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Investigating links between thermal tolerance and oxygen supply capacity in shark neonates from a hyperoxic tropical environment

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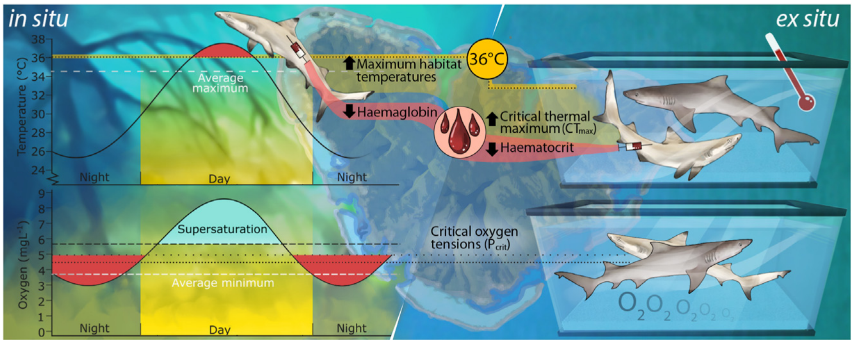
HIGHLIGHTS

- Blood-oxygen carrying capacity may predict thermal tolerance limits in some fishes.
- We tested this prediction in shark neonates living in hyperoxic tropical habitats.
- Haemoglobin concentration and maximum habitat temperature were inversely associated.
- Haematocrit and critical thermal maximum (CT_{Max}) were inversely associated *ex situ*.
- A role for oxygen supply capacity in haematocrit-CT_{Max} associations is equivocal.

GRAPHICAL ABSTRACT



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ABSTRACT

Temperature and oxygen limit the distribution of marine ectotherms. Haematological traits underlying blood-oxygen carrying capacity are thought to be correlated with thermal tolerance in certain fishes, and this relationship is hypothesised to be explained by oxygen supply capacity. We tested this hypothesis using reef shark neonates as experimental models because they live near their upper thermal limits and are physiologically sensitive to low oxygen conditions. We first described *in situ* associations between temperature and oxygen at the study site (Moorea, French Polynesia) and found that the habitats for reef shark neonates (*Carcharhinus melanopterus* and *Negaprion acutidens*) were hyperoxic at the maximum recorded temperatures. Next, we tested for *in situ* associations between thermal habitat characteristics and haematological traits of neonates. Contrary to predictions, we only demonstrated a negative association between haemoglobin concentration and maximum habitat temperatures in *C. melanopterus*. Next, we tested for *ex situ* associations between critical thermal maximum (CT_{Max}) and haematological traits, but only demonstrated a negative association between haematocrit and CT_{Max} in *C. melanopterus*. Finally, we measured critical oxygen tension (p_{crit}) *ex situ* and estimated its temperature sensitivity to predict oxygen-dependent values of CT_{Max}. Estimated temperature sensitivity of p_{crit} was similar to reported values for sharks and skates, and predicted values for CT_{Max} equalled maximum habitat temperatures.

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These data demonstrate unique associations between haematological traits and thermal tolerance in a reef shark that are likely not explained by oxygen supply capacity. However, a relationship between oxygen supply capacity and thermal tolerance remains to be demonstrated empirically.

1. Introduction

The way in which temperature affects physiological performance of ectotherms is linked to species' biogeography and the potential for local extirpations under climate change (Pörtner et al., 2017; Sunday et al., 2012). In response to temperature variation, species can physiologically acclimate to maintain performance of Darwinian fitness traits and behaviourally select favourable temperatures for performance (Angilletta Jr. et al., 2006). These physiological and behavioural strategies serve to establish a 'thermal safety margin' (TSM), which defines the difference between an organism's body temperature and its thermal tolerance limits (Sunday et al., 2014). Thermal safety margins decrease with increasing temperature, and tropical ectotherms appear to have the smallest TSMs (Payne and Smith, 2017; Sunday et al., 2019). Climate change is increasing air and sea surface temperatures and the frequency and duration of heatwaves globally (Bindoff et al., 2019). As such, global warming puts tropical ectotherms at a greater risk of shifts in distribution patterns or even mortality relative to temperate species (Pinsky et al., 2019). Because the capacity to tolerate high temperatures is an important fitness-related trait, thermal tolerance has been extensively investigated in laboratory studies.

Thermal tolerance can be quantified by measuring the critical thermal maximum (CT_{Max}) metric, which defines the upper temperature where an organism loses motor control following acute exposure (Lutterschmidt and Hutchison, 1997a; MacMillan, 2019). Much of what is known about CT_{Max} in ectotherms comes from studies on fishes (Beitinger and Bennett, 2000; Lutterschmidt and Hutchison, 1997a). While not a lethal endpoint, CT_{Max} is essentially 'ecological death', meaning that the organism would no longer be able to serve a functional ecological role in its environment, and survival is limited. Beyond laboratory approaches, CT_{Max} can be estimated from species' geographic ranges, where the warmest summer temperatures encountered at the equatorial extent are considered proxies for CT_{Max} (Sunday et al., 2019); although, this approach has highlighted that laboratory estimates are not always representative of *in situ* limits (Payne et al., 2016).

Oxygen availability is another important environmental factor that limits the distribution of water-breathing ectotherms. In water, oxygen concentrations are already quite low (*i.e.*, ~30% lower in water compared to air). Hypoxia (*i.e.*, low oxygen availability) is a widespread phenomenon in the oceans, such as oxygen minimum zones in the deep-sea (Seibel, 2011), and limits the distribution of water-breathers. Climate change is further reducing oxygen availability in aquatic ecosystems, partially because of global warming (Breitburg et al., 2018), and this could further affect species' distributions and survival (Wishner et al., 2018). However, the oxygen availability that constitutes hypoxia differs between species and is related to the sensitivity of an organism's physiological systems to changes in environmental oxygen. For instance, an often-cited hypoxia threshold of 2 mg O_2 L^{-1} would be fatal for the sandbar shark (*Carcharhinus plumbeus*; Crear et al., 2019), but not the clearnose skate (*Rostaraja eglanteria*; Schwieterman et al., 2019b). Thus, defining hypoxia tolerance is also key in predicting the effects of climate change on water-breathers.

A common measure of hypoxia tolerance, the critical oxygen tension (p_{crit}), defines the oxygen partial pressure (pO_2) below which metabolic rate declines with environmental pO_2 (Ultsch and Regan, 2019; Wood, 2018). Typically, p_{crit} is measured when an organism has achieved its standard metabolic rate (Rogers et al., 2016), which represents the metabolic rate of a postprandial organism at rest and at a stable temperature (Chabot et al., 2016). However, it has recently been suggested that p_{crit} is not an ecologically relevant measure of hypoxia tolerance (Wood, 2018) and simply represents a rate-specific measure of oxygen supply capacity (Seibel and Deutsch, 2020). Thus, when environmental oxygen reaches p_{crit} for a given metabolic rate, an organism's physiological oxygen supply capacity (α = metabolic rate $\cdot$ p_{crit}^{-1}) is being fully utilised (Seibel and Deutsch, 2020). As such, it has been suggested that the oxygen level at which maximum metabolic rate (MMR) is reduced (*i.e.*, $p_{crit\text{-}max}$) is a more ecologically relevant index of hypoxia tolerance (Seibel and Deutsch, 2020).

Temperature and oxygen act together on the physiology of marine ectotherms to set biogeographic range limits. This phenomenon reflects the temperature dependence of an organism's hypoxia tolerance (Deutsch et al., 2020). Conversely, the oxygen dependence of thermal tolerance is less clear (MacMillan, 2019). One hypothesis, oxygen- and capacity-limited thermal tolerance (OCLTT), posits that oxygen supply capacity limits thermal tolerance in ectotherms (Pörtner et al., 2017). One prediction of this hypothesis is that haematological traits (*e.g.*, haemoglobin concentration, haematocrit), which influence blood-oxygen carrying capacity and MMR, are positively correlated with CT_{Max} (Pörtner et al., 2017). Only a handful of studies have demonstrated intra-specific (Muñoz et al., 2018) and inter-specific (Beers and Sidell, 2011) associations between haematocrit (*i.e.*, the ratio of red blood cell volume to whole blood volume) and CT_{Max}, where others do not demonstrate such associations (Brijs et al., 2015; Wang et al., 2014). However, such associations do not suggest a mechanistic link, as even haematological traits do not always correlate with hypoxia tolerance traits (Mandic et al., 2009). Thus, future studies that continue to test this prediction ought to test whether oxygen supply capacity has a role in the presence or absence of such associations.

