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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
4 this relationship is hypothesised to be explained by oxygen supply capacity. We tested this hypothesis
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
10 haematological traits of neonates. Contrary to predictions, we only demonstrated a negative association
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

23 The way in which temperature affects physiological performance of ectotherms is linked to species'
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's body temperature and its
29 thermal tolerance limits (Sunday et al., 2014). Thermal safety margins decrease with increasing
30 temperature, and tropical ectotherms appear to have the smallest TSMs (Payne and Smith, 2017; Sunday
31 et al., 2019). Climate change is increasing air and sea surface temperatures and the frequency and
32 duration of heatwaves globally (Bindoff et al., 2019). As such, global warming puts tropical ectotherms at
33 a greater risk of shifts in distribution patterns or even mortality relative to temperate species (Pinsky et al.,
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,
40 1997a). While not a lethal endpoint, CT_{Max} is essentially 'ecological death', meaning that the organism
41 would no longer be able to serve a functional ecological role in its environment, and survival is limited.
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}
44 (Sunday et al., 2019); although, this approach has highlighted that laboratory estimates are not always
45 representative of *in situ* limits (Payne et al., 2016).

46 Oxygen availability is another important environmental factor that limits the distribution of water-
47 breathing ectotherms. In water, oxygen concentrations are already quite low (i.e., ~30% lower in water
48 compared to air). Hypoxia (i.e., low oxygen availability) is a widespread phenomenon in the oceans, such
49 as oxygen minimum zones in the deep-sea (Seibel, 2011), and limits the distribution of water-breathers.
50 Climate change is further reducing oxygen availability in aquatic ecosystems, partially because of global
51 warming (Breitburg et al., 2018), and this could further affect species' distributions and survival (Wishner
52 et al., 2018). However, the oxygen availability that constitutes hypoxia differs between species and is
53 related to the sensitivity of an organism's physiological systems to changes in environmental oxygen. For
54 instance, an often-cited hypoxia threshold of 2 mg O₂ L⁻¹ would be fatal for the sandbar shark
55 (*Carcharhinus plumbeus*; Crear et al., 2019), but not the clearnose skate (*Rostaraja eglanteria*;
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
59 pressure (pO_2) below which metabolic rate declines with environmental pO_2 (Ultsch and Regan, 2019;
60 Wood, 2018). Typically, p_{crit} is measured when an organism has achieved its standard metabolic rate
61 (Rogers et al., 2016), which represents the metabolic rate of a postprandial organism at rest and at a stable
62 temperature (Chabot et al., 2016). However, it has recently been suggested that p_{crit} is not an ecologically
63 relevant measure of hypoxia tolerance (Wood, 2018) and simply represents a rate-specific measure of
64 oxygen supply capacity (Seibel and Deutsch, 2020). Thus, when environmental oxygen reaches p_{crit} for a
65 given metabolic rate, an organism's physiological oxygen supply capacity ($\alpha = \text{metabolic rate} \cdot p_{crit}^{-1}$) is
66 being fully utilised (Seibel and Deutsch, 2020). As such, it has been suggested that the oxygen level at
67 which maximum metabolic rate (MMR) is reduced (i.e., $p_{crit-max}$) 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 biogeographic
70 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
77 and Sidell, 2011) associations between haematocrit (i.e., the ratio of red blood cell volume to whole blood
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
81 test this prediction ought to test whether oxygen supply capacity has a role in the presence or absence of
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
85 correlate with increases (McArley et al., 2018) or decreases (Fangue et al., 2001) in oxygen in shallow,
86 coastal habitats, such as rockpools. The relative amount of primary productivity and respiration occurring
87 at high temperatures determines whether these habitats become hypoxic (i.e., when respiration rates
88 exceed photosynthesis rates) or hyperoxic (i.e., when photosynthesis rates exceed respiration rates). In
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
97 because they have relatively high thermal tolerance (Bouyoucos et al., 2020a; Gervais et al., 2018) and
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
102 experimentally derived thermal tolerance and physiological oxygen supply capacity in the same
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*

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

114 *2.2. Study site*

115 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.

