 0.41 SE).

Discussion

Some species of shark have been found to show gender-specific variation in growth rates (Klimley, 1987; Parsons, 1993), which could correlate with differences in bioenergetic demands or in food acquisition. This may then be reflected by differences in condition between sexes in a population. Despite this, no sex-based differences in body condition were detected for either species studied here. Juvenile shark populations are not sexually segregated whilst using the St. Joseph nursery site (Weideli et al., 2019a), with sharks of both sexes using the same habitats and presumably utilizing the same resources. A previous study into the growth rates of *N. acutidens* suggests there is no significant difference in the growth rate of male and female lemon sharks during the first year of life (Hodgkiss et al., 2017).

Generally, the study found differing condition sensitivities between the two species' populations across season, size class and habitat zone. *Carcharhinus melanopterus* showed variation in body condition across all of these factors, whilst condition of *N. acutidens* remained consistent. *Carcharhinus melanopterus* displayed seasonal variations in condition with sharks generally in better condition during the northwest season. No significant differences in temperature were detected from our data, however these data are taken from instantaneous readings at different sub-habitat areas and at different times of day. Generally, there are well-known seasonal shifts in temperature at the site with warmer waters during the northwest season (Gadoutsis et al., 2019) and such shifts may influence condition in *C. melanopterus*. Differences in dissolved oxygen were detected at the site with higher dissolved oxygen levels prevalent in the northwest season. Dissolved oxygen is known to play a role in the movements of sharks (Parsons and Hoffmayer, 2005; Heithaus et al., 2009) and it is suggested that some sharks' movements reflect efforts to remain within optimal dissolved oxygen conditions (Heithaus et al., 2009). Few studies however have investigated the impacts of dissolved oxygen on physiology and bioenergetics in sharks. Findings here for *C. melanopterus* could relate to increased oxygen uptake during periods of higher dissolved oxygen in the northwest season. Further study into the relationship between dissolved oxygen levels and shark condition is needed to explore this in more detail. No distinct differences in condition of *N. acutidens* were detected between seasons. *Negaprion acutidens* has a larger size at birth (45-80 cm) than *C. melanopterus* (30-50 cm) (Ebert et al., 2013) which could indicate a better overall relative condition in this species from birth and this could be reflected in a greater resilience to seasonal variations in environmental conditions. Alternatively, species-specific differences in hypoxia tolerance (Carlson and Parsons, 2003) could explain different seasonal sensitivities in condition between these species. Differences in size at birth between the two species may also link to findings relating to size-class. Larger size at birth, and better condition at birth, may explain the consistent body condition found in sharks of all sizes for *N. acutidens.* Previous study of *N. acutidens* showed that there were no significant differences in growth rates of sharks in early life stages (Hodgkiss et al., 2017). Juvenile *C. melanopterus* were in the poorest condition at the smallest sizes, with condition improving as the sharks grew larger. This means that juvenile *C. melanopterus* are the most vulnerable in the first months of life.

Across habitat zones 1-5, no variations in body condition were found for either species. For *C. melanopterus*, sharks caught in zone 6 were in significantly better condition than sharks caught at all other sites. Numbers of long-term recaptures for sharks of both species in close vicinity to the original capture sites implies that sharks occupy relatively restricted home ranges in the early stages of life (Weideli et al., 2019a) and that regular movements across the study site are unlikely. Interestingly, though zone 6 is no different to the other habitat zones in terms of environmental conditions, the predominant benthos across zones 1-5 was sand, whereas at zone 6 the benthos was largely seagrass. Seagrass habitats may offer more food resources or beneficial foraging conditions that improve the condition of sharks using the area. Zone 6 is also the closest zone to the reef crest where large predators are found in greater numbers. Previous research has shown that individual differences in risk tolerance exist within a population, with shark sub-populations exposed to greater risk having a lower apparent survival but faster growth rates (Dhellemmes et al., 2021). In this study, catch per unit effort was lowest at zone 6. A risk versus reward trade-off may be reflected by the present findings, with fewer sharks occupying zone 6 (as a result of increased predation rates) but improved condition of sharks that are found there (as a result of improved foraging potential). Conclusions regarding spatial variations in condition here are limited due to restricted sampling at some sites. More data and research will be needed to explore this further.

Factors other than those explored here have the potential to impact shark health and body condition in the early stages of life. Both species are known to be parasitized by marine leeches at St. Joseph (Daly et al., 2019; Bullock et al., unpublished data 2022) and susceptibility to parasitization may vary intra or interspecifically. Evidence of reduced girth, relative to similarly-sized uninfected individuals, in parasitized blacktip reef sharks caught at St. Joseph, does indicate a potential impact of parasitization on body condition and fitness (Bullock et al., unpublished data 2022). An ulcerative skin condition known as 'fin rot,' 'gill rot,' or 'black patch necrosis (BPN)' is a prevalent ailment in wild sharks that is caused by bacterial pathogens (Devesa et al., 1989). This illness is often identified by white discoloured spots on the skin that advance to shedding of skin cells, which leads to haemorrhagic, ulcerative lesions on the skin in investigations with fish (Dippenaar et al., 2009). It remains unclear as to whether fin rot is linked to shark mortality and/or body condition and further study is required to better understand the effects of both parasites and bacterial pathogens in sharks.

Conclusion

Research into the health of shark populations is lacking. These findings contribute novel understanding to the health of two juvenile populations of threatened sharks using a nursery site. Findings highlight differences in sensitivities to environmental changes between the two species. *Carcharhinus melanopterus* were generally more vulnerable at birth, and sensitive to seasonal environmental changes. Condition in *N. acutidens* remained constant across all size classes, and sharks were unaffected by seasonal environmental changes. Some evidence for spatial variations in the condition, relative to differences in habitat zones occupied were also found for *C. melanopterus.*

This study provides baseline data on the relative condition of two threatened shark populations at a largely undisturbed tropical site. These data form the basis of long-term monitoring of population health at the site and could help to detect impacts to population health from potential future disturbances.

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