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Thermally insensitive physiological performance allows neonatal sharks to use coastal habitats as nursery areas

Ian A. Bouyoucos[1,2,*], Colin A. Simpfendorfer[3], Serge Planes[2,4], Gail D. Schwieterman[5], Ornella C. Weideli[2], Jodie L. Rummer[1,6]

[1]Australian Research Council Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, QLD 4811, Australia

[2]PSL Research University, EPHE-UPVD-CNRS, USR 3278 CRIOBE, Université de Perpignan, 66860 Perpignan Cedex, France

[3]Centre for Sustainable Tropical Fisheries and Aquaculture & College of Science and Engineering, James Cook University, Townsville, QLD 4811, Australia

[4]Laboratoire d'Excellence 'CORAIL', EPHE, PSL Research University, UPVD, USR 3278 CRIOBE, 98729 Papetoai, Moorea, French Polynesia

[5]Virginia Institute of Marine Science, William & Mary, Gloucester Point, VA 23062, USA

[6]College of Science and Engineering, James Cook University, Townsville, QLD 4811, Australia

ABSTRACT: Coastal sharks can use shallow, nearshore habitats as nursery areas, which is a behaviour that may increase fitness. The ecological benefits of shark nursery areas are well studied; yet the physiological mechanisms that enable sharks to exploit coastal habitats, especially those that experience extreme and dynamic temperatures, remain understudied. We hypothesised that neonatal sharks are able to use thermally dynamic coastal habitats as nursery areas because temperature does not strongly affect their physiology. To test this hypothesis, we defined patterns of nursery area use and temperature-dependent physiological performance in 2 reef shark species. First, we determined whether 10 sites around the island of Moorea, French Polynesia, satisfied nursery area criteria for neonate populations of blacktip reef sharks *Carcharhinus melanopterus* and sicklefin lemon sharks *Negaprion acutidens* using 5 consecutive years of abundance surveys. We then quantified effects of thermal exposure *in situ* on growth in recaptured individuals and quantified the temperature dependence of metabolic rate *ex situ* using respirometry. We found several potential *C. melanopterus* nursery areas, but during different sampling years, and identified 1 *N. acutidens* nursery area that remained consistent during the entire 5 yr study. In support of our hypothesis, growth and metabolic performance were not strongly affected by temperature in either species. Thus, thermally insensitive physiological performance may be a trait that elasmobranchs exhibit in thermally variable coastal habitats, including shark nursery areas. Together, this approach demonstrates how physiological and ecological concepts complement each other to improve our understanding of nursery area use in coastal shark populations.

KEY WORDS: Thermal performance · Metabolic rate · Q_{10} · Oxygen uptake rate · Growth · Growing degree day · Blacktip reef shark · Sicklefin lemon shark

1. INTRODUCTION

Nursery areas are key aspects of the ecology of many marine and estuarine fishes and invertebrates. Beck et al. (2001) hypothesised that nursery areas for juveniles of a species are habitats that must contribute a higher density of recruits to the adult, reproductive population when compared to other adjacent, non-nursery habitats. As such, using nursery areas is a fitness-maximising behaviour (Beck et al.

*Corresponding author: ian.bouyoucos@my.jcu.edu.au

2001, Fodrie et al. 2009, Nagelkerken et al. 2015). The nursery area concept has received particular attention among studies examining elasmobranch fishes (Heupel et al. 2007, 2019, Martins et al. 2018). Indeed, the nursery area concept proposed by Beck et al. (2001) has since been refined to identify shark nursery areas by Heupel et al. (2007): juvenile sharks are more abundant in nursery areas relative to other habitats; juveniles are resident within nursery areas for extended periods of time; and the use of nursery areas by juvenile sharks is stable over time. Key assumptions underlying the fitness benefits of shark nursery areas are that nursery areas provide ample prey abundance or refuge from predation to improve survival of juveniles during their first years of life (Heithaus 2007, Heupel et al. 2007). Prey and refuge benefits are not qualities of all nursery areas, per se, as some are either characterised by only one or the other. For example, Kaneohe Bay (Hawaii, USA) is a scalloped hammerhead shark *Sphyrna lewini* nursery area that provides refuge from predators, but is food-limited (Bush & Holland 2002, Lowe 2002, Duncan & Holland 2006). While it is probable that other unidentified benefits of nursery area use also contribute to fitness, many studies on shark nursery areas only go so far as to test the criteria to identify nursery areas (Heupel et al. 2019), but do not aim to evaluate the associated effects on fitness.

Temperature is a powerful controlling factor of the physiology of ectotherms (Fry 1947) and is therefore likely to influence the benefits associated with shark nursery areas. This idea was originally proposed by Heithaus (2007) as an important avenue for further research. Previous studies have quantified the effects of temperature on growth (e.g. Pistevos et al. 2015, Gervais et al. 2018, Izzo & Gillanders 2020) and metabolic rates (e.g. Lear et al. 2017, Schwieterman et al. 2019, Bouyoucos et al. 2020a) in several shark and ray species; yet, few have directly applied these data within the context of nursery area use. One example includes bioenergetic modelling to estimate consumption rates in juvenile sandbar sharks *Carcharhinus plumbeus* in a Chesapeake Bay (USA) nursery area (Dowd et al. 2006a,b). Biologging and respirometry were also used to define environmental tolerance thresholds of *C. plumbeus* from the same habitat (Crear et al. 2019). Bioenergetics modelling has also been applied to predict accelerated mass loss in freshwater largetooth sawfish *Pristis pristis* and euryhaline bull sharks *C. leucas* in response to higher water temperatures in nursery areas (Lear et al. 2020, 2021). Another study observed an association between temperature and abundance of juvenile *C. leucas* that revealed a potential shift in nursery area habitat to cooler waters (Bangley et al. 2018). Declines in activity levels have also been observed in juvenile sawfishes (*P. pectinata* and *P. pristis*), lemon sharks *Negaprion brevirostris*, nurse sharks *Ginglymostoma cirratum*, and *C. leucas* at supra-optimal temperatures in various marine and freshwater nursery areas (Lear et al. 2019, 2020). Indeed, understanding the sensitivity of physiological performance traits to temperature (i.e. thermal dependence) can explain habitat use in fishes (Gannon et al. 2014, Payne et al. 2016, 2018).

The ability of neonate and juvenile sharks to occupy thermally volatile environments like coastal nursery areas suggests that physiological performance traits of these sharks should not be strongly affected by temperature. Coastal environments can serve as important habitats for fish nursery areas (Lefcheck et al. 2019), but these shallow, nearshore habitats can be dynamic environments that experience extreme environmental conditions (Knip et al. 2010). For instance, the North Sound shark nursery area for *N. brevirostris* in Bimini (The Bahamas) experiences daily temperatures ranging from 14.5 to 36.5°C in the dry season and from 23.8 to 37.8°C in the wet season (DiGirolamo et al. 2012). In addition, neonate and juvenile sharks can exhibit site fidelity (Chapman et al. 2009) and small home ranges within nursery areas (George et al. 2019, Bouyoucos et al. 2020b). Therefore, it follows that these animals should be able to physiologically tolerate extreme abiotic conditions without abandoning the resources (e.g. refuge, ample prey densities) afforded by their nursery areas. For example, juvenile *P. pristis* in Fitzroy River (Western Australia) nursery areas experience temperatures that exceed their optimal temperature for activity during almost 40% of the year (Lear et al. 2019). Further, sharks and rays that are confined to nursery areas seem to exhibit low thermal sensitivity of activity levels and metabolic rate (Lear et al. 2019, 2020). Indeed, this low thermal sensitivity may be adaptive because high thermal sensitivity in variable thermal environments can be energetically costly (da Silva et al. 2019). This also broadly agrees with evidence that juvenile fishes have greater thermal tolerance than other ontogenetic stages (Dahlke et al. 2020). For instance, being able to tolerate environmental conditions that are not tolerated by potential predators would allow neonates to reduce their predation risk by using such habitats. Thus, examining thermal dependence of fitness-related traits (e.g. growth, metabolic rate) may help further understand how sharks exploit habitats as nursery areas.

