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1 Investigating links between thermal tolerance and oxygen supply capacity in shark neonates from a

2 hyperoxic tropical environment

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1 Abstract

2 Temperature and oxygen limit the distribution of marine ectotherms. Haematological traits underlying 3 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 4 5 using reef shark neonates as experimental models because they live near their upper thermal limits and are 6 physiologically sensitive to low oxygen conditions. We first described in situ associations between 7 temperature and oxygen at the study site (Moorea, French Polynesia) and found that the habitats for reef 8 shark neonates (Carcharhinus melanopterus and Negaprion acutidens) were hyperoxic at the maximum 9 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 10 11 between haemoglobin concentration and maximum habitat temperatures in C. melanopterus. Next, we 12 tested for ex situ associations between critical thermal maximum (CT_{Max}) and haematological traits, but 13 only demonstrated a negative association between haematocrit and CT_{Max} in C. melanopterus. Finally, we 14 measured critical oxygen tension (p_{crit}) ex situ and estimated its temperature sensitivity to predict oxygen-15 dependent values of CT_{Max} . Estimated temperature sensitivity of p_{crit} was similar to reported values for 16 sharks and skates, and predicted values for CT_{Max} equalled maximum habitat temperatures. These data 17 demonstrate unique associations between haematological traits and thermal tolerance in a reef shark that 18 are likely not explained by oxygen supply capacity. However, a relationship between oxygen supply 19 capacity and thermal tolerance remains to be demonstrated empirically.

20

Keywords: Critical thermal maximum, haematocrit, haemoglobin, oxygen supply capacity, p_{crit} , reef

21 sharks

22 **1. Introduction**

The way in which temperature affects physiological performance of ectotherms is linked to species' 23 24 biogeography and the potential for local extirpations under climate change (Pörtner et al., 2017; Sunday et 25 al., 2012). In response to temperature variation, species can physiologically acclimate to maintain 26 performance of Darwinian fitness traits and behaviourally select favourable temperatures for performance 27 (Angilletta et al., 2006). These physiological and behavioural strategies serve to establish a 'thermal 28 safety margin' (TSM), which defines the difference between an organism' body temperature and its 29 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 30 et al., 2019). Climate change is increasing air and sea surface temperatures and the frequency and 31 duration of heatwaves globally (Bindoff et al., 2019). As such, global warming puts tropical ectotherms at 32 a greater risk of shifts in distribution patterns or even mortality relative to temperate species (Pinsky et al., 33 34 2019). Because the capacity to tolerate high temperatures is an important fitness-related trait, thermal 35 tolerance has been extensively investigated in laboratory studies.

36 Thermal tolerance can be quantified by measuring the critical thermal maximum (CT_{Max}) metric, 37 which defines the upper temperature where an organism loses motor control following acute exposure 38 (Lutterschmidt and Hutchison, 1997a; MacMillan, 2019). Much of what is known about CT_{Max} in 39 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 40 would no longer be able to serve a functional ecological role in its environment, and survival is limited. 41 42 Beyond laboratory approaches, CT_{Max} can be estimated from species' geographic ranges, where the 43 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 44 45 representative of in situ limits (Payne et al., 2016).

46 Oxygen availability is another important environmental factor that limits the distribution of waterbreathing ectotherms. In water, oxygen concentrations are already quite low (i.e., ~30% lower in water 47 48 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. 49 Climate change is further reducing oxygen availability in aquatic ecosystems, partially because of global 50 51 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 52 related to the sensitivity of an organism's physiological systems to changes in environmental oxygen. For 53 instance, an often-cited hypoxia threshold of $2 \text{ mg } O_2 \text{ L}^{-1}$ would be fatal for the sandbar shark 54 (Carcharhinus plumbeus; Crear et al., 2019), but not the clearnose skate (Rostaraja eglanteria; 55 56 Schwieterman et al., 2019b). Thus, defining hypoxia tolerance is also key in predicting the effects of 57 climate change on water-breathers.