Coastal, tropical marine habitats are natural laboratories to study the role of oxygen-supply capacity in the relationship between haematological traits and thermal tolerance. Increases in water temperature correlate with increases (McArley et al., 2018) or decreases (Fangue et al., 2001) in oxygen in shallow, coastal habitats, such as rockpools. The relative amount of primary productivity and respiration occurring at high temperatures determines whether these habitats become hypoxic (*i.e.*, when respiration rates exceed photosynthesis rates) or hyperoxic (*i.e.*, when photosynthesis rates exceed respiration rates). In habitats that experience hyperoxia at high temperatures, it is thought that these oxygen conditions support high thermal tolerance by elevating aerobic performance (McArley et al., 2020). Therefore, if haematological traits and thermal tolerance are related, and this relationship is linked to oxygen supply capacity, supporting evidence should be observable in organisms from hot and supersaturated marine habitats (Giomi et al., 2019).

The purpose of this study was to test for associations between haematological traits and thermal tolerance and suggest a role for oxygen supply capacity in this relationship using species living near their thermal and oxygen limits. This was accomplished using reef shark neonates as experimental models because they have relatively high thermal tolerance (Bouyoucos et al., 2020a; Gervais et al., 2018) and relatively poor hypoxia tolerance (Routley et al., 2002). The first objective was to describe water temperatures and oxygen levels at the study site. The second objective was to test for *in situ* associations between thermal habitat characteristics and haematological traits in the two reef shark neonate populations at the study site. The third objective was to test for *ex situ* associations between experimentally derived thermal tolerance and physiological oxygen supply capacity in the same populations. Together, this study combines field and laboratory data to offer a novel perspective on whether haematological traits are reliable predictors of thermal tolerance as suggested in previous studies (Beers and Sidell, 2011; Muñoz et al., 2018). Further, these data can contribute to our understanding of the mechanistic basis of thermal tolerance in marine ectotherms and how tropical fishes might cope with ocean warming and deoxygenation now and in the future.

2. Material and methods

2.1. Ethical approval

All experimental procedures described herein were approved by the James Cook University Animal Ethics Committee (protocols A2089 and A2394). Permission to conduct research on protected shark species in French Polynesia was obtained from the Ministère de la Promotion des Langues, de la Culture, de la Communication, et de l'Environnement (Arrêtés N°9524, N°5129, and N°11491).

2.2. Study site

All research was conducted on Moorea, French Polynesia (S 17°30′; W 149°50′). Research effort was focused on ten sites (Apaura, Haapiti, Maharepa, Pointe de Paorea, Papetoai, Pihaena, Tiki, Vaiane, Vaiare, and Valorie) that span Moorea's 60-km coastline. These sites are typically less than 1 m deep, within 50 m of shore, and comprise a homogenous mix of corals, algal turfs, sandflats, and rock substrate. All sites are contained within Moorea's narrow lagoon system that experiences little variation in tide height (~20–30 cm).

2.3. Objective 1

Our first objective was to describe temperature and oxygen conditions of the ten study sites. This involved describing associations between water temperature and dissolved oxygen concentration (DO, in mg O_2 L^{-1}), rates of oxygen decline, and rates of warming. First, paired temperature and DO spot measurements were taken at each site with a YSI Pro20 (YSI Inc., Yellow Springs, OH, USA), approximately once every two weeks from November 2016 through March 2017. During this period, 5 ± 3 (mean ± standard deviation unless stated otherwise) measurements (range = 2–24) were taken in two-hour bins from 0630 to 1830. A simple linear model was then used to model DO by the interaction of site and temperature using the R '*Stats*' package (R Core Team, 2020). Next, three representative sites were selected from Moorea's western, northern, and eastern coastline (Apaura, Pihaena, and Valorie, respectively). Sites were sampled three times each from December 2016 through April 2017. Rates of oxygen decline were measured from 1800 to 0600 using spot measurements taken every 30 min. The highest linear rate of oxygen decline was identified over a four-hour window during each sampling night using a rolling regression function ('auto_resp()') in the '*respR*' package (Harianto et al., 2019). Finally, one or two temperature data-loggers (HOBO UA-002-64, Onset Computer Corporation, Bourne, MA, USA) were deployed at each site to record temperature every 10 min (accuracy = ±0.5 °C, resolution = 0.14 °C at 25 °C), yielding partial records from November 2015 through February 2019 (Bouyoucos et al., 2020a). Temperature data from four summers (November–February) were selected, and the previously mentioned rolling regression function was applied to identify the highest linear warming rate over a four-hour window for each site during each summer.

2.4. Objective 2

Our second objective was to test for associations between *in situ* thermal profiles of our study sites and haematological profiles of resident reef shark neonates. Thermal habitat profiles were generated for each site from temperature data-loggers (as described in section '2.3 Objective 1') and consisted of the average and maximum temperature. Rather than selecting the single highest measured value as an estimate of maximum habitat temperature, the mean temperature plus three standard deviations was used as a conservative estimate of maximum habitat temperature (Fig. S1). This rationale is also informed by the idea that an organism's upper thermal limits can be roughly estimated from thermal reaction norms of physiological performance traits as three standard deviations above the optimal temperature (Deutsch et al., 2008). Temperature data were available for all ten sites from November 2015 to February 2019.

Haematological profiles were generated for neonatal blacktip reef sharks (*Carcharhinus melanopterus*, Quoy & Gaimard 1824; $n = 170$ individuals from eight sites) and sicklefin lemon sharks (*Negaprion acutidens*, Rüppell 1837; $n = 54$ individuals from four sites). Both species were collected from eight sites around Moorea during summer parturition months. Sites were monospecific or hosted both species (Mourier et al., 2013a,b). Sharks were collected using gill-nets during two parturition seasons (October 2014 through March 2015 and November 2015 through March 2016). Gill-nets were set from 1700 to 2000 and were constantly monitored so that sharks could be removed from the gill-net as soon as they were captured to minimize capture-related stress. Blood samples were then collected immediately by inverting sharks and taking 1 mL of blood from the caudal vasculature with 21–23 gauge, heparin-washed needles. Samples were processed immediately in the field or put on ice and stored for up to 3 h and processed in the lab; this practice has been shown to not affect values of the haematological metrics (*i.e.*, haematocrit and haemoglobin concentration) that were measured (Schwieterman et al., 2019a). We measured haematocrit (Hct) by centrifuging blood samples in 70 μL microhaematocrit tubes for 2 min at 4400*g* using a portable centrifuge (ZIPocrit, LW Scientific, Lawrenceville, GA, USA), and calculated Hct as the ratio of packed cell volume to total sample volume. Next, haemoglobin concentration ([Hb], in g L^{-1}) was measured using a handheld point-of-care device (HemoCue Hb 201, Australia Pty Ltd., Victoria, Australia) and corrected using species-specific equations (Schwieterman et al., 2019a). Mean corpuscular haemoglobin concentration (MCHC, in g L^{-1}) was then calculated as [Hb] divided by Hct. Haematological profiles were generated for each species as the site-mean value of each metric, yielding eight values (*i.e.*, eight sites) for *C. melanopterus* ($n = 8$–36 samples per site) and four values (*i.e.*, four sites) for *N. acutidens* ($n = 4$–24 samples per site). Associations between thermal habitat profiles metrics (*i.e.*, average and maximum habitat temperatures) and haematological profiles (*i.e.*, [Hb], Hct, and MCHC) were tested for each species using simple linear regression. Statistical significance for regression was determined with a Bonferroni-corrected $\alpha = 0.008$ to account for multiple comparisons ($n = 6$ per species).

2.5. Objective 3

2.5.1. Experimental design

Our third objective was to test for associations between *ex situ* haematological traits, thermal tolerance, and physiological oxygen supply capacity. Individual sharks were sampled for every trait (*i.e.*, haematological traits, thermal tolerance, and physiological oxygen supply capacity), but in differing sampling orders. Sharks were fed between trials, allowing at least 48 h to recover and fast between trials (Bouyoucos et al., 2020a). Sharks were maintained at ambient temperatures to avoid possible confounding effects of thermal acclimation to a regulated tank temperature.