The temperature dependence of biological rates can be quantified as a temperature quotient (Q_{10}). This value represents the sensitivity of a biological rate to a temperature change, where a Q_{10} value of 2, for example, means that the biological rate will double in response to a 10°C increase in temperature. Passive thermodynamic effects on biochemical reactions are reflected by exponential increases in biological rates with temperature (Schulte 2015, Havird et al. 2020), as exemplified by Q_{10} values in the range of 2.0–3.0 that are observed across ectothermic taxa (Clarke & Johnston 1999, Seebacher et al. 2015), including elasmobranch fishes (Di Santo & Bennett 2011, Lear et al. 2017, Luongo & Lowe 2018). Biological rates that are highly sensitive to changes in temperature exhibit Q_{10} values >3; however, high Q_{10} values are also indicative of active depression of biological rates especially in cold environments (Hopkins & Cech 1994, Dabruzzi et al. 2013, Speers-Roesch et al. 2018). Conversely, traits that have Q_{10} values approaching 1 are temperature insensitive and possibly under active physiological control to restore or compensate the biological rate (Sandblom et al. 2014). Thus, for elasmobranchs living in confined habitats, one might predict Q_{10} values for biological rates occurring on the lower end (i.e. $Q_{10} < 2$).

The purpose of this study was to test the hypothesis that coastal elasmobranchs with restricted patterns of habitat use are characterised by temperature-insensitive physiological performance. We predicted that neonatal reef sharks found around the island of Moorea (French Polynesia) exhibit restricted patterns of habitat use (i.e. by using shark nursery areas) and that these sharks exhibit little to no thermal dependence of growth and metabolic rate. Together, these data have significance for advancing nursery area theory so that we may better understand why certain habitats function as nursery areas, the benefits sharks derive from these habitats, and whether environmental stressors (e.g. marine heatwaves, climate change) may ultimately affect the suitability of habitats as shark nursery areas.

2. MATERIALS AND METHODS

2.1. Ethical approvals

Experimental protocols were approved by the James Cook University Animal Ethics Committee (A2089 and A2394). Authorisation to collect, possess, and transport sharks and shark tissues in French Polynesia was obtained from the Ministère de la Promotion des Langues, de la Culture, de la Communication, et de l'Environnement of French Polynesia (Arrêté No. 9524).

2.2. Study site and species

The Centre de Recherches Insulaires et Observatoire de l'Environnement (CRIOBE) has conducted a fisheries-independent survey of neonate and juvenile sharks at 14 unique sites around Moorea since 2007 (Mourier & Planes 2013). Here, the 10 most productive sites were fished over 5 parturition seasons (October–March) during 2015–2020. The 10 sites (Apaura, Haapiti, Maharepa, Paorea, Papetoai, Pihaena, Tiki, Vaiane, Vaiare, and Valorie) are distributed around the entire perimeter of Moorea (Fig. 1). Two shark species occur in these sites: blacktip reef sharks *Carcharhinus melanopterus* and sicklefin lemon sharks *Negaprion acutidens*.

Throughout the study, individuals of both species were captured using monofilament gillnets (50 m long by 1.5 m depth; 5 cm mesh size, 10 cm stretch) set perpendicular from shore spanning the hours of approximately 17:00–20:00 h and fished most evenings throughout the parturition season (Mourier & Planes 2013). Water temperatures typically ranged from 28 to 31°C during evenings while fishing (see Bouyoucos et al. 2021 for temperature data at all sites from 2015 to 2019). Total fishing effort is reported in Table 1. All sharks that were captured throughout the study were

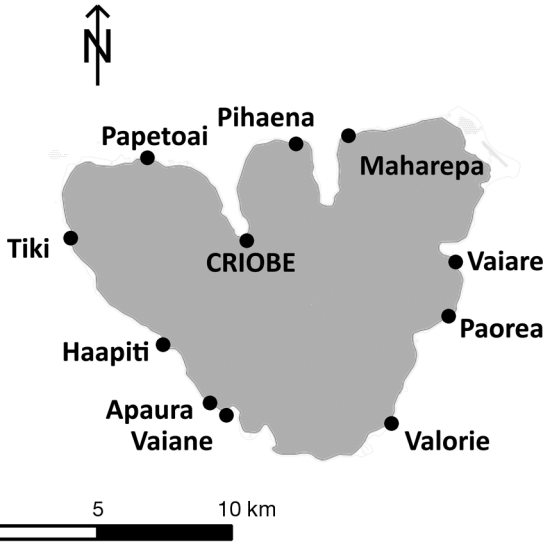

Fig. 1. Shark capture locations around the island of Moorea, French Polynesia (17° 30′ S, 149° 50′ W). Some animals were brought to the Centre de Recherches Insulaires et Observatoire de l'Environnement (CRIOBE) to characterise physiological performance

Table 1. Fishing effort and morphometrics of neonates of 2 reef sharks around Moorea, French Polynesia

Year	Effort (h)	Gillnet sets (n)	*Carcharhinus melanopterus*				*Negaprion acutidens*			
			Individuals caught (n)	Mass (kg; range)	Total length (mm; range)	Recaptured individuals (n)	Individuals caught (n)	Mass (kg; range)	Total length (mm; range)	Recaptured individuals (n)
2015	242	92	147	0.65–1.36	510–800	12	30		580–940	12
2016	247	90	152	0.72–1.97	513–750	24	43	0.94–3.65	582–872	4
2017	206	78	135	0.67–1.67	502–658	23	26	0.92–2.92	594–850	9
2018	221	93	94	0.54–1.88	476–684	15	77	0.88–6.66	566–1100	30
2019	118	55	99	0.43–1.56	490–702	17	60	0.92–2.49	582–782	20

tagged with a coloured T-bar (Hallprint) or a passive integrated transponder tag (Biolog-id SAS) for identification in case of recapture. The precaudal length (PCL; snout to caudal peduncle) and total length (TL; snout to upper caudal lobe tip) were recorded for all individuals. Sharks were also weighed to the nearest 10 g in a soft plastic bag suspended from a digital scale and measured to estimate body condition (Weideli et al. 2019a). Capture durations were typically under 5 min, and tagging, measuring, and weighing lasted 10–15 min ind.$^{-1}$.

All *C. melanopterus* collected for this study were found at all 10 sites and were neonates or juveniles aged 0–1 yr, as confirmed by the presence of open or recently closed umbilical scars (Chin et al. 2015). Although the sex of individuals can be visually identified, sex was not included in analyses or interpretation of the data because it was assumed that habitat usage by neonates and thermal dependence of physiological performance are independent of sex. Indeed, *C. melanopterus* do not mature sexually until 4–8 yr of age (Chin et al. 2013). Body mass and total length of *C. melanopterus* during each parturition season (survey year) are summarised in Table 1. A subset of *C. melanopterus* (n = 10) that weighed 0.92 ± 0.14 kg (mean ± SD; range = 0.60–1.12 kg) was transported from 2 sites, Papetoai and Vaiare, to the CRIOBE in October 2018 for estimation of metabolic rate. All *C. melanopterus* were released at their original capture sites.