58 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; 59 Wood, 2018). Typically, pcrit is measured when an organism has achieved its standard metabolic rate 60 (Rogers et al., 2016), which represents the metabolic rate of a postprandial organism at rest and at a stable 61 62 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 63 oxygen supply capacity (Seibel and Deutsch, 2020). Thus, when environmental oxygen reaches p_{crit} for a 64 given metabolic rate, an organism's physiological oxygen supply capacity (α = metabolic rate $\cdot p_{crit}^{-1}$) is 65 being fully utilised (Seibel and Deutsch, 2020). As such, it has been suggested that the oxygen level at 66 67 which maximum metabolic rate (MMR) is reduced (i.e., $p_{\text{crit-max}}$) is a more ecologically relevant index of 68 hypoxia tolerance (Seibel and Deutsch, 2020).

69 Temperature and oxygen act together on the physiology of marine ectotherms to set biogeographic70 range limits. This phenomenon reflects the temperature dependence of an organism's hypoxia tolerance

71 (Deutsch et al., 2020). Conversely, the oxygen dependence of thermal tolerance is less clear (MacMillan, 72 2019). One hypothesis, oxygen- and capacity-limited thermal tolerance (OCLTT), posits that oxygen 73 supply capacity limits thermal tolerance in ectotherms (Pörtner et al., 2017). One prediction of this 74 hypothesis is that haematological traits (e.g., haemoglobin concentration, haematocrit), which influence 75 blood-oxygen carrying capacity and MMR, are positively correlated with CT_{Max} (Pörtner et al., 2017). 76 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 77 78 volume) and CT_{Max}, where others do not demonstrate such associations (Brijs et al., 2015; Wang et al., 79 2014). However, such associations do not suggest a mechanistic link, as even haematological traits do not 80 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 81 82 such associations.

83 Coastal, tropical marine habitats are natural laboratories to study the role of oxygen-supply capacity 84 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, 85 coastal habitats, such as rockpools. The relative amount of primary productivity and respiration occurring 86 87 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 88 89 habitats that experience hyperoxia at high temperatures, it is thought that these oxygen conditions support 90 high thermal tolerance by elevating aerobic performance (McArley et al., 2020). Therefore, if 91 haematological traits and thermal tolerance are related, and this relationship is linked to oxygen supply 92 capacity, supporting evidence should be observable in organisms from hot and supersaturated marine 93 habitats (Giomi et al., 2019).

94 The purpose of this study was to test for associations between haematological traits and thermal
95 tolerance and suggest a role for oxygen supply capacity in this relationship using species living near their

96 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 97 98 relatively poor hypoxia tolerance (Routley et al., 2002). The first objective was to describe water 99 temperatures and oxygen levels at the study site. The second objective was to test for *in situ* associations 100 between thermal habitat characteristics and haematological traits in the two reef shark neonate 101 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 102 103 populations. Together, this study combines field and laboratory data to offer a novel perspective on 104 whether haematological traits are reliable predictors of thermal tolerance as suggested in previous studies 105 (Beers and Sidell, 2011; Muñoz et al., 2018). Further, these data can contribute to our understanding as to 106 the mechanistic basis of thermal tolerance in marine ectotherms and how tropical fishes might cope with 107 ocean warming and deoxygenation now and in the future.

108 2. Material and methods

109 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).

114 *2.2. Study site*

All research was conducted on Moorea, French Polynesia (S 17°30'; W 149°50'). Research effort was

116 focused on ten sites (Apaura, Haapiti, Maharepa, Pointe de Paorea, Papetoai, Pihaena, Tiki, Vaiane,

117 Vaiare, and Valorie) that span Moorea's 60-km coastline. These sites are typically less than 1 m deep,

118 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).

121 2.3. *Objective 1*

122 Our first objective was to describe temperature and oxygen conditions of the ten study sites. This 123 involved describing associations between water temperature and dissolved oxygen concentration (DO, in 124 mg O₂ L⁻¹), rates of oxygen decline, and rates of warming. First, paired temperature and DO spot 125 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, $5 \pm$ 126 3 measurements (range = 2-24) were taken in two-hour bins from 0630-1830. A simple linear model was 127 128 then used to model DO by the interaction of site and temperature using the R 'Stats' package (R Core Team, 2018). Next, three representative sites were selected from Moorea's western, northern, and eastern 129 130 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 1800-0600 using spot 131 132 measurements taken every 30 minutes. 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 133 134 'respR' package (Harianto et al., 2019). Finally, one or two temperature data-loggers (HOBOUA-002-64, Onset Computer Corporation, Bourne, MA, USA) were deployed at each site to record temperature every 135 ten minutes (accuracy = ± 0.5 °C, resolution = 0.14 °C at 25 °C), yielding partial records from November 136 137 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 138 139 applied to identify the highest linear warming rate over a four-hour window for each site during each 140 summer.