119 All sites are contained within Moorea's narrow lagoon system that experiences little variation in tide
120 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),
126 approximately once every two weeks from November 2016 through March 2017. During this period, 5 ±
127 3 measurements (range = 2-24) were taken in two-hour bins from 0630-1830. A simple linear model was
128 then used to model DO by the interaction of site and temperature using the R '*Stats*' package (R Core
129 Team, 2018). Next, three representative sites were selected from Moorea's western, northern, and eastern
130 coastline (Apaura, Pihaena, and Valorie, respectively). Sites were sampled three times each from
131 December 2016 through April 2017. Rates of oxygen decline were measured from 1800-0600 using spot
132 measurements taken every 30 minutes. The highest linear rate of oxygen decline was identified over a
133 four-hour window during each sampling night using a rolling regression function ('*auto_resp()*') in the
134 '*respR*' package (Harianto et al., 2019). Finally, one or two temperature data-loggers (HOBOUA-002-64,
135 Onset Computer Corporation, Bourne, MA, USA) were deployed at each site to record temperature every
136 ten minutes (accuracy = ± 0.5 °C, resolution = 0.14 °C at 25 °C), yielding partial records from November
137 2015 through February 2019 (Bouyoucos et al., 2020a). Temperature data from four summers
138 (November-February) were selected, and the previously mentioned rolling regression function was
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 sites
143 and haematological profiles of resident reef shark neonates. Thermal habitat profiles were generated for

144 each site from temperature data-loggers (as described in section ‘2.3. *Objective 1*’) and consisted of the
145 average and maximum temperature. Rather than selecting the single highest measured value as an
146 estimate of maximum habitat temperature, the mean temperature plus three standard deviations was used
147 as a conservative estimate of maximum habitat temperature (Fig. S1). This rationale is also informed by
148 the idea that an organism’s upper thermal limits can be roughly estimated from thermal reaction norms of
149 physiological performance traits as three standard deviations above the optimal temperature (Deutsch et
150 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*
152 *melanopterus*, Quoy & Gaimard 1824; $n = 170$ individuals from eight sites) and sicklefin lemon sharks
153 (*Negaprion acutidens*, Rüppell 1837; $n = 54$ individuals from four sites). Both species were collected
154 from eight sites around Moorea during summer parturition months. Sites were monospecific or hosted
155 both species (Mourier et al., 2013a, 2013b). Sharks were collected using gill-nets during two parturition
156 seasons (October 2014 through March 2015 and November 2015 through March 2016). Gill-nets were set
157 from 1700-2000 and were constantly monitored so that sharks could be removed from the gill-net as soon
158 as they were captured to minimize capture-related stress. Blood samples were then collected immediately
159 by inverting sharks and taking 1 mL of blood from the caudal vasculature with 21-23 gauge, heparin-
160 washed needles. Samples were processed immediately in the field or put on ice and stored for up to three
161 hours and processed in the lab; this practice has been shown to not affect values of the haematological
162 metrics (i.e., haematocrit and haemoglobin concentration) that were measured (Schwieterman et al.,
163 2019a). We measured haematocrit (Hct) by centrifuging blood samples in 70 μL microhaematocrit tubes
164 for two minutes at 4,400 g using a portable centrifuge (ZIPocrit, LW Scientific, Lawrenceville, GA,
165 USA), and calculated Hct as the ratio of packed cell volume to total sample volume. Next, haemoglobin
166 concentration ([Hb], in g L^{-1}) was measured using a handheld point-of-care device (HemoCue Hb 201,
167 Australia Pty Ltd, Victoria, Australia) and corrected using species-specific equations (Schwieterman et
168 al., 2019a). Mean corpuscular haemoglobin concentration (MCHC, in g L^{-1}) was then calculated as [Hb]

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 (n
175 = 6 per species).

176 2.5. Objective 3

177 2.5.1. Experimental design

178 Our third objective was to test for associations between *ex situ* haematological traits, thermal
179 tolerance, and physiological oxygen supply capacity. Individual sharks were sampled for every trait (i.e.,
180 haematological traits, thermal tolerance, and physiological oxygen supply capacity), but in differing
181 sampling orders. Sharks were fed between trials, allowing at least 48 hours to recover and fast between
182 trials (Bouyoucos et al., 2020a). Sharks were maintained at ambient temperatures to avoid possible
183 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
187 seawater (Bouyoucos et al., 2018). At the CRIOBE, sharks were housed in 1250 L (1.5 m diameter and 70
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
191 passive integrated transponder (PIT) tags (Biolog-id SAS, Paris, France). Sharks were fed *ad libitum*
192 every other day with fresh tuna (*Thunnus* spp.). *Carcharhinus melanopterus* neonates ($n = 12$; mass =
193 0.89 ± 0.15 kg, range = 0.67-1.16 kg) were maintained in groups of two or three per tank, and larger *N.*