Neonatal *N. acutidens* were captured the same way as *C. melanopterus* and were present at all sites except for Paorea and Vaiare. In addition, *N. acutidens* were observed to move between Apaura and Vaiane (I. A. Bouyoucos pers. obs.); these 2 sites are separated by approximately 800 m of shoreline and were therefore treated as 1 site ('Apaura-Vaiane') for this species. All *N. acutidens* collected for this study were neonates or juveniles. The age of juveniles could not be determined; however, these sharks were confirmed to be immature because size-at-maturity is approximately 2200 mm (Stevens 1984). Morphometrics of *N. acutidens* are summarised in Table 1. In October 2018, a subset of *N. acutidens* (n = 8) weighing 1.39 ± 0.21 kg (range = 1.00–1.63 kg) was transported from 2 sites, Pihaena and Tiki, to the CRIOBE for estimation of metabolic rate. All *N. acutidens* were released at their original capture sites.

2.3. Identifying shark nursery areas

The first study objective was to determine which shark habitats around Moorea serve an ecologically important role as shark nursery areas. It is important to note that the sampling areas in this study were originally selected (ca. 2007) because of accessibility and presence and abundance of the study species, which may bias our identification of sites as nursery areas. Indeed, our approach only allows for identification of nursery areas relative to the studied sites but not other, unobserved locations; thus, it is not possible for all studied sites to be designated as 'shark nursery areas'. Fishing survey data were used to quantify catch per unit effort (CPUE, sharks h^{-1}) for each species per gillnet set per site and per survey year (i.e. 2015, 2016, 2017, 2018, and 2019). These CPUE data were then used to test the 3 shark nursery area criteria proposed by Heupel et al. (2007, 2019) to identify which of the 10 sites function as shark nursery areas and which do not. We assessed nursery area criteria for sites across the entire study (i.e. 2015–2020) to define sites that satisfied all 3 criteria as 'shark nursery areas' and all other sites as 'non-nursery areas'. We also tested the criteria for sites within survey year (i.e. 2015, 2016, 2017, 2018, or 2019) to see if the roles of sites as nursery areas and non-nursery areas were consistent over time (Yates et al. 2012). Sites that met all 3 criteria within a survey year but did not meet the criteria across the entire study were labelled 'potential nursery areas' (more detail below).

Criterion 1 was satisfied if neonates and juveniles were more abundant in the target location relative to other locations (Heupel et al. 2007). Bootstrap hypothesis testing was used to test criterion 1 (Froeschke et al. 2010). For each site, the mean CPUE for each year (n = 5, i.e. 2015–2019) was sampled with replacement (n = 1000). Each sample was then scored '1' if its value was larger than the population mean CPUE (i.e. the average CPUE across all sites during the entire study); otherwise, each sample was scored '0' (Froeschke et al. 2010). Significance was determined by calculating p-values as the number of samples with values greater than or equal to the measured value (i.e. the sum of measured mean CPUE scores for each survey year) divided by the total number of samples. Criterion 1 could therefore be quantitatively assessed by determining which sites have significantly higher CPUE than the population mean for the entire study period. A p-value of 0.05 was selected to identify sites as candidate nursery areas. All other sites (i.e. $p > 0.05$) that did not satisfy criterion 1 could not be classified as nursery areas. Analyses were conducted in R (version 4.0.2; R Core Team 2020).

Criterion 2 was satisfied if individuals demonstrated residency by using the target area for extended periods of time (Heupel et al. 2007). This criterion was evaluated using recapture rate as a site-specific metric of residency and was tested using a linear model. Recapture rate was calculated for each site each year as the proportion of individuals that were recaptured at least once out of the total number of captured individuals during that same year at the same site. The recapture rate for each site in each year was log-ratio transformed and modelled as a function of site as a categorical variable, assuming the transformed recapture rate data followed a Gaussian distribution using the 'nlme' package (Pinheiro et al. 2018). Model assumptions were met, as assessed by visual inspection of quantile-quantile plots of model residuals (normality), model residuals plotted against fitted values (homogeneity of variances), and model residuals plotted against explanatory variables (independence; Zuur et al. 2009). Sites were only tested if sharks were recaptured during 3 or more survey years. Confidence intervals of effect size of fixed effects terms were generated from 1000 posterior simulations using the R package 'arm' (Gelman & Su 2018). Criterion 2 could therefore be quantitatively assessed by determining whether recapture rates of sharks at sites that did satisfy criterion 1 were greater than or not different from recapture rates of sharks at sites that did not satisfy criterion 1.

Criterion 3 was satisfied if abundance did not decline at the target location over time (Heupel et al. 2007). This criterion was tested for all sites using a generalised linear model with a Poisson distribution. Relative abundance (i.e. the number of individuals caught) per year per site per gillnet set was modelled as a function of the log of fishing effort in minutes as an offset term, site as a categorical variable, survey year as a continuous variable, and the interaction of site and survey year. Sites were only tested where sharks were captured for 3 or more survey years. Confidence intervals of effect size were produced for all levels of the interaction term so that criterion 3 could be quantitatively assessed by comparing mean effect sizes of the slopes. A positive effect size or an effect with a confidence interval overlapping zero suggests that CPUE was increasing or stable during the study period, respectively. A negative effect size would suggest that CPUE decreased during the study period and would not satisfy criterion 3.

Sites that are defined as shark nursery areas over sufficiently long time scales (i.e. years to decades), however, may not satisfy nursery area criteria during any one given year (Yates et al. 2012). To account for the possibility that our abovementioned analyses excluded sites as nursery areas because criteria were not met during most or all years of the 5 yr study, nursery area criteria were also tested for sites within each of the 5 survey years to identify 'potential nursery areas'. As describe above, criterion 1 was evaluated for each site using bootstrap hypothesis testing using CPUE values for each gill-net set compared to the population mean. Criterion 2 was evaluated for sites that satisfied criterion 1 by defining recapture rates for the entire survey year. Recapture rates were compared qualitatively between sites that did and did not satisfy criterion 1 in a similar manner as described above. Criterion 3 was evaluated for sites that satisfied criteria 1 and 2 using generalised linear models with a Poisson distribution, where relative shark abundance was modelled with the log of fishing effort in minutes as an offset term and chronological gill-net set number as a continuous factor.

2.4. Effects of temperature on growth

Next, we tested the thermal dependence of growth, *in situ*, during 2015–2019. Environmental temperature data were recorded using 1 or 2 temperature loggers (UA-002-64, Onset Computer) that were deployed up to 50 m from shore in representative microhabitats (e.g. in mangrove stands, between

coral structures, over sand substrate, etc.) at each site (Bouyoucos et al. 2020a). Loggers recorded temperature continuously every 10 min (accuracy = ±0.5°C, resolution = 0.14°C at 25°C), producing a 4 yr time series (2015–2019) at most sites. At sites where 2 loggers were deployed, an average was taken of each time-stamped value for subsequent analyses. Temperatures recorded by loggers were assumed to be representative of temperatures experienced by sharks because neonatal sharks around Moorea have very small home ranges; *C. melanopterus* neonates have an average core habitat size of 0.02 km^2 (Bouyoucos et al. 2020b). Further, recorded body temperatures of *C. melanopterus* neonates match average habitat temperatures and span the range of recorded environmental temperatures (Bouyoucos et al. 2020a). Growth was measured using precaudal length (PCL, in mm) data from shark recaptures. At the end of this part of the study (i.e. the 2018 survey year), the absolute change in PCL was calculated over an individual's longest time at liberty (*C. melanopterus*, median = 25 d, quartiles = 16, 73, range = 9–300 d; *N. acutidens*, median = 43 d, quartiles = 21, 64, range = 2–395 d). Change in PCL was then regressed against thermal exposure as growing degree days (GDDs, °C days), which is a commonly used temperature-derived metric to model growth performance in fishes (Neuheimer & Taggart 2007).