141 2.4. *Objective* 2

142 Our second objective was to test for associations between *in situ* thermal profiles of our study sites143 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.

151 Haematological profiles were generated for neonatal blacktip reef sharks (Carcharhinus *melanopterus*, Quoy & Gaimard 1824; n = 170 individuals from eight sites) and sicklefin lemon sharks 152 (Negaprion acutidens, Rüppell 1837; n = 54 individuals from four sites). Both species were collected 153 from eight sites around Moorea during summer parturition months. Sites were monospecific or hosted 154 both species (Mourier et al., 2013a, 2013b). Sharks were collected using gill-nets during two parturition 155 seasons (October 2014 through March 2015 and November 2015 through March 2016). Gill-nets were set 156 from 1700-2000 and were constantly monitored so that sharks could be removed from the gill-net as soon 157 as they were captured to minimize capture-related stress. Blood samples were then collected immediately 158 by inverting sharks and taking 1 mL of blood from the caudal vasculature with 21-23 gauge, heparin-159 160 washed needles. Samples were processed immediately in the field or put on ice and stored for up to three hours and processed in the lab; this practice has been shown to not affect values of the haematological 161 metrics (i.e., haematocrit and haemoglobin concentration) that were measured (Schwieterman et al., 162 163 2019a). We measured haematocrit (Hct) by centrifuging blood samples in 70 μ L microhaematocrit tubes for two minutes at 4,400 g using a portable centrifuge (ZIPocrit, LW Scientific, Lawrenceville, GA, 164 165 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, 166 167 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] 168

169 divided by Hct. Haematological profiles were generated for each species as the site-mean value of each 170 metric, yielding eight values (i.e., eight sites) for *C. melanopterus* (n = 8-36 samples per site) and four 171 values (i.e., four sites) for *N. acutidens* (n = 4-24 samples per site). Associations between thermal habitat 172 profiles metrics (i.e., average and maximum habitat temperatures) and haematological profiles (i.e., [Hb], 173 Hct, and MCHC) were tested for each species using simple linear regression. Statistical significance for 174 regression was determined with a Bonferroni-corrected $\alpha = 0.008$ to account for multiple comparisons (n175 = 6 per species).

176 2.5. *Objective 3*

177 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 hours 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.

184 2.5.2. Animal collection and husbandry

185 Neonatal C. melanopterus and N. acutidens were collected and transported to a laboratory facility at 186 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 70 187 188 cm deep) circular tanks that received a constant supply of seawater from an offshore pump. Holding tanks 189 were in a covered, open-air facility that exposed sharks to a natural photoperiod; although, tanks were 190 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 191 192 every other day with fresh tuna (*Thunnus* spp.). Carcharhinus melanopterus neonates (n = 12; mass = 0.89 ± 0.15 kg, range = 0.67-1.16 kg) were maintained in groups of two or three per tank, and larger N. 193

194 *acutidens* neonates (n = 10; mass = 1.30 ± 0.27 kg, range = 0.99-1.76 kg) were maintained in groups of 195 two per tank. Due to constraints on tank space at the CRIOBE, experiments on *C. melanopterus* were 196 conducted in October 2019 (ambient water temperature = $29 \,^{\circ}$ C) and experiments on *N. acutidens* were 197 conducted in January 2020 (ambient water temperature = $28 \,^{\circ}$ C). All sharks were released at their original 198 site of capture at the end of experimentation.