2.5.2. Animal collection and husbandry

Neonatal *C. melanopterus* and *N. acutidens* were collected and transported to a laboratory facility at the Centre de Recherches Insulaires et Observatoire de l'Environnement (CRIOBE) in coolers of aerated seawater (Bouyoucos et al., 2018). At the CRIOBE, sharks were housed in 1250 L

(1.5 m diameter and 70 cm deep) circular tanks that received a constant supply of seawater from an offshore pump. Holding tanks were in a covered, open-air facility that exposed sharks to a natural photoperiod; although, tanks were covered with 60% shade cloth to minimize disturbance. To identify individuals, sharks were tagged with passive integrated transponder (PIT) tags (Biolog-id SAS, Paris, France). Sharks were fed *ad libitum* every other day with fresh tuna (*Thunnus* spp.). *Carcharhinus melanopterus* neonates ($n = 12$; mass $= 0.89 \pm 0.15$ kg, range $= 0.67–1.16$ kg) were maintained in groups of two or three per tank, and larger *N. acutidens* neonates ($n = 10$; mass $= 1.30 \pm 0.27$ kg, range $= 0.99–1.76$ kg) were maintained in groups of two per tank. Due to constraints on tank space at the CRIOBE, experiments on *C. melanopterus* were conducted in October 2019 (ambient water temperature = 29 °C) and experiments on *N. acutidens* were conducted in January 2020 (ambient water temperature = 28 °C). All sharks were released at their original site of capture at the end of experimentation.

2.5.3. Haematological traits

Haematological traits were measured as described in section '2.4 Objective 2'. Sharks were individually captured from holding tanks and inverted so that up to 1 mL of blood could be drawn from the caudal vasculature using 21- or 23-gauge, heparin-washed needles. Handling and blood sampling were under 2 min so that all sharks within a tank could be sampled in rapid succession and on the same day. Blood samples were placed on ice for up to 30 min before being processed. Haematocrit was measured in duplicate, using 70 μL microhaematocrit tubes and a benchtop centrifuge to spin samples at 10,000 *g* for 5 min. Haemoglobin concentration was also measured in duplicate, where blood samples were incubated within microcuvettes for 9 min before being read by the HemoCue (Schwieterman et al., 2019a). Species-specific corrections were applied to raw [Hb] output (Schwieterman et al., 2019a). The mean Hct and [Hb] values were used to calculate a single MCHC value for each shark.

2.5.4. Thermal tolerance

Thermal tolerance was estimated by measuring the critical thermal maximum (CT_{Max}) metric. Individual sharks were removed from their holding tank and introduced to a 100 L, 1 m diameter circular tank containing five 300-watt aquarium heaters (Jager 300w, EHEIM GmbH & Co KG, Deizisau, Germany). The tank was continuously aerated with an air stone to maintain oxygen saturation above 80%. Following a five-minute habituation to the experimental tank, the water temperature was elevated from an ambient temperature of 29.4 ± 0.9 °C for *C. melanopterus* trials and 28.0 ± 1.5 °C for *N. acutidens* trials at a constant rate of 0.12 ± 0.02 °C min^{-1} using the aquarium heaters until the shark began to exhibit muscle spasms (Bouyoucos et al., 2020a). The temperature at which sharks exhibited the onset of muscle spasms (OS) was recorded as an individual's CT_{Max}, where a spasm was defined as any rapid convulsion originating from the animal's trunk. The temperature at OS is a repeatable, non-lethal endpoint for determining CT_{Max} (Lutterschmidt and Hutchison, 1997b). After reaching OS, the shark was immediately returned to its holding tank.

2.5.5. Oxygen supply capacity

Physiological oxygen supply capacity (α, in mg O_2 $kg^{-0.89}$ h^{-1} kPa^{-1}) was calculated by estimating sharks' routine metabolic rate (RMR, in mg O_2 $kg^{-0.89}$ h^{-1}) and critical oxygen tension (p_{crit}, in kPa). To estimate RMR and p_{crit}, we used intermittent-flow respirometry (Svendsen et al., 2016). Sharks were removed from their holding tanks and placed into individual static respirometry chambers. Up to four 32 L chambers (24 cm diameter and 70 cm long) were placed in a single holding tank that was filled to 530 L (1.5 m diameter and 30 cm deep) and received continuous water supply and aeration. Each chamber was fitted with two 2500 L h^{-1} pumps (EHEIM GmbH & Co KG, Deizisau, Germany): one was configured in a closed, recirculating loop to mix water within the chamber, and the other was configured to flush water from the holding tank through the chamber and out an overflow pipe above the water's surface. Oxygen was measured in each chamber with a FireSting Optical Oxygen Meter (PyroScience, GmbH, Aachen, Germany). Oxygen probes were placed within each chamber through the overflow pipe and measured DO every 2 s. A single temperature probe was connected to the oxygen meter and placed in the holding tank to correct DO readings for changes in ambient temperature. The average ambient water temperature recorded during respirometry trials was 29.8 ± 0.2 °C for *C. melanopterus* and 29.4 ± 0.1 °C for *N. acutidens*. Corrections for salinity were made by manually inputting a single value into the FireSting software, and barometric pressure was accounted for with a built-in sensor. Oxygen probes were calibrated to 100% and 0% saturation before each trial.

Routine metabolic rate was estimated during the first 4 h sharks spent in respirometry chambers (Heinrich et al., 2014). Classically, RMR was simply defined as any metabolic rate between the standard and maximum metabolic rates (Chabot et al., 2016). After introducing sharks to respirometry chambers, intermittent cycling of the flush pump was controlled with a custom data acquisition system and software (National Instruments, Austin, TX, USA). Flush pumps were programmed to flush water through chambers for 10 min, followed by 5 min of inactivity when oxygen uptake rates ($\dot{M}O_2$) could be measured. In total, 16 $\dot{M}O_2$ values were calculated for each shark. To approximate RMR of a minimally active animal, RMR in this study was estimated as the mean of the lowest three $\dot{M}O_2$ values (Norin et al., 2014). Oxygen uptake rates were calculated by multiplying the rate of linear oxygen decline (in mg O_2 L^{-1} s^{-1}; $R^2 > 0.95$) by the volume of water in the chamber (in L) and dividing by the mass of the shark (in kg). To account for the effects of variation in mass on $\dot{M}O_2$, all $\dot{M}O_2$ values were scaled to the mass of a 1 kg individual using an intra-specific mass scaling exponent of 0.89 (Jerde et al., 2019). All $\dot{M}O_2$ values used to estimate RMR were calculated using custom R script (A. Mercière and T. Norin, unpublished data).

The p_{crit} was estimated immediately after estimation of RMR. In this study, p_{crit} was defined as the oxygen partial pressure (pO_2, in kPa) at which a shark's RMR began a linear decline with ambient pO_2 (Rogers et al., 2016). After sharks spent 4 h in chambers for estimation of RMR, flush pumps were disabled to allow sharks to consume oxygen within the chambers and experience progressive hypoxia (Speers-Roesch et al., 2012). This 'closed respirometry' technique is an acceptable method of measuring p_{crit} relative to other methods (*e.g.*, intermittent-flow or flow-through respirometry), and possibly gives more ecologically relevant estimates of p_{crit} because low-oxygen environments are typically high in carbon dioxide as a by-product of respiration (Ultsch and Regan, 2019; Wood, 2018). Experiments were terminated when DO measurements began to flatten over time, as determined by visual inspection of data in real time, or when DO reached 2.0 mg O_2 L^{-1}, which is a threshold when neonatal *C. melanopterus* exhibit muscle spasms (Bouyoucos et al., 2020a). Upon termination of the experiment, sharks were then removed from chambers and returned to their holding tanks. To estimate p_{crit}, we first calculated an $\dot{M}O_2$ value every 5 min during the closed respirometry phase using LabChart software (7.3.8, ADInstruments, Dunedin, New Zealand). Then, simple linear regression was used to fit a line to these $\dot{M}O_2$ values against the first DO reading at which they were measured for each shark. The DO value at which each sharks' RMR intersected this line was recorded as a sharks' p_{crit} and converted to kPa using temperature, salinity, and barometric pressure data (Reemeyer and Rees, 2019).

Physiological oxygen supply capacity could be calculated after estimating RMR and p_{crit}. In this study, α was defined as the maximum ability of the cardiorespiratory system to provide oxygen to metabolically demanding tissues (Ern et al., 2016), and is realized at the p_{crit} of a given metabolic rate (Kielland et al., 2019). In other words, α represents the relationship between a given metabolic rate and its critical oxygen tension, and can be calculated by dividing RMR by its p_{crit} (Seibel and Deutsch, 2020). RMR and p_{crit} values used to calculate α for *C. melanopterus* and *N. acutidens* are presented in Table 1 and Table 2, respectively.