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 °C) and experiments on *N. acutidens* were
197 conducted in January 2020 (ambient water temperature = 28 °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
202 the caudal vasculature using 21- or 23-gauge, heparin-washed needles. Handling and blood sampling
203 were under two minutes so that all sharks within a tank could be sampled in rapid succession and on the
204 same day. Blood samples were placed on ice for up to 30 minutes before being processed. Haematocrit
205 was measured in duplicate, using 70 μ L microhaematocrit tubes and a benchtop centrifuge to spin
206 samples at 10,000 g for five minutes. Haemoglobin concentration was also measured in duplicate, where
207 blood samples were incubated within microcuvettes for nine minutes before being read by the HemoCue
208 (Schwieterman et al., 2019a). Species-specific corrections were applied to raw [Hb] output
209 (Schwieterman et al., 2019a). The mean Hct and [Hb] values were used to calculate a single MCHC value
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
214 tank containing five 300-watt aquarium heaters (Jager 300w, EHEIM GmbH & Co KG, Deizisau,
215 Germany). The tank was continuously aerated with an air stone to maintain oxygen saturation above 80%.
216 Following a five-minute habituation to the experimental tank, the water temperature was elevated from an
217 ambient temperature of 29.4 ± 0.9 °C for *C. melanopterus* trials and 28.0 ± 1.5 °C for *N. acutidens* trials
218 at a constant rate of 0.12 ± 0.02 °C min^{-1} using the aquarium heaters until the shark began to exhibit

219 muscle spasms (Bouyoucos et al., 2020a). The temperature at which sharks exhibited the onset of muscle
220 spasms (OS) was recorded as an individual's CT_{Max} , where a spasm was defined as any rapid convulsion
221 originating from the animal's trunk. The temperature at OS is a repeatable, non-lethal endpoint for
222 determining CT_{Max} (Lutterschmidt and Hutchison, 1997b). After reaching OS, the shark was immediately
223 returned to its holding tank.

224 2.5.5. Oxygen supply capacity

225 Physiological oxygen supply capacity (α , in $mg\ O_2\ kg^{-0.89}\ h^{-1}\ kPa^{-1}$) was calculated by estimating
226 sharks' routine metabolic rate (RMR, in $mg\ O_2\ kg^{-0.89}\ h^{-1}$) and critical oxygen tension (p_{crit} , in kPa). To
227 estimate RMR and p_{crit} , we used intermittent-flow respirometry (Svendsen et al., 2016). Sharks were
228 removed from their holding tanks and placed into individual static respirometry chambers. Up to four 32
229 L chambers (24 cm diameter and 70 cm long) were placed in a single holding tank that was filled to 530 L
230 (1.5 m diameter and 30 cm deep) and received continuous water supply and aeration. Each chamber was
231 fitted with two 2,500 $L\ h^{-1}$ pumps (EHEIM GmbH & Co KG, Deizisau, Germany): one was configured in
232 a closed, recirculating loop to mix water within the chamber, and the other was configured to flush water
233 from the holding tank through the chamber and out an overflow pipe above the water's surface. Oxygen
234 was measured in each chamber with a FireSting Optical Oxygen Meter (PyroScience, GmbH, Aachen,
235 Germany). Oxygen probes were placed within each chamber through the overflow pipe and measured DO
236 every two seconds. A single temperature probe was connected to the oxygen meter and placed in the
237 holding tank to correct DO readings for changes in ambient temperature. The average ambient water
238 temperature recorded during respirometry trials was $29.8 \pm 0.2\ ^\circ C$ for *C. melanopterus* and $29.4 \pm 0.1\ ^\circ C$
239 for *N. acutidens*. Corrections for salinity were made by manually inputting a single value into the
240 FireSting software, and barometric pressure was accounted for with a built-in sensor. Oxygen probes were
241 calibrated to 100% and 0% saturation before each trial.

242 Routine metabolic rate was estimated during the first four hours sharks spent in respirometry
243 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
245 chambers, intermittent cycling of the flush pump was controlled with a custom data acquisition system
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
248 ($\dot{M}O_2$) could be measured. In total, 16 $\dot{M}O_2$ values were calculated for each shark. To approximate RMR
249 of a minimally active animal, RMR in this study was estimated as the mean of the lowest three $\dot{M}O_2$
250 values (Norin et al., 2014). Oxygen uptake rates were calculated by multiplying the rate of linear oxygen
251 decline (in $\text{mg O}_2 \text{ L}^{-1} \text{ s}^{-1}$; $R^2 > 0.95$) by the volume of water in the chamber (in L) and dividing by the
252 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
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).