199 2.5.3. Haematological traits

200 Haematological traits were measured as described in section '2.4. Objective 2'. Sharks were 201 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 202 were under two minutes so that all sharks within a tank could be sampled in rapid succession and on the 203 204 same day. Blood samples were placed on ice for up to 30 minutes before being processed. Haematocrit was measured in duplicate, using 70 µL microhaematocrit tubes and a benchtop centrifuge to spin 205 samples at 10,000 g for five minutes. Haemoglobin concentration was also measured in duplicate, where 206 blood samples were incubated within microcuvettes for nine minutes before being read by the HemoCue 207 (Schwieterman et al., 2019a). Species-specific corrections were applied to raw [Hb] output 208 (Schwieterman et al., 2019a). The mean Hct and [Hb] values were used to calculate a single MCHC value 209 210 for each shark.

211 2.5.4. Thermal tolerance

212 Thermal tolerance was estimated by measuring the critical thermal maximum (CT_{Max}) metric.

213 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.

224 2.5.5. Oxygen supply capacity

Physiological oxygen supply capacity (α , in mg O₂ kg^{-0.89} h⁻¹ kPa⁻¹) was calculated by estimating 225 sharks' routine metabolic rate (RMR, in mg O₂ kg^{-0.89} h⁻¹) and critical oxygen tension (p_{crit} , in kPa). To 226 estimate RMR and p_{crit} , we used intermittent-flow respirometry (Svendsen et al., 2016). Sharks were 227 removed from their holding tanks and placed into individual static respirometry chambers. Up to four 32 228 L chambers (24 cm diameter and 70 cm long) were placed in a single holding tank that was filled to 530 L 229 (1.5 m diameter and 30 cm deep) and received continuous water supply and aeration. Each chamber was 230 fitted with two 2,500 L h⁻¹ pumps (EHEIM GmbH & Co KG, Deizisau, Germany): one was configured in 231 232 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 233 was measured in each chamber with a FireSting Optical Oxygen Meter (PyroScience, GmbH, Aachen, 234 235 Germany). Oxygen probes were placed within each chamber through the overflow pipe and measured DO every two seconds. A single temperature probe was connected to the oxygen meter and placed in the 236 237 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 238 for N. acutidens. Corrections for salinity were made by manually inputting a single value into the 239 240 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. 241

Routine metabolic rate was estimated during the first four hours sharks spent in respirometry
chambers (Heinrich et al., 2014). Classically, RMR was simply defined as any metabolic rate between the

244 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 245 246 and software (National Instruments, Austin, TX, USA). Flush pumps were programmed to flush water 247 through chambers for ten minutes, followed by five minutes of inactivity when oxygen uptake rates (\dot{MO}_2) could be measured. In total, 16 \dot{MO}_2 values were calculated for each shark. To approximate RMR 248 of a minimally active animal, RMR in this study was estimated as the mean of the lowest three $\dot{M}O_2$ 249 250 values (Norin et al., 2014). Oxygen uptake rates were calculated by multiplying the rate of linear oxygen decline (in mg O₂ L⁻¹ s⁻¹; $R^2 > 0.95$) by the volume of water in the chamber (in L) and dividing by the 251 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 252 253 scaled to the mass of a 1 kg individual using an intra-specific mass scaling exponent of 0.89 (Jerde et al., 254 2019). All $\dot{M}O_2$ values used to estimate RMR were calculated using custom R script (A. Mercière and T. 255 Norin, unpublished data).

The p_{crit} was estimated immediately after estimation of RMR. In this study, p_{crit} was defined as the 256 oxygen partial pressure (pO_2 , in kPa) at which a shark's RMR began a linear decline with ambient pO_2 257 (Rogers et al., 2016). After sharks spent four hours in chambers for estimation of RMR, flush pumps were 258 disabled to allow sharks to consume oxygen within the chambers and experience progressive hypoxia 259 260 (Speers-Roesch et al., 2012). This 'closed respirometry' technique is an acceptable method of measuring $p_{\rm crit}$ relative to other methods (e.g., intermittent-flow or flow-through respirometry), and possibly gives 261 262 more ecologically relevant estimates of p_{crit} because low-oxygen environments are typically high in 263 carbon dioxide as a by-product of respiration (Ultsch and Regan, 2019; Wood, 2018). Experiments were 264 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 265 exhibit muscle spasms (Bouyoucos et al., 2020a). Upon termination of the experiment, sharks were then 266 267 removed from chambers and returned to their holding tanks. To estimate p_{crit} , we first calculated an $\dot{M}O_2$ value every five minutes during the closed respirometry phase using LabChart software (7.3.8, 268

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).