2.5.6. Data analyses

Associations between *ex situ* haematological traits and thermal tolerance were tested for using simple linear regressions. Specifically, $\mathrm{CT}_{\mathrm{Max}}$ was modelled as a function of Hct, [Hb], and MCHC in individual models for each species. All analyses were conducted using the '*Stats*' R package. Statistical significance for regression was determined with a Bonferroni-corrected $\alpha = 0.016$ to account for multiple comparisons ($n = 3$ comparisons per species).

An additional series of analyses were undertaken to estimate the temperature sensitivity of p_{crit} under the assumption that thermal tolerance and physiological oxygen supply are related. These analyses were performed to test the prediction that, if $\mathrm{CT}_{\mathrm{Max}}$ and α are related, then p_{crit} should equal 21 kPa (*i.e.*, air-saturation and $p_{\mathrm{crit\text{-}max}}$) at the measured $\mathrm{CT}_{\mathrm{Max}}$ (Deutsch et al., 2020; Seibel and Deutsch, 2020). Data for both *C. melanopterus* and *N. acutidens* were tested. To do this, we used the average temperature sensitivity for p_{crit} ($E_{\mathrm{pcrit}} = 0.4$ eV) reported in Deutsch et al. (2020) to estimate $\mathrm{CT}_{\mathrm{Max}}$ as the temperature where $p_{\mathrm{crit}} = 21$ kPa using p_{crit} estimates for both species from our study. Then, we estimated the temperature sensitivity required for measured values of p_{crit} to equal 21 kPa at the measured temperatures for $\mathrm{CT}_{\mathrm{Max}}$ in this study. Next, we used previously published standard (SMR) and maximum (MMR) metabolic rates measured in *C. melanopterus* at 28 and 31 °C (Bouyoucos et al., 2020a) to calculate temperature sensitivities for SMR and MMR (E_{SMR} and E_{MMR}, respectively) so that E_{pcrit} could be estimated using the equation $E_{\mathrm{SMR}} = E_{\mathrm{MMR}} + E_{\mathrm{pcrit}}$ (Seibel and Deutsch, 2020). Finally, estimated E_{pcrit} from Bouyoucos et al. (2020a) was used to estimate $\mathrm{CT}_{\mathrm{Max}}$ for *C. melanopterus* using p_{crit} measurements from the current study. For reference, E_{pcrit} was calculated from the only other available data from elasmobranch fishes (Butler and Taylor, 1975; Crear et al., 2019; Schwieterman et al., 2019b).

3. Results

3.1. Objective 1

The average temperature calculated from spot measurements across all ten sites was 29.9 ± 0.2 °C (range = 26.5–37.0 °C) and the average DO was 6.32 ± 1.54 mg O_2 L^{-1} (range = 3.10–11.39 mg O_2 L^{-1}). Oxygen was positively associated with temperature at each site (Fig. 1; Table S1). Maximum heating rates occurred during 0610–1530 and were 1.4 ± 0.6 °C h^{-1} (Fig. S2). The average of the maximum temperatures that were recorded across all sites was 34.1 ± 1.7 °C. Maximum rates of oxygen decline between 1800 and 0600 were 0.45 ± 0.18 mg L^{-1} h^{-1} (Fig. S3). The average of the minimum DO values that were recorded across three sampling sites across all nine sampling nights was 4.18 ± 0.62 mg O_2 L^{-1}.

Table 1
Routine metabolic rates (RMR, mg O_2 $kg^{-0.89}$ h^{-1}) and critical oxygen tensions (p_{crit}, kPa) used to calculate physiological oxygen supply capacity (α, mg O_2 $kg^{-0.89}$ h^{-1} kPa^{-1}) in *Carcharhinus melanopterus*.

ID	Mass (kg)	RMR	p_{crit}	α	Temperature (°C)
1	1.11	171.15	17.20	9.95	29.6
2	0.93	122.07	18.19	6.71	29.6
3	0.75	95.53	15.57	6.13	29.6
4	0.76	95.89	20.03	4.79	29.6
5	0.95	114.13	16.29	7.00	30.1
6	0.67	83.63	15.91	5.26	29.9
7	1.16	151.96	18.71	8.12	29.5
8	0.98	160.55	15.68	10.24	29.5
9	0.93	123.10	19.35	6.36	29.6
10	0.8	137.07	18.33	7.48	30.0
11	0.77	105.68	16.18	6.53	29.9
12	1.08	200.03	18.09	11.05	29.9

Table 2
Routine metabolic rates (RMR, mg O_2 $kg^{-0.89}$ h^{-1}) and critical oxygen tensions (p_{crit}, kPa) used to calculate physiological oxygen supply capacity (α, mg O_2 $kg^{-0.89}$ h^{-1} kPa^{-1}) in *Negaprion acutidens*.

ID	Mass (kg)	RMR	p_{crit}	α	Temperature (°C)
1	1.06	176.52	13.76	12.83	29.6
2	1.26	191.22	18.82	10.16	29.6
3	1.52	284.53	16.49	17.25	29.4
4	1.35	283.73	14.36	19.75	29.4
5	0.99	221.25	17.08	12.95	29.4
6	1	161.77	16.95	9.54	29.4
7	1.35	222.71	16.47	13.53	29.3
8	1.12	142.55	17.28	8.249	29.3
9	1.76	335.99	13.93	24.13	29.3
10	1.63	307.12	15.07	20.38	29.3

3.2. Objective 2

The average temperature from 2015 to 2019 calculated from temperature data loggers across sites was 28.9 ± 0.3 °C, and the average maximum habitat temperature was 34.4 ± 0.8 °C. For *C. melanopterus*, there were no associations between average habitat temperature and [Hb], Hct, or MCHC (Fig. 2A–C; Table S2). However, maximum habitat temperature was negatively associated with [Hb] but not with Hct or MCHC (Fig. 2D–F; Table S2). For *N. acutidens*, neither average habitat temperature (Fig. 3A–C; Table S2) nor maximum habitat temperature (Fig. 3D–F; Table S2) were associated with [Hb], Hct, or MCHC.

3.3. Objective 3

Average $\mathrm{CT}_{\mathrm{Max}}$ was 35.9 ± 0.4 °C for *C. melanopterus* and 35.8 ± 0.6 °C for *N. acutidens*. The average p_{crit} measured in resting sharks was 17.46 ± 1.53 kPa (5.32 ± 0.47 mg O_2 L^{-1}) for *C. melanopterus* and 16.02 ± 1.66 kPa (4.90 ± 0.51 mg O_2 L^{-1}) for *N. acutidens*. Raw traces of $\dot{M}O_2$ used for determining p_{crit} are presented in the supplementary materials (*C. melanopterus*, Figs. S4–S5; *N. acutidens*, Figs. S6–S7). For *C. melanopterus*, Hct was negatively associated with $\mathrm{CT}_{\mathrm{Max}}$ (Fig. 4B; Table S3), whereas [Hb] and MCHC were not associated with $\mathrm{CT}_{\mathrm{Max}}$ (Fig. 4A and C; Table S3). For *N. acutidens*, $\mathrm{CT}_{\mathrm{Max}}$ was not associated with [Hb], Hct, or MCHC (Fig. 4D–F; Table S3).

Using our measured p_{crit} values of 17.4 kPa at 29.8 °C for *C. melanopterus* and 16.0 kPa at 29.4 °C for *N. acutidens*, a temperature sensitivity for p_{crit} (E_{pcrit}) of 0.4 eV yielded $\mathrm{CT}_{\mathrm{Max}}$ estimates of 33.6 °C and 34.8 °C, respectively. Conversely, achieving $\mathrm{CT}_{\mathrm{Max}}$ at measured temperatures (*i.e.*, 35.9 °C for *C. melanopterus* and 35.8 °C for *N. acutidens*) would necessitate E_{pcrit} values of 0.22 and 0.32 eV, respectively. Using previously published data for *C. melanopterus*, temperature sensitivities of SMR and MMR were estimated to be 0.739 and 0.727 eV, respectively. From these values, E_{pcrit} was estimated at 0.012 eV for *C. melanopterus*. However, this temperature sensitivity did not predict an ecologically realistic value of $\mathrm{CT}_{\mathrm{Max}}$. Out of all elasmobranch species for which temperature scaling of p_{crit} could be calculated from the literature ($n = 4$; Fig. 5; Table S4), E_{pcrit} was 0.43 ± 0.22.