For each 24 h period (i.e. 12:00–12:00 h), a degree day (DD) was calculated from temperature time series data recorded by temperature loggers deployed at each site using the following equation:

$$\mathrm{DD} = \left(\frac{T_{\mathrm{Max}} + T_{\mathrm{Min}}}{2}\right) - T_{\mathrm{o}} \quad (1)$$

where T_{Max} and T_{Min} are the maximum and minimum daily temperature, respectively (Chezik et al. 2014). A lower temperature threshold (T_{o}) of 18°C was used following the '10°C rule' of Charnov & Gillooly (2003) that would predict a temperature of zero growth/development at ~10°C below the mean developmental temperature of ~28°C. However, the lower temperature at which growth ceases is unknown for the study populations. For each recaptured shark, a GDD value was calculated as the sum of DD during the shark's time at liberty at the relevant site. Owing to variability in recapture rates between sites, change in PCL was modelled against GDD for each species across sites following a simple linear relationship (Neuheimer & Taggart 2007). Assumptions for linear regression were assessed through visual inspection of quantile-quantile plots, plots of residuals against fitted values, and plots of explanatory variables against fitted values as per Zuur et al. (2009). Change in PCL was also regressed against time at liberty for comparison to determine if GDD or time at liberty were better predictors of change in PCL. To account for possible effects of capture stress on growth, we excluded data for individuals with <14 d at liberty between captures. Further, owing to very small sample sizes (n < 3), we excluded individuals with >150 d at liberty between captures as outliers. We hypothesised that growth would demonstrate thermal dependence if GDD was a better linear predictor of growth than time at liberty. Coefficients of determination (R^2) and Akaike's information criterion (AIC; ΔAIC > 2) scores were used to compare GDD and time at liberty as linear predictors of change in PCL.

2.5. Effects of temperature on metabolic rate

For laboratory experiments, a subset of sharks was transported to a wetlab holding facility at the CRIOBE. Prior to departing, sharks were maintained in individual vinyl bags (0.2 m diameter and 1.0 m long), with mesh ends to allow water flow, for no more than 30 min post-capture. Sharks were then transported by car in 200 l insulated coolers in groups of 1–3 sharks per cooler. Water in the coolers was continuously aerated, and transport from the farthest site took approximately 30 min. At the CRIOBE, sharks were maintained in 1250 l circular holding tanks. Each species was housed in separate tanks; *C. melanopterus* were held in groups of 5 individuals and *N. acutidens* were held in groups of 4 individuals. At the wet lab holding facility, tanks were supplied continuously with sand-filtered seawater from Opunohu Bay. Holding tanks were covered with 60% shade cloth that exposed animals to a natural photoperiod. After approximately 1 wk of habituation, water temperatures were adjusted from ambient (~27°C at the time of experimentation) in 0.5°C d^{-1} increments using chillers (TK-1000/2000, TECO) to a target temperature of 28°C to facilitate comparison with previous studies by our group (Bouyoucos et al. 2020a); this temperature was maintained for 2 wk. Sharks were fed 5% of their body mass in fresh tuna (*Thunnus* spp.) supplied from a local restaurant every other day but were fasted for 48 h prior to experimentation to ensure that metabolic rate measurements were not influenced by digestion. After 22–32 d in captivity and following experimentation, sharks were released at their original capture sites using the transport methods described previously.

Captive sharks underwent intermittent-flow respirometry so that oxygen uptake rates ($\dot{M}O_2$, in mg O_2 kg^{-1} h^{-1}) could be measured *ex situ* as a proxy for metabolic rates (Svendsen et al. 2016). Specifically, routine $\dot{M}O_2$ ($\dot{M}O_{2Routine}$) is analogous to the routine metabolic rate (RMR) of an ectotherm, which is simply any metabolic rate measured from resting to maximum metabolic rate. However, for the purposes of this study, $\dot{M}O_{2Routine}$ reflects the average $\dot{M}O_2$ measured during a specified time at a given temperature (see below). Temperature-scaling was investigated by exposing sharks to rapid changes in ambient temperature simulating an extreme diel temperature cycle and measuring $\dot{M}O_{2Routine}$ at 5 sequential temperature steps: 28, 25, 28, 33, and 28°C (Di Santo & Bennett 2011). A representative trace of the experimental design is presented in Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m682p137_supp.pdf. The 8°C difference is representative of the maximum daily temperature range observed *in situ* (~10°C; environmental temperature range = 26–36°C). In addition, 25°C was the lower limit for the chilling capacity of our equipment, and animals could not be kept safely above 33°C (Bouyoucos et al. 2020a). Further, 33°C only provides a thermal safety margin of 3°C below the upper thermal limits of both species (Bouyoucos et al. 2021). This experimental design also made it possible to test for effects of heating and cooling on $\dot{M}O_2$ by consistently returning to 28°C.

After acclimating to 28°C for 2 wk, sharks were transferred from holding tanks to respirometry chambers and habituated at 28°C for 6 h. Oxygen uptake rates were then recorded over the final 2 h at 28°C. Water temperature was then cooled to 25°C at a rate of 0.88 ± 0.18°C h^{-1} (mean ± SD) using the same heater/chiller described above. Oxygen uptake rates were measured for 2 h once the system stabilised at 25°C, after which water temperature was heated to 28°C at a rate of 1.15 ± 0.44°C h^{-1}. Oxygen uptake rates were measured for 2 h upon reaching 28°C, after which water temperature was further heated to 33°C at a rate of 1.12 ± 0.27°C h^{-1}. Oxygen uptake rates were measured for 2 h at 33°C, after which water temperature was cooled back to 28°C at a rate of 1.35 ± 0.25°C h^{-1} for a final 2 h of measurement. Overall, heating (1.14 ± 0.34°C h^{-1}) and cooling rates (1.12 ± 0.32°C h^{-1}) were similar, but greater than maximum *in situ* rates of temperature change (0.53 ± 0.33°C h^{-1}). In total, $\dot{M}O_2$ was measured in three 2 h periods at 28°C, for 2 h at 25°C, and for 2 h at 33°C, for a total trial duration (excluding habituation) of 20.5–25 h.

Static, intermittent-flow respirometry chambers (32 l volume, including recirculating loop tubing; 70 cm long, 24 cm in diameter) were submerged in an aerated, covered water bath (550 l) that was temperature-controlled using an aquarium chiller (TK-1000/2000, TECO). Up to 4 chambers were tested at a time in the same water bath; all *C. melanopterus* (n = 10) were tested in 3 trials, whereas all *N. acutidens* (n = 8) were tested in 2 trials. Chambers were configured with a 2500 l h^{-1} aquarium pump (EHEIM) connected to the chamber in a closed, recirculating loop to ensure homogeneous mixing of oxygenated water. A second pump was configured to flush water from the external bath into individual chambers and out through overflow tubing above the water surface. Dissolved oxygen (DO, in mg O_2 l^{-1}) concentrations were measured using robust fibre-optic probes (PyroScience) that were directed into chambers through the overflow tubing. Probes were connected to a Firesting Optical Oxygen Meter (PyroScience). A single temperature probe was connected to the oxygen meter and placed into the water bath to allow for temperature compensation of DO measurements; the oxygen meter compensated for barometric pressure with an internal meter and salinity with a manually input value. The flush pump was controlled by a relay device that was automated using custom software on a laptop computer (National Instruments). The relay turned the flush pumps off for 5 min to yield a measurable decline in DO inside chambers (i.e. the 'measurement' phase). Flush pumps were then turned on for 10 min to reintroduce oxygenated water from the bath and restore DO inside chambers to saturation (i.e. the 'flush' phase). A single flush-measurement phase cycle lasted 15 min, and 2 h of $\dot{M}O_2$ measurement at each target temperature yielded 8 $\dot{M}O_2$ values per shark. In total, 40 $\dot{M}O_2$ determinations were made per shark, with up to 4 sharks tested per trial. At the conclusion of trials, sharks were returned to their holding tanks, acclimatised to ambient conditions over several days, and subsequently released at their original site of capture.