273 Physiological oxygen supply capacity could be calculated after estimating RMR and p_{crit} . In this 274 study, α was defined as the maximum ability of the cardiorespiratory system to provide oxygen to 275 metabolically demanding tissues (Ern et al., 2016), and is realized at the p_{crit} of a given metabolic rate 276 (Kielland et al., 2019). In other words, α represents the relationship between a given metabolic rate and its 277 critical oxygen tension, and can be calculated by dividing RMR by its p_{crit} (Seibel and Deutsch, 2020).

278 2.5.6. Data analyses

Associations between *ex situ* haematological traits and thermal tolerance were tested for using simple linear regressions. Specifically, CT_{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 284 285 the assumption that thermal tolerance and physiological oxygen supply are related. These analyses were 286 performed to test the prediction that, if CT_{Max} and α are related, then p_{crit} should equal 21 kPa (i.e., airsaturation and p_{crit-max}) at the measured CT_{Max} (Deutsch et al., 2020; Seibel and Deutsch, 2020). Data for 287 288 both C. melanopterus and N. acutidens were tested. To do this, we used the average temperature 289 sensitivity for $p_{\text{crit}}(E_{\text{pcrit}} = 0.4 \text{ eV})$ reported in Deutsch et al. (2020) to estimate CT_{Max} as the temperature 290 where $p_{\text{crit}} = 21$ 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 291 for CT_{Max} in this study. Next, we used previously published standard (SMR) and maximum (MMR) 292 metabolic rates measured in C. melanopterus at 28 and 31 °C (Bouyoucos et al., 2020a) to calculate 293

temperature sensitivities for SMR and MMR (E_{SMR} and E_{MMR} , respectively) so that E_{pcrit} could be estimated using the equation $E_{SMR} = E_{MMR} + E_{pcrit}$ (Seibel and Deutsch, 2020). Finally, estimated E_{pcrit} from Bouyoucos et al. (2020a) was used to estimate CT_{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).

299 **3. Results**

300 *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 O₂ L⁻¹ (range = 3.10-11.39 mg O₂ 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 O₂ L⁻¹.

308 *3.2. Objective 2*

The average temperature from 2015-2019 calculated from temperature data loggers across sites was 28.9 \pm 0.3 °C, and the average maximum habitat temperature was 34.4 \pm 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.

315 *3.3. Objective 3*

316 Average CT_{Max} was 35.9 ± 0.4 °C for *C. melanopterus* and 35.8 ± 0.6 °C for *N. acutidens*. The 317 average p_{crit} measured in resting sharks was 17.46 ± 1.53 kPa (5.32 ± 0.47 mg O₂ L⁻¹) for *C. melanopterus*

and 16.02 ± 1.66 kPa (4.90 ± 0.51 mg O₂ L⁻¹) for *N. acutidens*. Raw traces of \dot{M} O₂ 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 CT_{Max} (Fig. 4B; Table S3), whereas [Hb] and MCHC were not associated with CT_{Max} (Fig. 4A and C; Table S3). For *N. acutidens*, CT_{Max} was not associated with [Hb], Hct, or MCHC (Fig. 4D-F; Table S3).

- 323 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 CT_{Max} estimates of 33.6 °C and
- 325 34.8 °C, respectively. Conversely, achieving CT_{Max} at measured temperatures (i.e., 35.9 °C for *C*.
- 326 *melanopterus* and 35.8 °C for *N. acutidens*) would necessitate E_{pcrit} values of 0.22 and 0.32 eV,
- 327 respectively. Using previously published data for *C. melanopterus*, temperature sensitivities of SMR and

328 MMR were estimated to be 0.739 and 0.727 eV, respectively. From these values, E_{pcrit} was estimated at

329 0.012 eV for *C. melanopterus*. However, this temperature sensitivity did not predict an ecologically

realistic value of CT_{Max} . Out of all elasmobranch species for which temperature scaling of p_{crit} could be

calculated from the literature (n = 4; Fig. 6; Table S4), E_{pcrit} was 0.43 ± 0.22 .