4. Discussion

Our goal was to test for associations between haematological traits and thermal tolerance in reef shark neonates, and to investigate the possibility of a role for oxygen supply capacity in these associations. Here, we demonstrated that, when habitats approached the sharks' $\mathrm{CT}_{\mathrm{Max}}$, these habitats were also hyperoxic; therefore, environmental oxygen availability should not be limiting for physiological processes. However, this would only be true if oxygen supply capacity has not been compromised. Second, we demonstrated negative associations between haematological traits and upper thermal limits, both *in situ* and *ex situ*, but only in *C. melanopterus* neonates. This finding suggests that blood-oxygen carrying capacity and $\mathrm{CT}_{\mathrm{Max}}$ are not correlated, and that

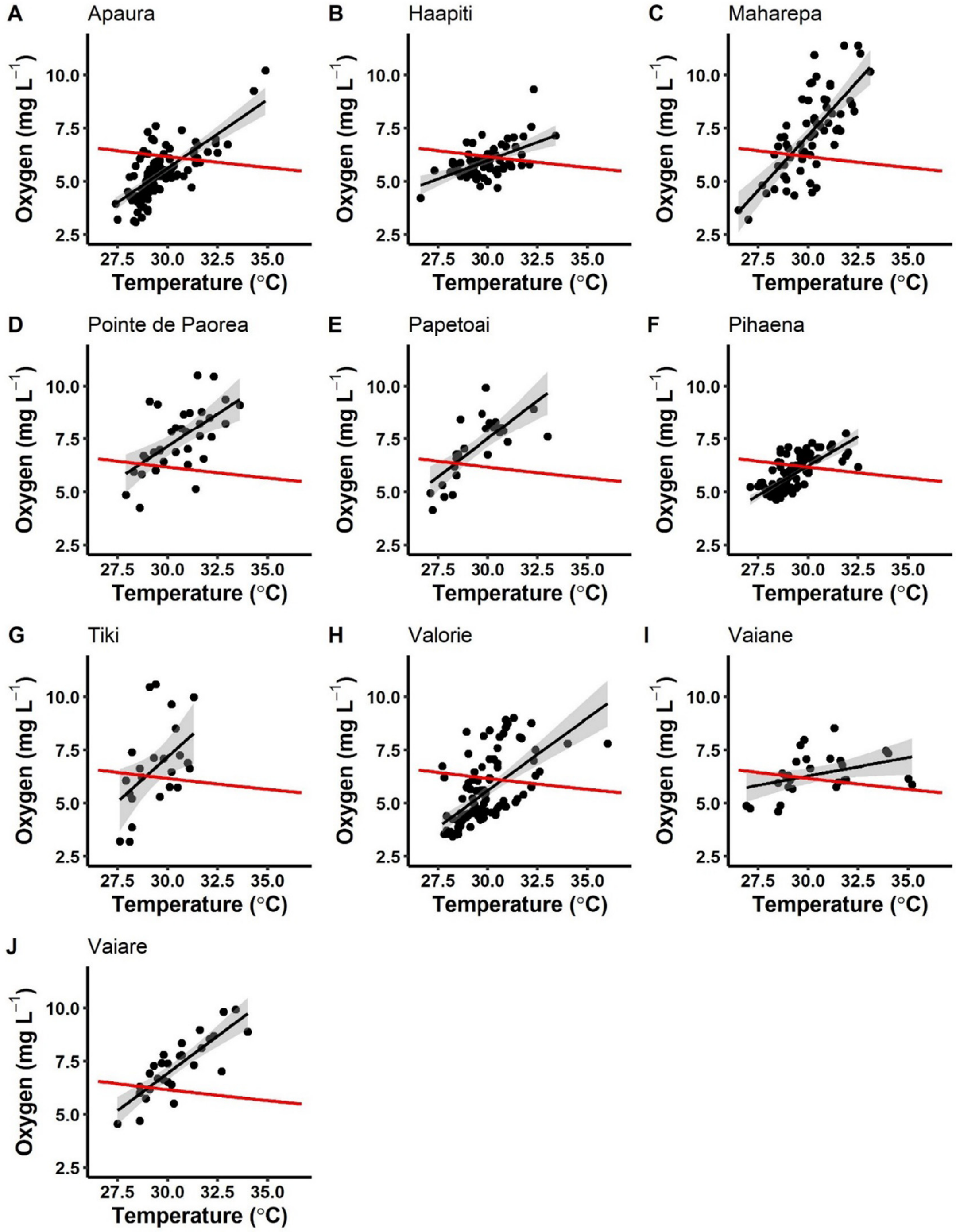

Fig. 1. Associations between water temperature and dissolved oxygen at ten locations (A–J) around Moorea, French Polynesia. Shading around regression lines represents 95% confidence intervals. The red line represents 100% oxygen saturation; oxygen values above this line denote supersaturation.

laboratory estimates of CT$_{Max}$ do not always approximate maximum habitat temperatures. Yet, associations observed in *C. melanopterus* neonates appear genuine, and these unique associations could reflect the effects of temperature on blood viscosity and haemoglobin isoform expression. Third, we estimated the temperature sensitivity of p_{crit} in sharks at rest, under the assumption that thermal tolerance and oxygen supply capacity are related. We found that the average reported temperature sensitivity predicts maximum habitat temperatures for Moorea and the estimated species-specific values fall within the reported range for other elasmobranch species. This provides support for a theoretical relationship between thermal tolerance and oxygen supply capacity in these species; however, we also emphasise that this link has not been demonstrated empirically. Indeed, the temperature sensitivity estimated from previously published data for *C. melanopterus* do not support the hypothesis of a relationship between CT$_{Max}$ and oxygen supply capacity. Therefore, investigating haematological traits that are related to blood-oxygen carrying capacity alone is likely not sufficient to test links between thermal tolerance and oxygen (see Beers and Sidell, 2011; Brijs et al., 2015; Muñoz et al., 2018; Wang et al., 2014). Future studies investigating the thermal dependence of oxygen supply capacity (*i.e.*, p_{crit}) may shed light on mechanistic links between thermal tolerance and oxygen (Deutsch et al., 2020; Seibel and Deutsch, 2020).