256 The p_{crit} was estimated immediately after estimation of RMR. In this study, p_{crit} was defined as the
257 oxygen partial pressure (pO_2 , in kPa) at which a shark's RMR began a linear decline with ambient pO_2
258 (Rogers et al., 2016). After sharks spent four hours in chambers for estimation of RMR, flush pumps were
259 disabled to allow sharks to consume oxygen within the chambers and experience progressive hypoxia
260 (Speers-Roesch et al., 2012). This 'closed respirometry' technique is an acceptable method of measuring
261 p_{crit} relative to other methods (e.g., intermittent-flow or flow-through respirometry), and possibly gives
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
265 in real time, or when DO reached $2.0 \text{ mg O}_2 \text{ L}^{-1}$, which is a threshold when neonatal *C. melanopterus*
266 exhibit muscle spasms (Bouyoucos et al., 2020a). Upon termination of the experiment, sharks were then
267 removed from chambers and returned to their holding tanks. To estimate p_{crit} , we first calculated an $\dot{M}O_2$
268 value every five minutes during the closed respirometry phase using LabChart software (7.3.8,

269 ADInstruments, Dunedin, New Zealand). Then, simple linear regression was used to fit a line to these
270 $\dot{M}O_2$ values against the first DO reading at which they were measured for each shark. The DO value at
271 which each sharks' RMR intersected this line was recorded as a sharks' p_{crit} and converted to kPa using
272 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

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

284 An additional series of analyses were undertaken to estimate the temperature sensitivity of p_{crit} under
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., air-
287 saturation and $p_{crit-max}$) at the measured CT_{Max} (Deutsch et al., 2020; Seibel and Deutsch, 2020). Data for
288 both *C. melanopterus* and *N. acutidens* were tested. To do this, we used the average temperature
289 sensitivity for p_{crit} ($E_{pcrit} = 0.4$ eV) reported in Deutsch et al. (2020) to estimate CT_{Max} as the temperature
290 where $p_{crit} = 21$ kPa using p_{crit} estimates for both species from our study. Then, we estimated the
291 temperature sensitivity required for measured values of p_{crit} to equal 21 kPa at the measured temperatures
292 for CT_{Max} in this study. Next, we used previously published standard (SMR) and maximum (MMR)
293 metabolic rates measured in *C. melanopterus* at 28 and 31 °C (Bouyoucos et al., 2020a) to calculate

294 temperature sensitivities for SMR and MMR (E_{SMR} and E_{MMR} , respectively) so that E_{pcrit} could be
295 estimated using the equation $E_{SMR} = E_{MMR} + E_{pcrit}$ (Seibel and Deutsch, 2020). Finally, estimated E_{pcrit}
296 from Bouyoucos et al. (2020a) was used to estimate CT_{Max} for *C. melanopterus* using p_{crit} measurements
297 from the current study. For reference, E_{pcrit} was calculated from the only other available data from
298 elasmobranch fishes (Butler and Taylor, 1975; Crear et al., 2019; Schwieterman et al., 2019b).

299 3. Results

300 3.1. Objective 1

301 The average temperature calculated from spot measurements across all ten sites was 29.9 ± 0.2 °C
302 (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).
303 Oxygen was positively associated with temperature at each site (Fig. 1; Table S1). Maximum heating
304 rates occurred during 0610-1530 and were 1.4 ± 0.6 °C h⁻¹ (Fig. S2). The average of the maximum
305 temperatures that were recorded across all sites was 34.1 ± 1.7 °C. Maximum rates of oxygen decline
306 between 1800 and 0600 were 0.45 ± 0.18 mg L⁻¹ h⁻¹ (Fig. S3). The average of the minimum DO values
307 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

309 The average temperature from 2015-2019 calculated from temperature data loggers across sites was
310 28.9 ± 0.3 °C, and the average maximum habitat temperature was 34.4 ± 0.8 °C. For *C. melanopterus*,
311 there were no associations between average habitat temperature and [Hb], Hct, or MCHC (Fig. 2A-C;
312 Table S2). However, maximum habitat temperature was negatively associated with [Hb] but not with Hct
313 or MCHC (Fig. 2D-F; Table S2). For *N. acutidens*, neither average habitat temperature (Fig. 3A-C; Table
314 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*