332 **4. Discussion**

333 Our goal was to test for associations between haematological traits and thermal tolerance in reef shark 334 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' CT_{Max} , these habitats were also 335 336 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. 337 Second, we demonstrated negative associations between haematological traits and upper thermal limits, 338 339 both in situ and ex situ, but only in C. melanopterus neonates. This finding suggests that blood-oxygen carrying capacity and CT_{Max} are not correlated, and that laboratory estimates of CT_{Max} do not always 340 341 approximate maximum habitat temperatures. Yet, associations observed in C. melanopterus neonates

342 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 343 344 rest, under the assumption that thermal tolerance and oxygen supply capacity are related. We found that 345 the average reported temperature sensitivity predicts maximum habitat temperatures for Moorea and the 346 estimated species-specific values fall within the reported range for other elasmobranch species. This 347 provides support for a theoretical relationship between thermal tolerance and oxygen supply capacity in 348 these species; however, we also emphasise that this link has not been demonstrated empirically. Indeed, 349 the temperature sensitivity estimated from previously published data for C. melanopterus do not support 350 the hypothesis of a relationship between CT_{Max} and oxygen supply capacity. Therefore, investigating 351 haematological traits that are related to blood-oxygen carrying capacity alone is likely not sufficient to 352 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 353 capacity (i.e., p_{crit}) may shed light on mechanistic links between thermal tolerance and oxygen (Deutsch et 354 355 al., 2020; Seibel and Deutsch, 2020).

356 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) 357 358 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 359 360 when these sharks are born, they have a thermal safety margin (TSM) of ~ 6 °C. While both species 361 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 362 363 exhibit changes in maximal maximum metabolic rates (MMR) in situ between 28 and 32.5 °C 364 (Bouyoucos et al., 2018), but mortality may be associated with exercise stress at temperatures above 31 365 °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 366

367 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 368 369 water that is supersaturated with oxygen, suggesting that thermal tolerance should not be impaired by 370 ambient oxygen levels, as long as oxygen supply capacity is not compromised. Heating rate is also known 371 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 372 measure CT_{Max} ex situ (7.2 °C h⁻¹), suggesting that, in situ, shark neonates may have greater TSMs than 373 laboratory results might suggest. Yet, TSMs should decrease as summer temperatures increase (McArley 374 375 et al., 2017; Sandblom et al., 2016), and indeed, this has been demonstrated, ex situ, with thermal 376 acclimation in C. melanopterus (Bouyoucos et al., 2020a). It is still not clear as to whether heatwaves 377 and/or ocean warming will expose reef shark neonates around Moorea to temperatures exceeding their 378 CT_{Max}, but this is an area important for future investigation.

379 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 380 pcrit of C. melanopterus in 68% of measurements (~8.1 hours) and in 45% of measurements (~5.4 hours) 381 for N. acutidens. Indeed, neonatal N. acutidens exhibited lower p_{crit} and higher α than C. melanopterus. 382 383 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 384 385 animals, as was the case in this study, and in animals that are forced to swim. Both C. melanopterus and 386 *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 387 388 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 389 390 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, 391