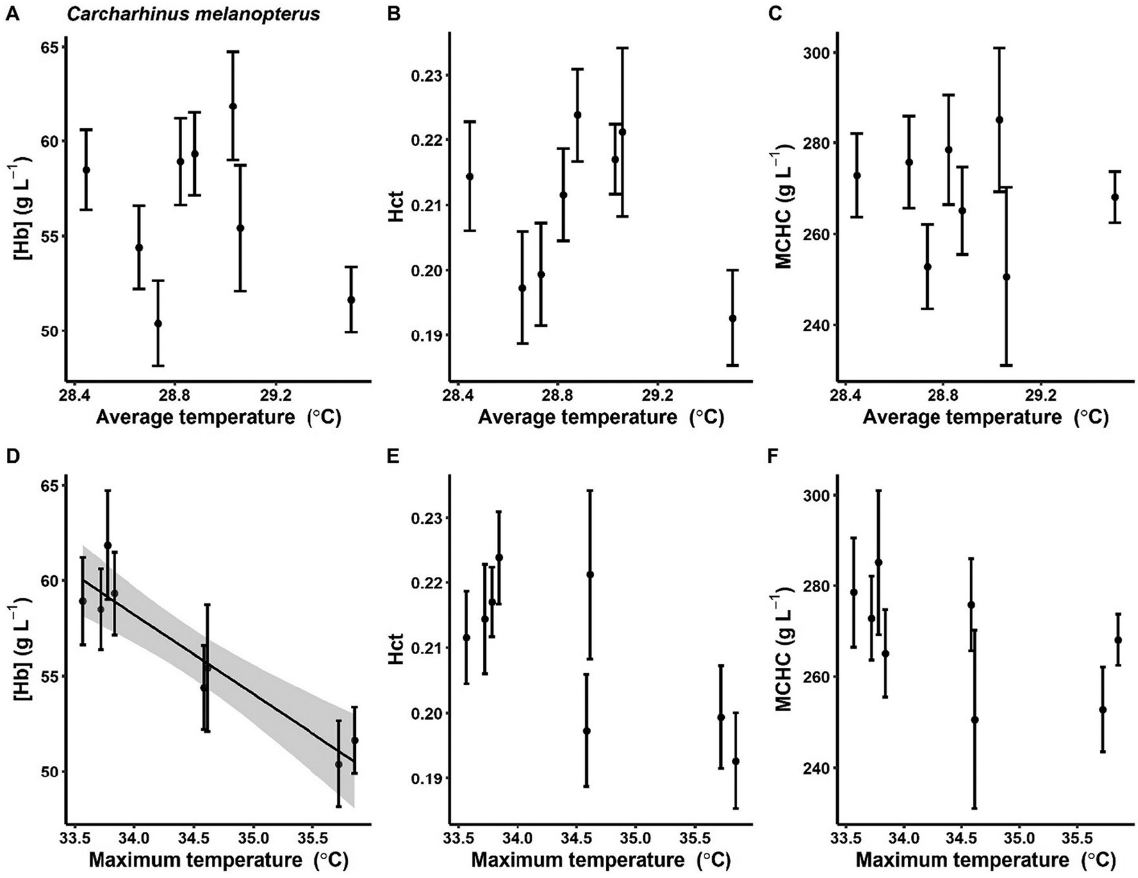

Fig. 2. Associations between haematological traits of blacktip reef shark (*Carcharhinus melanopterus*) neonates and thermal habitat profiles. Haemoglobin concentration ([Hb]; A, D), haematocrit (Hct; B, E), and mean corpuscular haemoglobin concentration (MCHC; C, F) are presented in response to either the average temperature (A–C) or maximum temperature (D–F) measured at eight sites. For panels A-C, individual observations represent the average temperature at a site from 2015 to 2019 and the average value of each haematological trait measured in sharks from 2014 to 2016 at the same sites ($n = 8$ sites). Error bars represent standard error of the mean. For panels D–F, individual observations represent the maximum habitat temperature at each site ($n = 8$ sites), calculated as the average from 2015 to 2019 plus three standard deviations, and the same data for sharks from panels A–C. Regression lines with 95% confidence intervals (*i.e.*, shading) represent statistically significant associations between variables.

Around Moorea, the reef shark neonate habitats approached but did not exceed the two experimental species' upper thermal limits. While the average maximum habitat temperatures across all sites (34.4 °C) did not exceed sharks' measured CT_{Max} (~36 °C), individual maximum habitat temperature estimates did match sharks' CT_{Max} at two of the ten sites. Together, this suggests that, during Austral summer months when these sharks are born, they have a thermal safety margin (TSM) of ~6 °C. While both species appear equally thermally tolerant, very little is known about the effects of acute temperature changes on the physiological performance of *N. acutidens* and *C. melanopterus*. For instance, *C. melanopterus* do not exhibit changes in maximal maximum metabolic rates (MMR) *in situ* between 28 and 32.5 °C (Bouyoucos et al., 2018), but mortality may be associated with exercise stress at temperatures above 31 °C (Bouyoucos et al., 2020a). In addition, *in vitro* studies on *C. melanopterus* revealed that haemoglobin's affinity for oxygen decreases with increasing temperatures; moreover, *in situ*, this species is also capable of behaviourally avoiding unfavourable temperatures (Bouyoucos et al., 2020a). However, when temperatures approach their CT_{Max} in their natural habitats, these sharks are predicted to experience water that is supersaturated with oxygen, suggesting that thermal tolerance should not be impaired by ambient oxygen levels, as long as oxygen supply capacity is not compromised. Heating rate is also known to affect CT_{Max} in fishes (Illing et al., 2020; Mora and Maya, 2006); indeed, maximum environmental heating rates measured *in situ* (1.4 °C h^{-1}) were much slower than experimental heating rates used to measure CT_{Max} *ex situ* (7.2 °C h^{-1}), suggesting that, *in situ*, shark neonates may have greater TSMs than laboratory results might suggest. Yet, TSMs should decrease as summer temperatures increase (McArley et al., 2017; Sandblom et al., 2016), and indeed, this has been demonstrated, *ex situ*, with thermal acclimation in *C. melanopterus* (Bouyoucos et al., 2020a). It is still not clear as to whether heatwaves and/or ocean warming will expose reef shark neonates around Moorea to temperatures exceeding their CT_{Max}, but this is an important area for future investigation.

Dissolved oxygen levels among the reef shark habitats were lower than measured p_{crit} values for both species. Overnight measurements at three of the ten sites demonstrated that DO fell below the measured p_{crit} of *C. melanopterus* in 68% of measurements (~8.1 h) and in 45% of measurements (~5.4 h) for *N. acutidens*. Indeed, neonatal *N. acutidens* exhibited lower p_{crit} and higher α than *C. melanopterus*. However, a more ecologically relevant p_{crit} would account for the effects of sharks' activity level and body temperature. Indeed, it is important to consider how p_{crit} and α vary when measured in immobile animals, as was the case in this study, and in animals that are forced to swim. Both *C. melanopterus* and *N. acutidens* typically swim to ventilate their gills but are also known to rest on the substrate and ventilate their gills through buccal pumping. If the capacity of these sharks to ventilate their gills while swimming is greater than their capacity to ventilate their gills through buccal pumping, then it is conceivable that swimming reef sharks could exhibit lower p_{crit} and higher α, despite their increased metabolic rates relative to resting. Another consideration is the effect of diel temperature fluctuations on p_{crit} in reef sharks *in situ*. Moorea's habitats experience declining DO at night when water temperatures are lowest, when p_{crit} would seemingly decrease, as has been demonstrated in other elasmobranchs (Butler

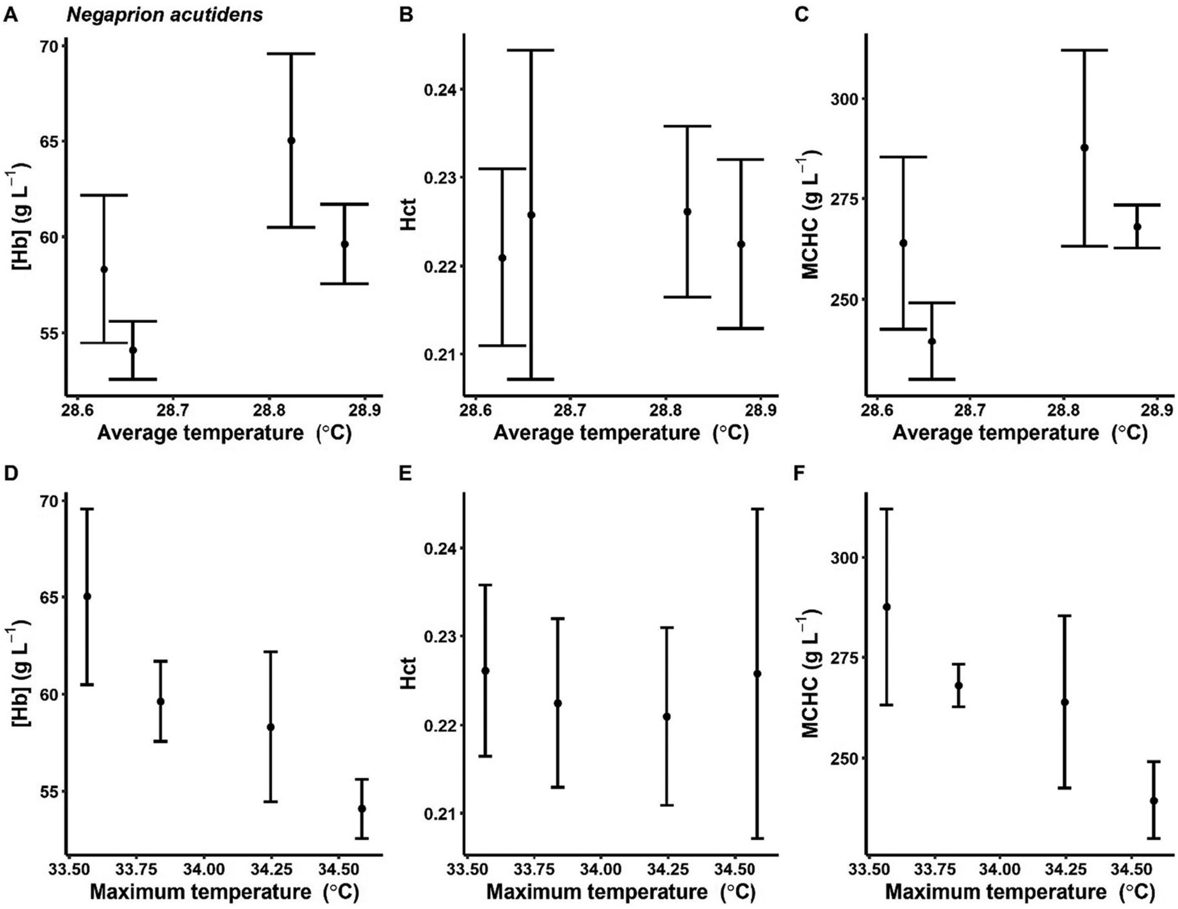

Fig. 3. Associations between haematological traits of sicklefin lemon shark (*Negaprion acutidens*) neonates and thermal habitat profiles. Haemoglobin concentration ([Hb]; A, D), haematocrit (Hct; B, E), and mean corpuscular haemoglobin concentration (MCHC; C, F) are presented in response to either the average temperature (A–C) or maximum temperature (D–F) measured at eight sites. For panels A–C, individual observations represent the average temperature at a site from 2015 to 2019 and the average value of each haematological trait measured in sharks from 2014 to 2016 at the same sites ($n = 4$ sites). Error bars represent standard error of the mean. For panels D–F, individual observations represent the maximum habitat temperature at each site ($n = 4$ sites), calculated as the average from 2015 to 2019 plus three standard deviations, and the same data for sharks from panels A–C.