318 and 16.02 ± 1.66 kPa (4.90 ± 0.51 mg O₂ L⁻¹) for *N. acutidens*. Raw traces of $\dot{M}O_2$ used for determining
319 p_{crit} are presented in the supplementary materials (*C. melanopterus*, Figs. S4-S5; *N. acutidens*, Figs. S6-
320 S7). For *C. melanopterus*, Hct was negatively associated with CT_{Max} (Fig. 4B; Table S3), whereas [Hb]
321 and MCHC were not associated with CT_{Max} (Fig. 4A and C; Table S3). For *N. acutidens*, CT_{Max} was not
322 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
324 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
330 realistic value of CT_{Max}. Out of all elasmobranch species for which temperature scaling of p_{crit} could be
331 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.
335 Here, we demonstrated that, when habitats approached the sharks' CT_{Max}, these habitats were also
336 hyperoxic; therefore, environmental oxygen availability should not be limiting for physiological
337 processes. However, this would only be true if oxygen supply capacity has not been compromised.
338 Second, we demonstrated negative associations between haematological traits and upper thermal limits,
339 both *in situ* and *ex situ*, but only in *C. melanopterus* neonates. This finding suggests that blood-oxygen
340 carrying capacity and CT_{Max} are not correlated, and that laboratory estimates of CT_{Max} do not always
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
343 and haemoglobin isoform expression. Third, we estimated the temperature sensitivity of p_{crit} in sharks at
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
353 al., 2018; Wang et al., 2014). Future studies investigating the thermal dependence of oxygen supply
354 capacity (i.e., p_{crit}) may shed light on mechanistic links between thermal tolerance and oxygen (Deutsch et
355 al., 2020; Seibel and Deutsch, 2020).

356 Around Moorea, the reef shark neonate habitats approached but did not exceed the two experimental
357 species' upper thermal limits. While the average maximum habitat temperatures across all sites (34.4 °C)
358 did not exceed sharks' measured CT_{Max} (~ 36 °C), individual maximum habitat temperature estimates did
359 match sharks' CT_{Max} at two of the ten sites. Together, this suggests that, during Austral summer months
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
362 the physiological performance of *N. acutidens* and *C. melanopterus*. For instance, *C. melanopterus* do not
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
366 haemoglobin's affinity for oxygen decreases with increasing temperatures; moreover, *in situ*, this species

367 is also capable of behaviourally avoiding unfavourable temperatures (Bouyoucos et al., 2020a). However,
368 when temperatures approach their CT_{Max} in their natural habitats, these sharks are predicted to experience
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
372 heating rates measured *in situ* ($1.4\text{ }^{\circ}\text{C h}^{-1}$) were much slower than experimental heating rates used to
373 measure CT_{Max} *ex situ* ($7.2\text{ }^{\circ}\text{C h}^{-1}$), suggesting that, *in situ*, shark neonates may have greater TSMs than
374 laboratory results might suggest. Yet, TSMs should decrease as summer temperatures increase (McArley
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
380 species. Overnight measurements at three of the ten sites demonstrated that DO fell below the measured
381 p_{crit} of *C. melanopterus* in 68% of measurements (~ 8.1 hours) and in 45% of measurements (~ 5.4 hours)
382 for *N. acutidens*. Indeed, neonatal *N. acutidens* exhibited lower p_{crit} and higher α than *C. melanopterus*.
383 However, a more ecologically relevant p_{crit} would account for the effects of sharks' activity level and
384 body temperature. Indeed, it is important to consider how p_{crit} and α vary when measured in immobile
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
387 their gills through buccal pumping. If the capacity of these sharks to ventilate their gills while swimming
388 is greater than their capacity to ventilate their gills through buccal pumping, then it is conceivable that
389 swimming reef sharks could exhibit lower p_{crit} and higher α , despite their increased metabolic rates
390 relative to resting. Another consideration is the effect of diel temperature fluctuations on p_{crit} in reef
391 sharks *in situ*. Moorea's habitats experience declining DO at night when water temperatures are lowest,

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-O₂) affinity, which is often reported as the pO_2 at which 50% of
395 the Hb is bound with O₂ (p_{50} ; Speers-Roesch et al., 2012). In this light, increasing Hb-O₂ 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 *in situ* and *ex situ* 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
418 between field and laboratory trends for different neonate sub-populations around Moorea also suggests
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*
422 in *C. melanopterus* suggests that CT_{Max} may not be a meaningful proxy for maximum habitat temperature,
423 and the mismatch in the haematological traits between *C. melanopterus* and *N. acutidens* suggests that
424 such traits may not reliably predict CT_{Max} .