392	when p_{crit} would seemingly decrease, as has been demonstrated in other elasmobranchs (Butler and
393	Taylor, 1975; Crear et al., 2019; Schwieterman et al., 2019b). In other tropical elasmobranchs, p_{crit} is also
394	associated with haemoglobin-oxygen (Hb-O ₂) affinity, which is often reported as the pO_2 at which 50% of
395	the Hb is bound with O_2 (p_{50} ; Speers-Roesch et al., 2012). In this light, increasing Hb-O ₂ affinity at lower
396	temperatures – as demonstrated in vitro in C. melanopterus (Bouyoucos et al., 2020a) – should be
397	accompanied by increasing p_{crit} . Thus, accounting for effects of activity level, gill ventilation, and diel
398	temperature changes would produce different p_{crit} estimates than what we characterized. Finally, prior
399	exposure to supersaturated oxygen conditions during the day could confer greater tolerance to acute
400	declining oxygen conditions that are experienced overnight (Giomi et al., 2019). It is probable that
401	neonatal C. melanopterus and N. acutidens use the shallow, nearshore habitats around Moorea for refuge
402	from predators and even thought to stay within these sites without venturing to deeper, possibly well
403	oxygenated waters at night (Bouyoucos et al., 2020b). Thus, C. melanopterus and N. acutidens must be
404	able to tolerate environmental oxygen levels that decline below their estimated p_{crit} for an immobile
405	animal for at least five hours if they are to remain within the protection of their shallow habitats at night.
406	Haematological traits varied with <i>in situ</i> and <i>ex situ</i> estimates of upper thermal limits in C.
407	melanopterus neonates, but none of the haematological traits we measured were associated with
408	maximum habitat temperature or CT _{Max} in N. acutidens. In C. melanopterus, [Hb] was negatively
409	associated with maximum habitat temperature while Hct was negatively associated with CT_{Max} . In
410	notothenioid fishes and Chinook salmon (Oncorhynchus tshawytscha), Hct has been found to be
411	positively associated with CT _{Max} (Beers and Sidell, 2011; Muñoz et al., 2018), and this finding has been
412	debated as evidence in support of a link between oxygen supply capacity and thermal tolerance (Brijs et
413	al., 2015; Pörtner et al., 2017; Wang et al., 2014). Finding the opposite trend in only C. melanopterus and
414	not in its congener, N. acutidens, suggests that associations between Hct and CT _{Max} are variable, species-
415	specific, and are likely not the best metrics for testing links between thermal tolerance and oxygen supply.
416	Further, such trends have only been demonstrated over narrow temperature ranges, such as ~ 1 °C in the

417 present study and ~3 °C in Beers et al. (2011) and Muñoz et al. (2018). The mismatch we observe between field and laboratory trends for different neonate sub-populations around Moorea also suggests 418 419 disagreement between laboratory estimates of CT_{Max} and maximum habitat temperatures. If CT_{Max} was a 420 reliable proxy for maximum habitat temperature, then we would predict that traits associated with one 421 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, 422 and the mismatch in the haematological traits between C. melanopterus and N. acutidens suggests that 423 424 such traits may not reliably predict CT_{Max}.

425 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, 426 427 coefficients of determination for Hct and CT_{Max} ($R^2 = 0.63$) and for [Hb] and maximum habitat 428 temperature ($R^2 = 0.88$) were relatively strong. Negative associations between these traits have, to the best 429 of our knowledge, not been previously demonstrated in fishes and suggest a unique, as-of-yet unexplained phenomenon. A negative association between Hct and CT_{Max} could reflect the thermal dependence of 430 blood viscosity. Depending on shear rate, blood viscosity increases as Hct increases (Baldwin and Wells, 431 1990; Wells and Baldwin, 1990), while increasing temperature reduces blood viscosity (Brill and Jones, 432 433 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 434 435 this has not been directly tested in C. melanopterus. However, it is important to note that Hct in C. 436 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 437 438 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 439 440 *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 441

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].

449 The average temperature sensitivity for p_{crit} reported by Deutsch et al. (2020) predicted oxygendependent values of CT_{Max} for C. melanopterus (33.6 °C) and N. acutidens (34.8 °C). This exercise relied 450 on the assumption that CT_{Max} and oxygen supply capacity are related, such that CT_{Max} is defined as the 451 temperature where p_{crit} equals the prevailing environmental pO_2 (i.e., 21 kPa). While these values 452 underestimate CT_{Max} as measured in this study, the predicted values align very closely with measured 453 maximum habitat temperatures in reef shark neonate habitats around Moorea. Further, the estimated 454 455 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 pcrit 456 457 that is needed to yield the CT_{Max} values that were measured for C. melanopterus and N. acutidens 458 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 459 deviation for p_{crit} of 0.40 ± 0.28 eV (range = -0.2-1.3 eV) for marine ectotherms. In comparison, available 460 data for elasmobranch species (n = 4) produces a temperature sensitivity of 0.43 ± 0.22 (range = 0.16-461 0.69) (Butler and Taylor, 1975; Crear et al., 2019; Schwieterman et al., 2019b), and we estimated 462 463 temperature sensitivities of 0.22 and 0.32 eV for p_{crit} in C. melanopterus and N. acutidens, respectively. 464 Conversely, the very low temperature sensitivity predicted from thermal sensitivities of SMR and MMR 465 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., 466