and Taylor, 1975; Crear et al., 2019; Schwieterman et al., 2019b). In other tropical elasmobranchs, p_{crit} is also associated with haemoglobin-oxygen (Hb-O_2) affinity, which is often reported as the pO_2 at which 50% of the Hb is bound with O_2 (p_{50}; Speers-Roesch et al., 2012). In this light, increasing Hb-O_2 affinity at lower temperatures – as demonstrated *in vitro* in *C. melanopterus* (Bouyoucos et al., 2020a) – should be accompanied by decreasing p_{crit}. Thus, accounting for effects of activity level, gill ventilation, and diel temperature changes would produce different p_{crit} estimates than what we characterized. Finally, prior exposure to supersaturated oxygen conditions during the day could confer greater tolerance to acute declining oxygen conditions that are experienced overnight (Giomi et al., 2019). It is probable that neonatal *C. melanopterus* and *N. acutidens* use the shallow, nearshore habitats around Moorea for refuge from predators and are even thought to stay within these sites without venturing to deeper, possibly well oxygenated waters at night (Bouyoucos et al., 2020b). Thus, *C. melanopterus* and *N. acutidens* must be able to tolerate environmental oxygen levels that decline below their estimated p_{crit} for an immobile animal for at least 5 h if they are to remain within the protection of their shallow habitats at night.

Haematological traits varied with *in situ* and *ex situ* estimates of upper thermal limits in *C. melanopterus* neonates, but none of the haematological traits we measured were associated with maximum habitat temperature or CT_{Max} in *N. acutidens*. In *C. melanopterus*, [Hb] was negatively associated with maximum habitat temperature while Hct was negatively associated with CT_{Max}. In notothenioid fishes and Chinook salmon (*Oncorhynchus tshawytscha*), Hct has been found to be positively associated with CT_{Max} (Beers and Sidell, 2011; Muñoz et al., 2018), and this finding has been debated as evidence in support of a link between oxygen supply capacity and thermal tolerance (Brijs et al., 2015; Pörtner et al., 2017; Wang et al., 2014). Finding the opposite trend in only *C. melanopterus* and not in its congener, *N. acutidens*, suggests that associations between Hct and CT_{Max} are variable, species-specific, and are likely not the best metrics for testing links between thermal tolerance and oxygen supply. Further, such trends have only been demonstrated over narrow temperature ranges, such as ~1 °C in the present study and ~3 °C in Beers and Sidell (2011) and Muñoz et al. (2018). The mismatch we observe between field and laboratory trends for different neonate sub-populations around Moorea also suggests disagreement between laboratory estimates of CT_{Max} and maximum habitat temperatures. If CT_{Max} was a reliable proxy for maximum habitat temperature, then we would predict that traits associated with one metric might also be associated with the proxy. Therefore, disparity in trends observed *in situ* and *ex situ* in *C. melanopterus* suggests that CT_{Max} may not be a meaningful proxy for maximum habitat temperature, and the mismatch in the haematological traits between *C. melanopterus* and *N. acutidens* suggests that such traits may not reliably predict CT_{Max}.

The strength of observed associations between haematological traits, CT_{Max}, and maximum habitat temperatures in *C. melanopterus*, however, suggest that these trends were not spurious. Indeed, coefficients of determination for Hct and CT_{Max} ($R^2 = 0.63$) and for [Hb] and maximum habitat temperature ($R^2 = 0.88$) were relatively strong. Negative associations between these traits have, to the best of our knowledge, not been previously demonstrated in fishes and suggest a unique, as-of-yet unexplained phenomenon. A negative association

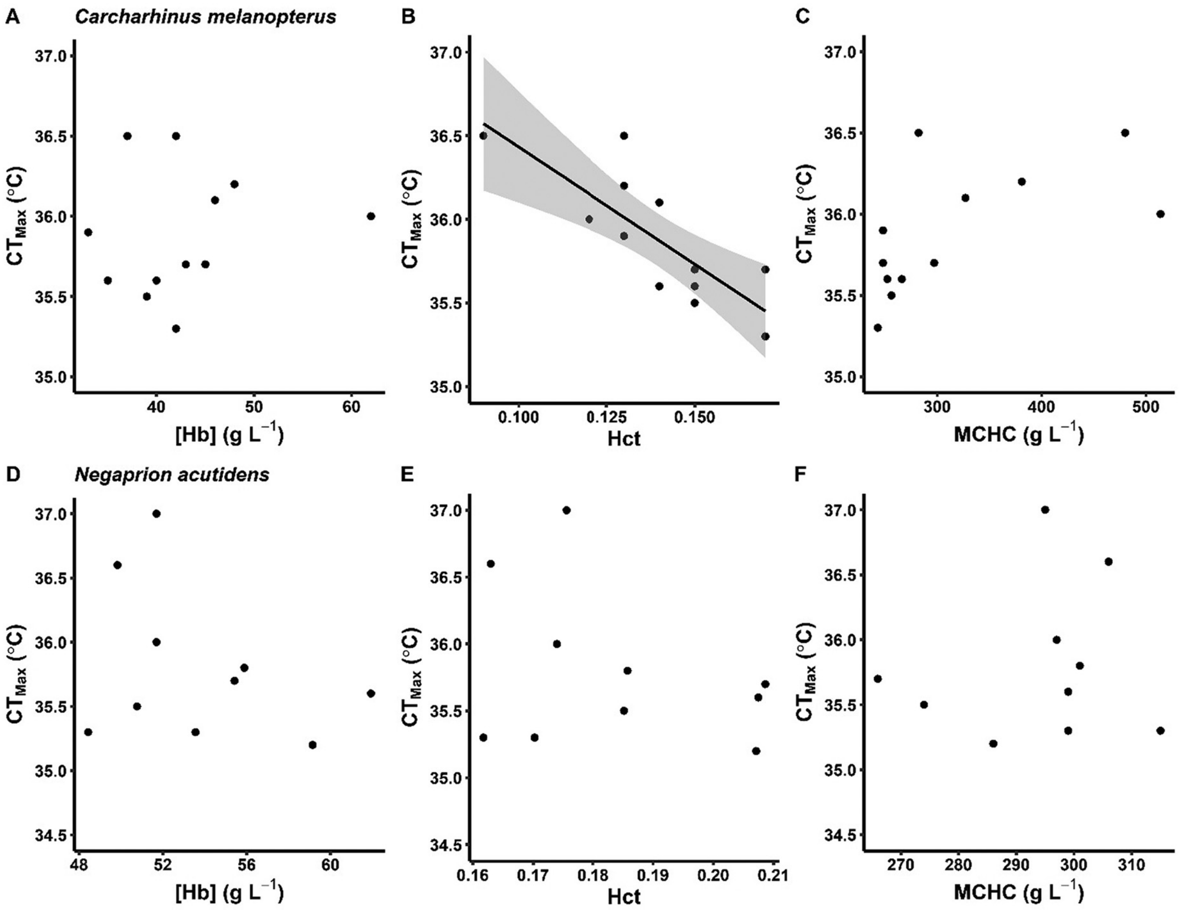

Fig. 4. Associations between critical thermal maximum (CT$_{Max}$) and haemoglobin concentration ([Hb]; A, D), haematocrit (Hct; B, E), and mean corpuscular haemoglobin concentration (MCHC; C, F) of blacktip reef shark (*Carcharhinus melanopterus*; A–C) and sicklefin lemon shark (*Negaprion acutidens*; D–F) neonates. Points represent observations for individual sharks (*C. melanopterus*, $n = 12$; *N. acutidens*, $n = 10$), and regression lines with 95% confidence intervals (*i.e.*, shading) represent statistically significant associations between variables.