425 The strength of observed associations between haematological traits, CT_{Max} , and maximum habitat
426 temperatures in *C. melanopterus*, however, suggest that these trends were not spurious. Indeed,
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
430 phenomenon. A negative association between Hct and CT_{Max} could reflect the thermal dependence of
431 blood viscosity. Depending on shear rate, blood viscosity increases as Hct increases (Baldwin and Wells,
432 1990; Wells and Baldwin, 1990), while increasing temperature reduces blood viscosity (Brill and Jones,
433 1994). Thus, it would be expected that *C. melanopterus* experience reductions in blood viscosity during
434 acute temperature increases during CT_{Max} experiments. It is possible that Hct increases during heating, but
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
437 (Schwieterman et al., 2019a). On the other hand, a negative association between [Hb] and maximum
438 habitat temperature could reflect the expression of fewer, more temperature resistant Hb isoforms in
439 habitats that achieve higher maximum temperatures. For instance, in *C. melanopterus* living at 27 °C, *in*
440 *vitro* experiments suggest a loss of Hb pH sensitivity when temperatures approaching the animal's CT_{Max}
441 (Bouyoucos et al., 2020a). This logic assumes differences in the costs of expressing temperature resistant

442 isoforms. Alternatively, because habitats around Moorea exhibited strong positive associations between
443 temperature and oxygen, lower [Hb] in habitats that achieve higher maximum temperatures could reflect
444 the degree of hyperoxia in those habitats. Clearly, further research is warranted to determine the
445 mechanistic underpinnings and biological significance of the negative associations between
446 haematological traits and thermal habitat characteristics in *C. melanopterus*. If these are, indeed, robust
447 trends, as they seem to be, increases in maximum habitat temperatures with ocean warming could result in
448 pathologies in sharks with very low Hct and [Hb].

449 The average temperature sensitivity for p_{crit} reported by Deutsch et al. (2020) predicted oxygen-
450 dependent values of CT_{Max} for *C. melanopterus* (33.6 °C) and *N. acutidens* (34.8 °C). This exercise relied
451 on the assumption that CT_{Max} and oxygen supply capacity are related, such that CT_{Max} is defined as the
452 temperature where p_{crit} equals the prevailing environmental pO_2 (i.e., 21 kPa). While these values
453 underestimate CT_{Max} as measured in this study, the predicted values align very closely with measured
454 maximum habitat temperatures in reef shark neonate habitats around Moorea. Further, the estimated
455 CT_{Max} value for *C. melanopterus* is close to the maximum body temperature of 34.1 °C that was measured
456 *in situ* for *C. melanopterus* (Bouyoucos et al., 2020a). Next, predicting the temperature sensitivity of p_{crit}
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
459 Deutsch et al. (2020). Indeed, Deutsch et al. (2020) report a mean temperature sensitivity and standard
460 deviation for p_{crit} of 0.40 ± 0.28 eV (range = -0.2-1.3 eV) for marine ectotherms. In comparison, available
461 data for elasmobranch species ($n = 4$) produces a temperature sensitivity of 0.43 ± 0.22 (range = 0.16-
462 0.69) (Butler and Taylor, 1975; Crear et al., 2019; Schwieterman et al., 2019b), and we estimated
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
466 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., 2020a, 2020c, 2018; 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, $\alpha \cdot 21 \text{ kPa} = 136\text{-}219 \text{ mg O}_2 \text{ kg}^{-0.89} \text{ h}^{-1}$, whereas measured values range $278\text{-}561 \text{ mg O}_2 \text{ kg}^{-0.89} \text{ h}^{-1}$ (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.