Oxygen uptake rate was calculated following:

$$\dot{M}O_2 = SV_{Resp}M^{-1} \quad (2)$$

where S is the absolute value of the slope of the linear decline in DO during a 5 min measurement phase with a coefficient of determination >0.95 (in mg O_2 l^{-1} s^{-1}), V_{Resp} is the volume of water (in litres) in the chamber (i.e. excluding the volume of the shark), and M is the mass of the shark (in kg), measured upon removal from the respirometry chamber. Values of S were extracted from raw Firesting meter output in R using custom code (A. Merciere and T. Norin unpub-

lished data). At each temperature (i.e. 25, 28, and 33°C), a single $\dot{M}O_{2Routine}$ value was calculated for each shark as the mean value across all measurements. To account for exogenous sources of respiration inside chambers (i.e. background respiration), $\dot{M}O_2$ measurements were made in empty chambers immediately before and after trials with sharks. Background $\dot{M}O_2$ was accounted for by fitting a line to the 2 background $\dot{M}O_2$ values that were measured at a known time, predicting the value of background $\dot{M}O_2$ at the time of measurements when sharks were inside chambers, and subtracting background $\dot{M}O_2$ from shark $\dot{M}O_2$ (Rummer et al. 2016).

This approach made it possible to calculate a temperature-scaling quotient (Q_{10}) for $\dot{M}O_{2Routine}$ that characterises the change in $\dot{M}O_{2Routine}$ over a 10°C change in temperature and is calculated using the following equation:

$$Q_{10} = (K_2 \times K_1^{-1})^{[10 \times (t_2 - t_1)^{-1}]} \quad (3)$$

where K_1 and K_2 are $\dot{M}O_2$ at temperatures t_1 and t_2, respectively. Temperature-scaling quotients were calculated for individuals of each species between each temperature step at 28 to 25°C, 25 to 28°C, 28 to 33°C, and 33 to 28°C. In addition, overall Q_{10} values were calculated from 25 to 33°C.

Temperature effects on $\dot{M}O_{2Routine}$ were investigated using linear mixed effects models. For each species, $\dot{M}O_{2Routine}$ was modelled as a function of temperature step (i.e. 28°C [1], 25°C, 28°C [2], 33°C, and 28°C [3]) as a categorical fixed effect with shark ID as a random effect. Models were validated through visual inspection of plots of model residuals and fitted values (to test homogeneity), model residuals and fixed effects (to test independence), and quantile-quantile plots of model residuals (to test normality); model assumptions were deemed reasonable. Similarly, Q_{10} was investigated for each species by modelling Q_{10} as a function of temperature range (i.e. 28 to 25°C, 25 to 28°C, 23 to 33°C, and 33 to 28°C) as a categorical fixed effect with shark ID as a random effect. Significance was determined by generating confidence intervals of effect size for fixed effects terms with posterior simulations (n = 1000).

3. RESULTS

3.1. Identifying shark nursery areas

No sites exhibited above-average *Carcharhinus melanopterus* CPUE from 2015 to 2020, and Apaura-Vaiane was the only site where CPUE of *Negaprion acutidens* was significantly higher than the population mean and therefore satisfied criterion 1 (Fig. 2A,B). According to the first ecological criterion, no other sites (Haapiti, Maharepa, Paorea, Papetoai, Pihaena, Tiki, Vaiare, or Valorie) were considered consistent nursery areas for either species. Recapture rates were highest for *C. melanopterus* at Maharepa (Table 2, Fig. 2C), whereas recapture rates for *N. acutidens* did not differ among sites (Table 2, Fig. 2D). There was no effect of survey year on relative abundance of *C. melanopterus* and no interactions between survey year and site (Table 3, Fig. 2E). Conversely, there was a positive effect of survey year on relative abundance of *N. acutidens*, which was largely driven by the Apaura-Vaiane site (Table 3, Fig. 2F). Therefore, Apaura-Vaiane consistently met all 3 shark nursery area criteria for *N. acutidens*, but no site consistently met all 3 ecological criteria for being a nursery for *C. melanopterus*.

Evaluating the nursery area criteria within each survey year revealed potential nursery areas for *C. melanopterus*. Sites that satisfied criterion 1 for this species were Haapiti in 2018, Maharepa in 2016 and 2019, Paorea in 2017 and 2018, and Valorie in 2018 (Fig. S2). Only Maharepa in 2016 and 2019, and Paorea in 2017 and 2018 met criterion 2 (Fig. S3). Of these, Maharepa in 2016 and 2019 and Paorea in 2018 met criterion 3 (Table S1, Fig. S4). Therefore, Maharepa was a potential nursery area for *C. melanopterus* in 2016 and 2019, and Paorea was a potential nursery area for *C. melanopterus* in 2018.

There were no potential nursery areas identified for *N. acutidens*. Apaura-Vaiane satisfied criterion 1 during each survey year (Fig. S5) and criterion 2 in each season except for 2016 when only a single individual was recaptured (Fig. S6). The relative abundance of *N. acutidens* decreased during the 2015, 2018, and 2019 survey years (Table S1, Fig. S7). Only Apaura-Vaiane in 2017 met all 3 criteria to be classified as a potential nursery area; however, this site is still considered a shark nursery area for *N. acutidens* because all 3 criteria were satisfied on an interannual basis across the full 5 yr study.

3.2. Effects of temperature on growth

There was a strong positive relationship between GDD and growth (i.e. increase in PCL during time at liberty) in *C. melanopterus* (mean effect size = 0.048 mm of PCL per GDD, 95% confidence interval = 0.031–0.066; R^2 = 0.409, Fig. 3A) and *N. acuti-*