between Hct and CT$_{Max}$ could reflect the thermal dependence of blood viscosity. Depending on shear rate, blood viscosity increases as Hct increases (Baldwin and Wells, 1990; Wells and Baldwin, 1990), while increasing temperature reduces blood viscosity (Brill and Jones, 1994). Thus, it would be expected that *C. melanopterus* experience reductions in blood viscosity during acute temperature increases during CT$_{Max}$ experiments. It is possible that Hct increases during heating, but this has not been directly tested in *C. melanopterus*. However, it is important to note that Hct in *C. melanopterus* does increase with thermal acclimation (Bouyoucos et al., 2020c) and upon exercise stress (Schwieterman et al., 2019a). On the other hand, a negative association between [Hb] and maximum habitat temperature could reflect the expression of fewer, more temperature resistant Hb isoforms in habitats that achieve higher maximum temperatures. For instance, in *C. melanopterus* living at 27 °C, *in vitro* experiments suggest a loss of Hb pH sensitivity when temperatures approaching the animal's CT$_{Max}$ (Bouyoucos et al., 2020a). This logic assumes differences in the costs of expressing temperature resistant isoforms. Alternatively, because habitats around Moorea exhibited strong positive associations between temperature and oxygen, lower [Hb] in habitats that achieve higher maximum temperatures could reflect the degree of hyperoxia in those habitats. Clearly, further research is warranted to determine the mechanistic underpinnings and biological significance of the negative associations between haematological traits and thermal habitat characteristics in *C. melanopterus*. If these are, indeed, robust trends, as they seem to be, increases in maximum habitat temperatures with ocean warming could result in pathologies in sharks with very low Hct and [Hb].

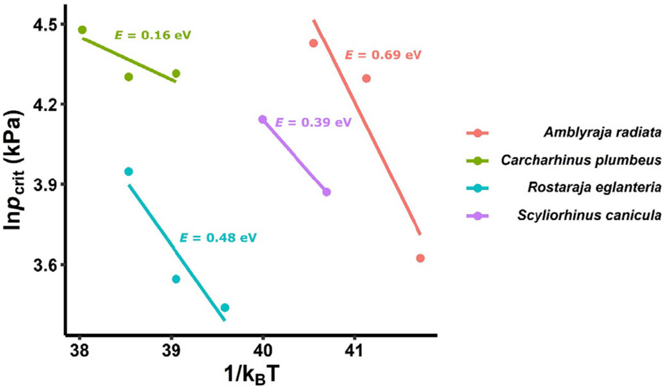

Fig. 5. Temperature sensitivity of critical oxygen tension (p_{crit}) of four elasmobranch species. The natural logarithm of p_{crit} (kPa) is plotted against the inverse of temperature (°Kelvin) multiplied by the Boltzmann constant. The slope of this line is the temperature sensitivity (E, in eV). Available data from the literature were collected for the thorny skate, *Amblyraja radiata* (Schwieterman et al., 2019b), sandbar shark, *Carcharhinus plumbeus* (Crear et al., 2019), clearnose skate, *Rostaraja eglanteria* (Schwieterman et al., 2019b), and small-spotted catshark, *Scyliorhinus canicula* (Butler and Taylor, 1975).

The average temperature sensitivity for p_{crit} reported by Deutsch et al. (2020) predicted oxygen-dependent values of CT$_{Max}$ for *C. melanopterus* (33.6 °C) and *N. acutidens* (34.8 °C). This exercise relied on the assumption that CT$_{Max}$ and oxygen supply capacity are related, such that CT$_{Max}$ is defined as the temperature where p_{crit} equals the prevailing environmental pO_2 (*i.e.*, 21 kPa). While these values underestimate CT$_{Max}$ as measured in this study, the predicted values align very closely with measured maximum habitat temperatures in reef shark neonate habitats around Moorea. Further, the estimated CT_{Max} value for *C. melanopterus* is close to the maximum body temperature of 34.1 °C that was measured *in situ* for *C. melanopterus* (Bouyoucos et al., 2020a). Next, predicting the temperature sensitivity of p_{crit} that is needed to yield the CT_{Max} values that were measured for *C. melanopterus* and *N. acutidens* produced values within one standard deviation of the average value for marine ectotherms presented in Deutsch et al. (2020). Indeed, Deutsch et al. (2020) report a mean temperature sensitivity and standard deviation for p_{crit} of 0.40 ± 0.28 eV (range $= -0.2$–1.3 eV) for marine ectotherms. In comparison, available data for elasmobranch species ($n = 4$) produces a temperature sensitivity of 0.43 ± 0.22 (range $= 0.16$–0.69) (Butler and Taylor, 1975; Crear et al., 2019; Schwieterman et al., 2019b), and we estimated temperature sensitivities of 0.22 and 0.32 eV for p_{crit} in *C. melanopterus* and *N. acutidens*, respectively. Conversely, the very low temperature sensitivity predicted from thermal sensitivities of SMR and MMR in *C. melanopterus* that predicted an unrealistic value for CT_{Max} could reflect the effects of long-term thermal acclimation (Bouyoucos et al., 2020a), as opposed to short-term acclimation (Slesinger et al., 2019). Alternatively, these empirical data for *C. melanopterus* could refute the notion of an association between CT_{Max} and oxygen supply capacity. Unfortunately, CT_{Max} data were not available for the other elasmobranch species for which E_{pcrit} could be calculated. We emphasise, however, that our study does not empirically demonstrate whether CT_{Max} is limited by oxygen supply capacity. Further, p_{crit} and α values may not reflect these reef sharks' full oxygen supply capacity, and instead reflect p_{crit} and α for an immobile, buccal-pumping animal. Both of our study species swim nearly continuously *in situ* (Wells et al., 1992); although, both species rest on the benthos and are capable of buccal pumping to ventilate their gills. Notably, MMR can be estimated from α at the prevailing environmental pO_2 (Seibel and Deutsch, 2020), which was ≥21 kPa in this study; this exercise underestimated MMR for *C. melanopterus* based on values previously reported by our group (Bouyoucos et al., 2018, 2020a,c; Rummer et al., 2020). Thus, a swimming reef shark could be able to better ventilate their gills relative to a resting animal, which would theoretically yield a lower p_{crit} and a higher α despite sharks' having higher metabolic rates. For instance, $\alpha \cdot 21$ kPa $= 136$–219 mg O_2 $kg^{-0.89}$ h^{-1}, whereas measured values range 278–561 mg O_2 $kg^{-0.89}$ h^{-1} (Bouyoucos et al., 2020c). However, MMR, as reported in these previous studies was measured using the identical respirometry technique described here. Thus, while these data do not preclude the possibility of a relationship between oxygen supply capacity and CT_{Max} in these reef sharks, these data cannot empirically demonstrate such a relationship.

5. Conclusions

In conclusion, our investigations using reef sharks in a model experimental system do not support the notion that haematological traits can be used to predict intra-specific variation in thermal tolerance. However, our results do not preclude the possibility of a mechanistic link between thermal tolerance and oxygen. To accomplish this fully, in the future, it will be necessary to define the oxygen dependence of CT_{Max} (Ern et al., 2016) and the temperature dependence of the metabolic phenotype (*i.e.*, SMR, MMR, and p_{crit}) over a species' full ecologically relevant temperature range (Seibel and Deutsch, 2020). Indeed, there are only two fish species for which sufficient data exist to test the latter: the goldfish (*Carassius auratus*; Fry and Hart, 1948) and black seabass (*Centropristis striata*; Slesinger et al., 2019). Future studies should also consider testing for a link between oxygen and thermal tolerance between different ontogenetic stages (Dahlke et al., 2020) and investigating links between thermal tolerance and different physiological systems (Vornanen, 2020). Thus, our data suggest some capacity of reef shark neonates to tolerate further ocean warming, but our findings do not indicate how reef shark neonates will fare under the combined stress of ocean warming and deoxygenation. Additional research into thermal tolerance and oxygen supply capacity in sharks and other water-breathing ectotherms is needed, and future studies can benefit from testing the multiple available hypotheses.

CRediT authorship contribution statement

Ian A. Bouyoucos: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization, Project administration. **José E. Trujillo:** Investigation, Writing – review & editing. **Ornella C. Weideli:** Investigation, Writing – review & editing. **Nao Nakamura:** Investigation, Writing – review & editing. **Johann Mourier:** Investigation, Writing – review & editing. **Serge Planes:** Resources, Writing – review & editing, Supervision, Funding acquisition. **Colin A. Simpfendorfer:** Writing – review & editing, Supervision. **Jodie L. Rummer:** Conceptualization, Methodology, Investigation, Resources, Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data statement

Data presented in this manuscript are available at the Research Data Repository (Tropical Data Hub) at James Cook University: doi:10.25903/JX5T-8794.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.146854.

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