467 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 468 469 elasmobranch species for which E_{pcrit} could be calculated. We emphasise, however, that our study does 470 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 471 immobile, buccal-pumping animal. Both of our study species swim nearly continuously in situ (Wells et 472 al., 1992); although, both species rest on the benthos and are capable of buccal pumping to ventilate their 473 gills. Notably, MMR can be estimated from α at the prevailing environmental pO₂ (Seibel and Deutsch, 474 475 2020), which was ≥ 21 kPa in this study; this exercise underestimated MMR for *C. melanopterus* based 476 on values previously reported by our group (Bouyoucos et al., 2020a, 2020c, 2018; Rummer et al., 2020). 477 Thus, a swimming reef shark could be able to better ventilate their gills relative to a resting animal, which 478 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 O₂ kg^{-0.89} h⁻¹, whereas measured values range 278-561 mg O₂ kg^{-0.89} h⁻¹ 479 (Bouyoucos et al., 2020c). However, MMR, as reported in these previous studies was measured using the 480 identical respirometry technique described here. Thus, while these data do not preclude the possibility of 481 a relationship between oxygen supply capacity and CT_{Max} in these reef sharks, these data cannot 482 483 empirically demonstrate such a relationship.

484 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*) 492 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 493 494 ontogenetic stages (Dahlke et al., 2020) and investigating links between thermal tolerance and different 495 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 496 the combined stress of ocean warming and deoxygenation. Additional research into thermal tolerance and 497 oxygen supply capacity in sharks and other water-breathing ectotherms is needed, and future studies can 498 499 benefit from testing the multiple available hypotheses.

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513 Data statement

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

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713 Tables

Table 1. Routine metabolic rates (RMR, mg O_2 kg^{-0.89} h⁻¹) and critical oxygen tensions (p_{crit} , kPa) used to

715	calculate physiological oxyge	n supply capacity (α, n	ng O ₂ kg ^{-0.89} h ⁻¹ kl	Pa ⁻¹) in <i>Carcharhir</i>	nus melanopterus.
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ID	Mass (kg)	RMR	$p_{\rm crit}$	a	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

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ID	Mass (kg)	RMR	<i>p</i> _{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

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*.

721 Figure captions



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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.



727 Fig. 2. Associations between haematological traits of blacktip reef shark (Carcharhinus melanopterus) 728 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 729 average temperature (A-C) or maximum temperature (D-F) measured at eight sites. For panels A-C, 730 individual observations represent the average temperature at a site from 2015-2019 and the average value 731 of each haematological trait measured in sharks from 2014-2016 at the same sites (n = 8 sites). Error bars 732 733 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 2015-2019 plus three standard 734 deviations, and the same data for sharks from panels A-C. Regression lines with 95% confidence intervals 735 736 (i.e., shading) represent statistically significant associations between variables.



738 Fig. 3. Associations between haematological traits of sicklefin lemon shark (Negaprion acutidens) 739 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 740 average temperature (A-C) or maximum temperature (D-F) measured at eight sites. For panels A-C, 741 742 individual observations represent the average temperature at a site from 2015-2019 and the average value of each haematological trait measured in sharks from 2014-2016 at the same sites (n = 4 sites). Error bars 743 744 represent standard error of the mean. For panels D-F, individual observations represent the maximum 745 habitat temperature at each site (n = 4 sites), calculated as the average from 2015-2019 plus three standard 746 deviations, and the same data for sharks from panels A-C.



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.



Fig. 5. Temperature sensitivity of critical oxygen tension (p_{crit}) of four elasmobranch species. The natural**103**logarithm of p_{crit} (kPa) is plotted against the inverse of temperature (°Kelvin) multiplied by the Boltzmann**103**constant. The slope of this line is the temperature sensitivity (E, in eV). Available data from the literature**103**were collected for the thorny skate, *Amblyraja radiata* (Schwieterman et al., 2019b), sandbar shark,**100***Carcharhinus plumbeus* (Crear et al., 2019), clearnose skate, *Rostaraja eglanteria* (Schwieterman et al.,

761 2019b), and small-spotted catshark, *Scyliorhinus canicula* (Butler and Taylor, 1975).