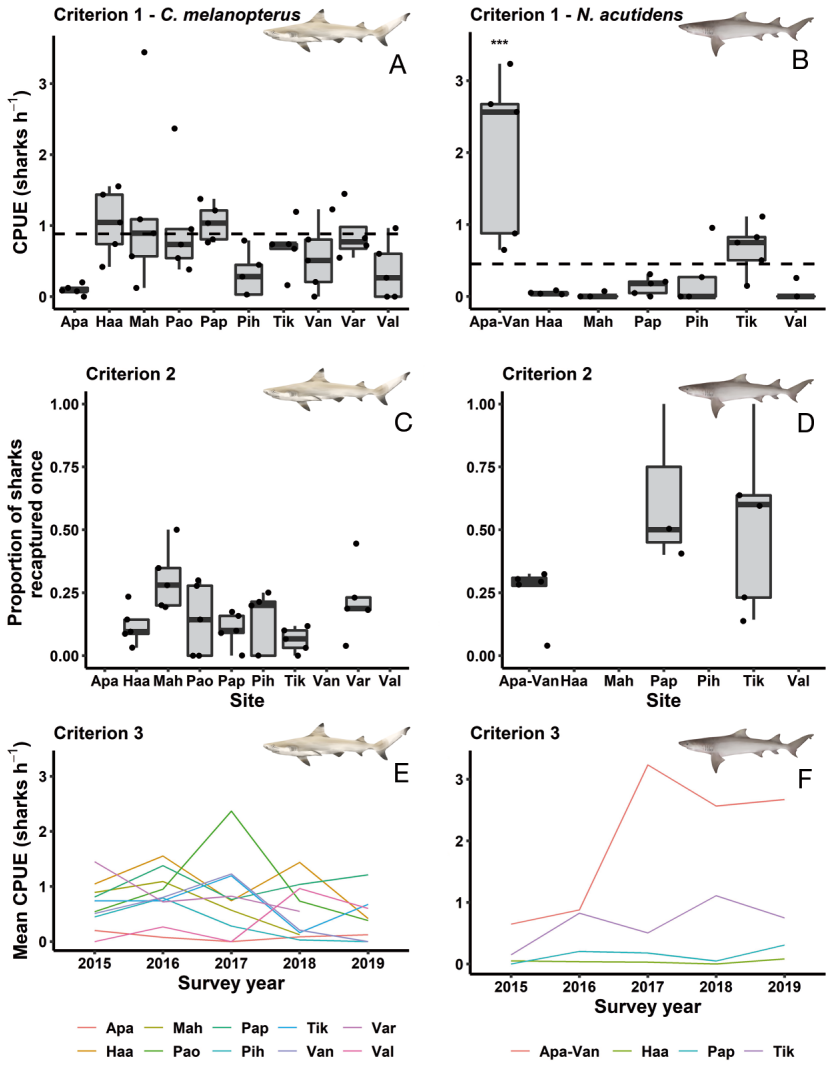

Fig. 2. Testing shark nursery area criteria for (A,C,E) *Carcharhinus melanopterus* and (B,D,F) *Negaprion acutidens* around Moorea, French Polynesia. To qualify as a shark nursery area, habitats must exhibit higher relative neonate abundance (criterion 1; A,B), neonates must exhibit residency (criterion 2; C,D), and habitat use must be stable through time (criterion 3; E,F). Abundance surveys were conducted over 5 consecutive parturition seasons from 2015 to 2020. Criterion 1 was assessed relative to the mean catch per unit effort (CPUE) of the entire study (horizontal dashed line), where asterisks denote sites that differ significantly in CPUE from that mean. Box plots represent the median and quartiles and whiskers represent the most extreme observations within 1.5 times the interquartile range. Individual observations of CPUE (sharks h^{-1}) represent mean values per survey year within a site (n = 5 observations per site). Criterion 2 was assessed between sites. Individual observations represent the proportion of individuals that were recaptured at least once each survey year within each site (n = 4 observations per site for *C. melanopterus*; n = 3–5 observations per site for *N. acutidens*). Finally, criterion 3 was assessed between survey years (n = 10 sites yr^{-1} for *C. melanopterus*; n = 4 sites yr^{-1} for *N. acutidens*). Individuals of *N. acutidens* were recaptured between 2 sites (Apaura and Vaiane), and so these were combined into one for analysis (i.e. Apaura-Vaiane). Apa: Apaura; Apa-Van: Apaura-Vaiane; Haa: Haapiti; Mah: Maharepa; Pao: Paorea; Pap: Papetoai; Pih: Pihaena; Tik: Tiki; Van: Vaiane; Var: Vaiare; Val: Valorie. Illustrations by Erin Walsh

dens (mean effect size = 0.052 mm of PCL per GDD, 95% confidence interval = 0.030–0.074; R^2 = 0.427; Fig. 3B). Similarly, time at liberty was positively correlated with growth (*C. melanopterus*, mean effect size = 0.818 mm of PCL d^{-1}, 95% confidence interval = 0.633–1.003, R^2 = 0.630, Fig. 3C; *N. acutidens*, mean effect size = 0.621 mm of PCL d^{-1}, 95% confidence interval = 0.361–0.881, R^2 = 0.424, Fig. 3D). For *C. melanopterus*, AIC scores indicated that time at liberty (AIC = 383.88) was a better linear predictor of change in mass than GDD (AIC = 405.95); conversely, time at liberty (AIC = 291.78) was an equally good predictor of change in mass relative to GDD (AIC = 291.57) in *N. acutidens*.

3.3. Effects of temperature on metabolic rate

Temperature step had significant effects on $\dot{M}O_{2Routine}$ in *C. melanopterus* and *N. acutidens* (Table 4). In *C. melanopterus* (Fig. 4A), $\dot{M}O_{2Routine}$ decreased upon initially lowering the temperature to 25°C, and then increased until 33°C. In *N. acutidens*, temperature effects were most apparent between the 25 and 33°C steps (Fig. 4B). Overall, mean $\dot{M}O_{2Routine}$ was 112.9 mg O_2 kg^{-1} h^{-1} at 25°C, 140.7 mg O_2 kg^{-1} h^{-1} at 28°C, and 167.6 mg O_2 kg^{-1} h^{-1} at 33°C for *C. melanopterus* and 138.9 mg O_2 kg^{-1} h^{-1} at 25°C, 170.2 mg O_2 kg^{-1} h^{-1} at 28°C, and 195.29 mg O_2 kg^{-1} h^{-1} at 33°C for *N. acutidens*. In general, Q_{10} did not differ whether heating or cooling was occurring. Instead, Q_{10} for *C. melanopterus* was greater within the cooler range of temperatures (Q_{10} = 2.92 from 28 to 25°C and 2.44 from 25 to 28°C) than within the warmer range of temperatures (Q_{10} = 1.43 from 28 to 33°C and 1.71 from 33 to 28°C). No trend with Q_{10} and temperature range was apparent for *N. acutidens*. Temperature quotients (Q_{10}) across the entire

Table 2. Generalised linear model output to test shark nursery area criterion 2 (i.e. neonates exhibit residency). Mean effect size and 2.5 and 97.5% confidence interval limits are presented

Response	*Carcharhinus melanopterus*				*Negaprion acutidens*			
	Site	Mean	2.5%	97.5%	Site	Mean	2.5%	97.5%
Proportion of individuals recaptured once	Haapiti	−1.68	−2.41	−0.94	Apaura-Vaiane	−0.96	−1.87	−0.05
	Maharepa	−0.63	−1.37	0.10	Papetoai	0.00	−1.44	1.44
	Paorea	−1.73	−2.47	−0.99	Tiki	−0.24	−1.26	0.77
	Papetoai	−1.81	−2.55	−1.08				
	Pihaena	−1.77	−2.51	−1.03				
	Tiki	−2.15	−2.88	−1.41				
	Vaiare	−1.13	−1.87	−0.40				

Table 3. Weighted least-squares regression output to test shark nursery area criterion 3 (i.e. consistent habitat use over time). Mean effect size and 2.5 and 97.5% confidence interval limits are presented

Carcharhinus melanopterus				*Negaprion acutidens*			
Parameter	Mean	2.5%	97.5%	Parameter	Mean	2.5%	97.5%
Apaura	−3.07	−4.68	−1.76	Apaura-Vaiane	−1.97	−2.34	−1.62
Haapiti	−0.63	−1.03	−0.25	Haapiti	−4.72	−7.47	−2.76
Maharepa	−1.49	−1.99	−1.04	Papetoai	−4.61	−6.48	−3.22
Paorea	−0.99	−1.53	−0.50	Tiki	−2.69	−3.28	−2.16
Papetoai	−1.19	−1.63	−0.80	Survey year	0.29	0.19	0.39
Pihaena	−1.11	−1.81	−0.46	Survey year × Haapiti	−0.22	−0.90	0.48
Tiki	−1.39	−1.81	−0.99	Survey year × Papetoai	0.06	−0.32	0.50
Vaiane	−1.08	−1.71	−0.50	Survey year × Tiki	0.00	−0.18	0.19
Vaiare	−1.14	−1.66	−0.67				
Valorie	−4.05	−5.58	−2.83				
Survey year	−0.19	−0.77	0.32				
Survey year × Haapiti	−0.02	−0.55	0.56				
Survey year × Maharepa	0.19	−0.35	0.78				
Survey year × Paorea	0.10	−0.44	0.70				
Survey year × Papetoai	0.12	−0.41	0.71				
Survey year × Pihaena	−0.31	−0.91	0.33				
Survey year × Tiki	0.08	−0.46	0.67				
Survey year × Vaiane	−0.25	−0.83	0.37				
Survey year × Vaiare	0.12	−0.42	0.72				
Survey year × Valorie	0.72	0.11	1.39				

25–33°C range were 1.72 and 1.61 in *C. melanopterus* and *N. acutidens*, respectively.