484 **Conclusions**

485 In conclusion, our investigations using reef sharks in a model experimental system do not support the
486 notion that haematological traits can be used to predict intra-specific variation in thermal tolerance.
487 However, our results do not preclude the possibility of a mechanistic link between thermal tolerance and
488 oxygen. To accomplish this fully, in the future, it will be necessary to define the oxygen dependence of
489 CT_{Max} (Ern et al., 2016) and the temperature dependence of the metabolic phenotype (i.e., SMR, MMR,
490 and p_{crit}) over a species' full ecologically relevant temperature range (Seibel and Deutsch, 2020). Indeed,
491 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
493 studies should also consider testing for a link between oxygen and thermal tolerance between different
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
496 tolerate further ocean warming, but our findings do not indicate how reef shark neonates will fare under
497 the combined stress of ocean warming and deoxygenation. Additional research into thermal tolerance and
498 oxygen supply capacity in sharks and other water-breathing ectotherms is needed, and future studies can
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
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516

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712

713 **Tables**

714 **Table 1.** Routine metabolic rates (RMR, mg O₂ kg^{-0.89} h⁻¹) and critical oxygen tensions (p_{crit} , kPa) used to
715 calculate physiological oxygen supply capacity (α , mg O₂ 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

716

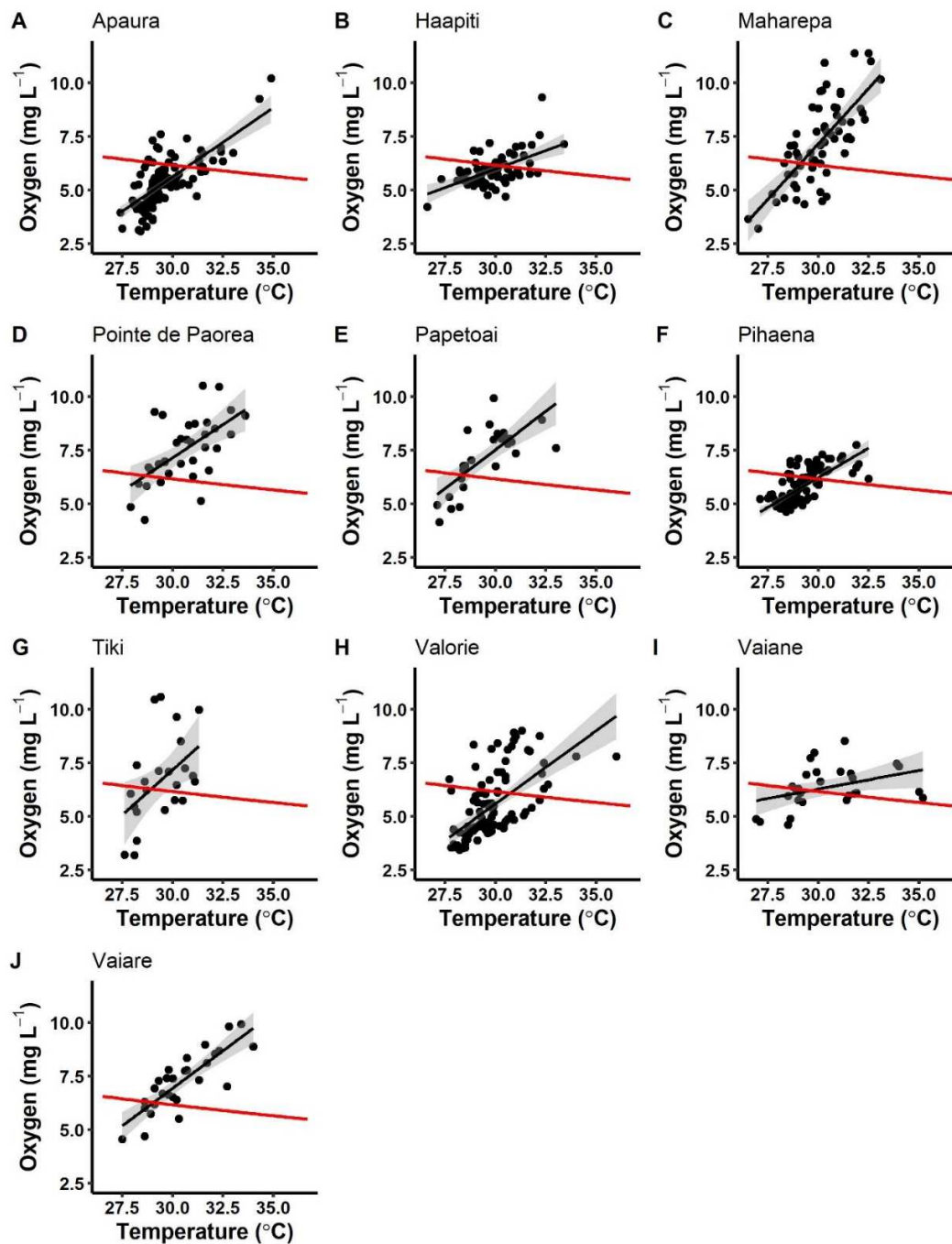
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718 **Table 2.** Routine metabolic rates (RMR, mg O₂ kg^{-0.89} h⁻¹) and critical oxygen tensions (p_{crit} , kPa) used to
 719 calculate physiological oxygen supply capacity (α , mg O₂ kg^{-0.89} h⁻¹ kPa⁻¹) in *Negaprion acutidens*.