4. DISCUSSION

The purpose of this study was to combine ecological and physiological approaches to better understand nursery area use in 2 sympatric reef shark populations. Our findings demonstrated that 2 reef shark neonate populations use shallow, coastal habitats as nursery areas to various degrees, and that both populations exhibit minimal thermal dependence of physiological performance, which corroborates previous findings on thermal physiology in *Carcharhinus melanopterus* (Bouyoucos et al. 2020a). Indeed, these data support the hypothesis that populations that reside in coastal environments for considerable periods of time, such as neonates that rely on nursery areas, should exhibit lower thermal dependence of performance than more transient populations (Lear et al. 2019); however, this hypothesis is likely quite species- and habitat-specific (e.g. pelagic vs. coastal species). Temperature may therefore be an important driver underlying the 3 proposed ecological nursery area criteria through its effects on physiological performance and possibly abundance in some species.

Both study species relied, to some extent, on habitats around the island of Moorea as nursery areas. This approach led us to conclude that *Negaprion acutidens* consistently use one site as a nurs-

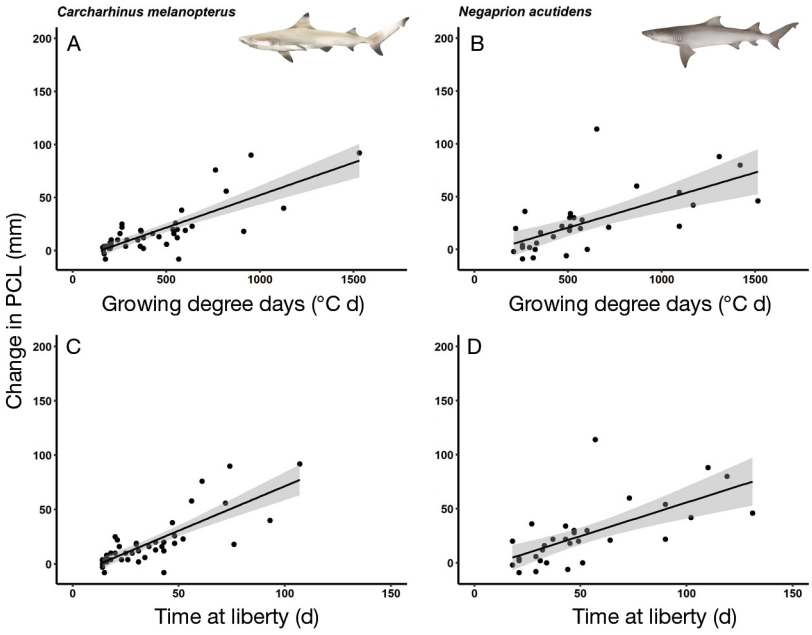

Fig. 3. Growth (i.e. change in precaudal length; PCL) as a function of (A,B) growing degree days and (C,D) time at liberty in neonatal *Carcharhinus melanopterus* (n = 47) and *Negaprion acutidens* (n = 32). Growth was measured in free-ranging sharks that were recaptured after 14 to 150 d at liberty. Growing degree days (GDDs) were calculated as the sum of degree days during the time at liberty of an individual shark, where a degree day equals the average of the minimum and maximum environmental temperature during a single calendar day within the habitat of a shark its time at liberty. Individual observations represent growth and GDDs or time at liberty for individual sharks. Shading around regression lines represents 95% confidence interval. Illustrations by Erin Walsh

ery area, but all remaining other sites were non-nursery areas for this species. We could not identify any single site as a consistent nursery area for *C. melanopterus*, although we did identify 2 different sites as potential *C. melanopterus* nursery areas during different years of the study. However, although we observed evidence of nursery areas for *N. acutidens* and *C. melanopterus* over various temporal and spatial scales, the ecological benefits that these species derive from Moorea's nursery areas remain unclear. The Apaura-Vaiane nursery area for *N. acutidens* is likely not unique in offering refuge from predation, as all habitats where sharks were collected in this study were shallow enough to exclude predators. Moreover, the Apaura-Vaiane nursery area likely does not provide unique access to ample food resources, as foraging success of *C. melanopterus* caught at this site was deemed low (Weideli et al. 2019a). Nursery area use is also not necessarily associated with prey availability or quality, as has been demonstrated in some populations of scalloped hammerhead (Bush & Holland 2002, Duncan & Holland 2006) and blacktip sharks (Heupel & Hueter 2002). Alternatively, *C. melanopterus* and *N. acutidens* neonates could derive benefits associated with social learning interactions within nursery areas (Jacoby et al. 2012); however, no study has quantified sociality in *N. acutidens* or in *C. melanopterus* neonates. Thus, the potential ecological benefits that neonatal *C. melanopterus* and *N. acutidens* would derive from using nursery areas around Moorea require further examination.

Reef shark neonates exhibited minimal temperature dependence of growth *in situ*. Changes in body length (PCL) of sharks recaptured up to over 1 yr at

Table 4. Model outputs of the effects of temperature on oxygen uptake rates of reef sharks. For mixed effects models, mean effect size, and 2.5 and 97.5% confidence interval limits are presented. $\dot{M}O_{2Routine}$: routine oxygen uptake rate; Q_{10}: temperature quotient

Model	Parameter	*Carcharhinus melanopterus*			*Negaprion acutidens*		
		Mean	2.5%	97.5%	Mean	2.5%	97.5%
$\dot{M}O_{2Routine}$ ~	28°C [1]	148.24	129.32	167.16	194.75	157.91	233.67
Temperature	25°C	112.49	92.78	132.13	139.72	98.97	177.18
Step + Shark ID	28°C [2]	142.65	123.55	162.39	149.60	113.32	187.37
(random)	33°C	167.19	147.96	187.68	195.73	159.04	232.96
	28°C [3]	130.34	111.46	147.96	166.24	128.09	203.99
Q_{10} ~	25 to 28°C	2.46	1.90	3.09	1.62	0.86	2.43
Temperature	28 to 25°C	2.90	2.29	3.50	3.86	3.09	4.70
Range + Shark ID	28 to 33°C	1.42	0.83	1.98	1.77	0.98	2.52
(random)	33 to 28°C	1.70	2.32	1.12	1.42	0.69	2.15

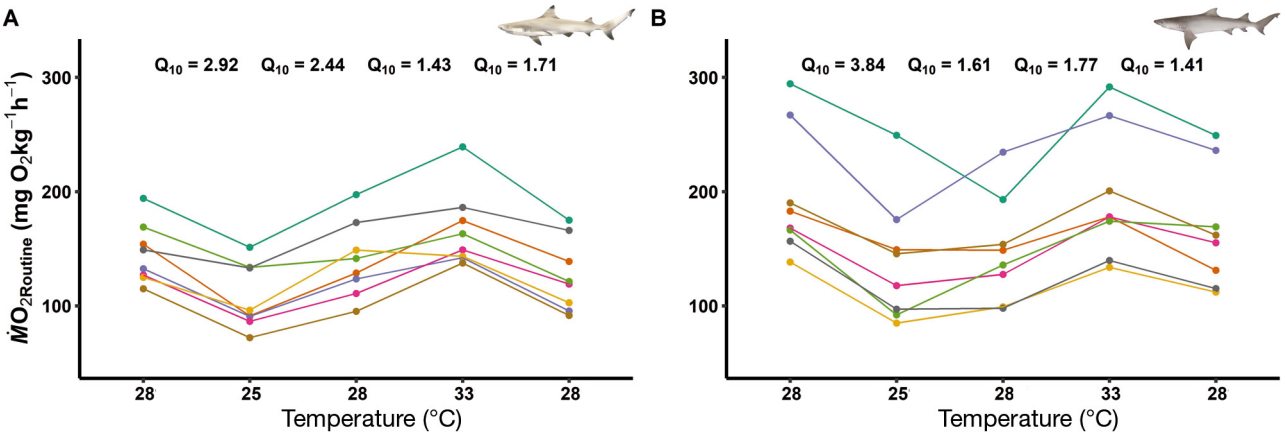

Fig. 4. Temperature-scaling of routine oxygen uptake rates ($\dot{M}O_{2Routine}$) in (A) *Carcharhinus melanopterus* (n = 10) and (B) *Negaprion acutidens* (n = 8). Differing colours denote different individuals within each species. Temperature quotients (Q_{10}) are presented for each change in temperature (i.e. 28–25, 25–28, 28–33, and 33–28°C) and were calculated from average $\dot{M}O_{2Routine}$. Individual observations represent values for individual sharks. Illustrations by Erin Walsh

liberty did not vary with thermal exposure, demonstrating that there was no measurable effect of temperature on growth in either species *in situ*. In species that exhibit temperature-dependent growth, the GDD metric is a better linear predictor of growth than time (Neuheimer & Taggart 2007). In our study, GDD was not a better linear predictor of changes in PCL than time at liberty, which does not support the notion of temperature-dependent growth in either species. These data corroborate absent effects of temperature on growth rates observed in *C. melanopterus*, *ex situ*, between 28 and 31°C (Bouyoucos et al. 2020a); however, this relatively small temperature range and variable growth rates may have obfuscated possible temperature effects on growth, despite the experimental duration in Bouyoucos et al. (2020a) approximating the median time at liberty for *C. melanopterus* in the present study. It is possible that average summer temperatures did not vary enough (28–32°C) for growth to exhibit strong temperature dependence, despite daily temperature fluctuations ranging from 22 to 37°C. Another explanation could be that highly variable growth rates, as documented in both species in a nursery area in Seychelles (Weideli et al. 2019b), could confound possible thermal effects. A third explanation could be related to foraging success, given that food availability is also known to interact with temperature to affect growth rate in fishes (McLeod et al. 2013, Cominassi et al. 2020). Indeed, gastric lavage of neonatal *C. melanopterus* around Moorea demonstrated that only 47% of neonates had stomach contents at the time of capture, which suggests low foraging success in this population (Weideli et al. 2019a). Reduced thermal sensitivity of growth could be an adaptive response to high variability in daily temperatures. For example, high growth rates are selected against in a congener of *N. acutidens*, *N. brevirostris*, owing to increased predation risk for larger, younger, and more naïve juveniles (DiBattista et al. 2007, Hussey et al. 2017).

Minimal temperature sensitivity of *ex situ* routine metabolic rates across an ecologically relevant temperature range further supports the prediction of similar patterns of habitat use in *C. melanopterus* and *N. acutidens* neonates. The Q_{10} values were 1.72 and 1.61 in *C. melanopterus* and *N. acutidens*, respectively, across the entire 25–33°C range. Comparatively, juvenile *C. leucas* and *Pristis pristis* exhibited similarly low Q_{10} values of 1.88 and 1.58, respectively; however, routine metabolic rates were measured *in situ* using a static, annular respirometry system that allows for swimming, and animals experienced diel temperatures ranging from 20 to 32°C (Lear et al. 2020). Low temperature sensitivity of routine metabolic rates in neonatal *C. melanopterus* and *N. acutidens* could reflect some capacity to restore $\dot{M}O_{2Routine}$ to pre-heating/cooling levels (i.e. $\dot{M}O_{2Routine}$ at 28°C), possibly because these animals can experience as much as 8°C in diel temperature fluctuations (Bouyoucos et al. 2020a). Here, neonatal *C. melanopterus* and *N. acutidens* could exhibit low thermal sensitivity of metabolic rates because diel temperature variability is greater than seasonal variability (da Silva et al. 2019). Indeed, diel temperature range can be as high as 8°C, whereas the average seasonal temperature range is less than 2°C (average wet season temperature = 29.6°C, average dry sea-

son temperature = 28.0°C). Differences in diel and seasonal temperature variation are reflected in observed differences in Q_{10} with duration of thermal exposure in *C. melanopterus*. For instance, longer exposures (i.e. 4 wk) to increased temperatures yielded higher Q_{10} values of 2.24 and 2.07 for standard and maximum metabolic rates, respectively (Bouyoucos et al. 2020a), although metabolic rate may be differentially sensitive to exposure to static temperature regimes versus fluctuating regimes (Morash et al. 2018). Indeed, the timescale and stability of thermal exposure used in laboratory studies should receive careful consideration when attempting to make claims about metabolic rates in the field. Therefore, although seasonal temperatures around Moorea are stable and within a narrow range, highly variable daily temperatures should produce phenotypes among neonatal *C. melanopterus* and *N. acutidens* that exhibit a small thermal acclimation response (Healy & Schulte 2012).

Routine metabolic rates of neonatal *C. melanopterus* were less sensitive to temperature changes at higher temperatures relative to lower temperatures. This suggests a physiological mechanism by which neonatal *C. melanopterus* — and possibly *N. acutidens* — tolerate temperatures approaching their upper thermal limits whilst remaining in nursery area habitat. In *C. melanopterus*, routine metabolic rates were less sensitive to temperature changes between higher temperatures (i.e. 28–33°C; Q_{10} = 1.70) than between lower temperatures (i.e. 25–28°C; Q_{10} = 2.92); yet, there was no evidence of directional warming or cooling effects on Q_{10}. Conversely, there were no apparent effects of temperature range or the direction of temperature change on Q_{10} in *N. acutidens*. A lower Q_{10} at higher temperatures (i.e. 28–33°C) relative to lower temperatures (i.e. 25–28°C) in *C. melanopterus* could reflect metabolic compensation, where sharks are actively regulating their metabolic rate to counteract thermodynamic effects of temperature change on their metabolism. However, the lowest Q_{10} for *C. melanopterus* (1.43, measured at 28–33°C) was still considerably greater than 1 (Sandblom et al. 2014). Further, differences in spontaneous activity at different temperatures during respirometry may have been confounding (Speers-Roesch et al. 2018). For instance, *C. melanopterus* are generally more active than *N. acutidens* (Baldwin & Wells 1990), which could explain the difference in trends between the 2 species. Moreover, Q_{10} in *C. melanopterus* may have been lower at 28–33°C relative to 25–28°C because of reduced activity at 33°C, which is close to the maximum habitat temperature at some sites where *C. melanopterus* occur around Moorea. Finally, confounding effects of stress must be considered, because static respirometry systems are known to induce stress in fishes (Murray et al. 2017). Whilst the nuances of thermal acclimation capacity and sensitivity in sharks warrant further investigation, this study corroborates an overall diminished sensitivity of metabolic rates to high temperatures in sharks confined to nursery areas.

In conclusion, this study demonstrates how physiological concepts can complement ecological concepts to better understand how fishes use coastal habitats such as nursery areas. Indeed, this study advances ecological nursery area concepts by offering physiological explanations of how reef shark neonates exploit shallow, nearshore environments, including nursery areas. Moving forward, it will be imperative to define eco-physiological mechanisms that link abiotic conditions and physiological performance to fitness to predict effects of climate change for shark nursery areas (e.g. Crear et al. 2019, 2020). This will involve phenomenological investigations of shark ecology complemented by reductionist physiological approaches (e.g. Lear et al. 2019, 2020). However, as more research emerges to suggest new anthropogenic pathways to reduce fitness in sharks, conservation issues, like climate change, will urgently need to be addressed.

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Data availability. Data presented in this manuscript are available from the Research Data Repository (Tropical Data Hub) at James Cook University: https://doi.org/10.25903/5eb256b915630

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