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

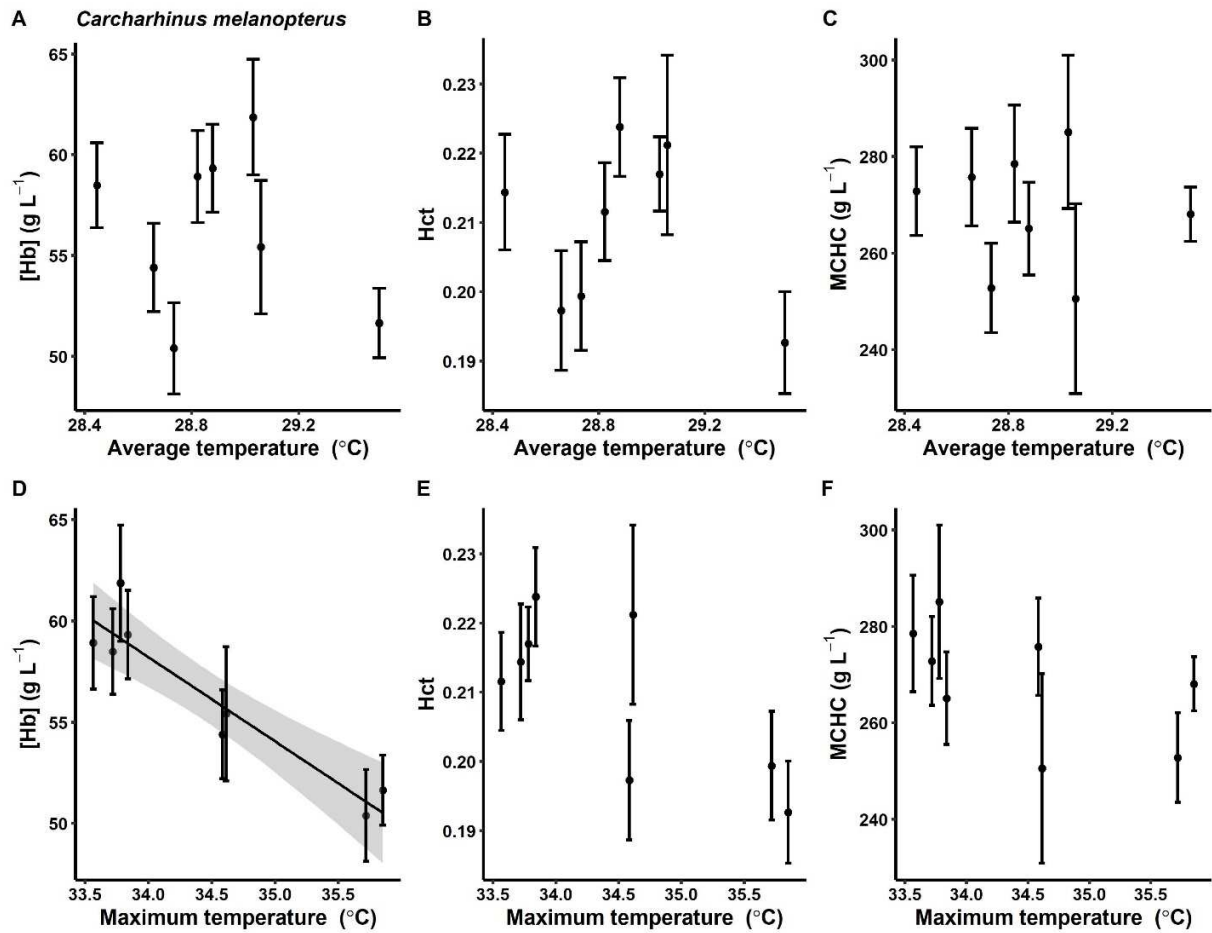
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721 **Figure captions**



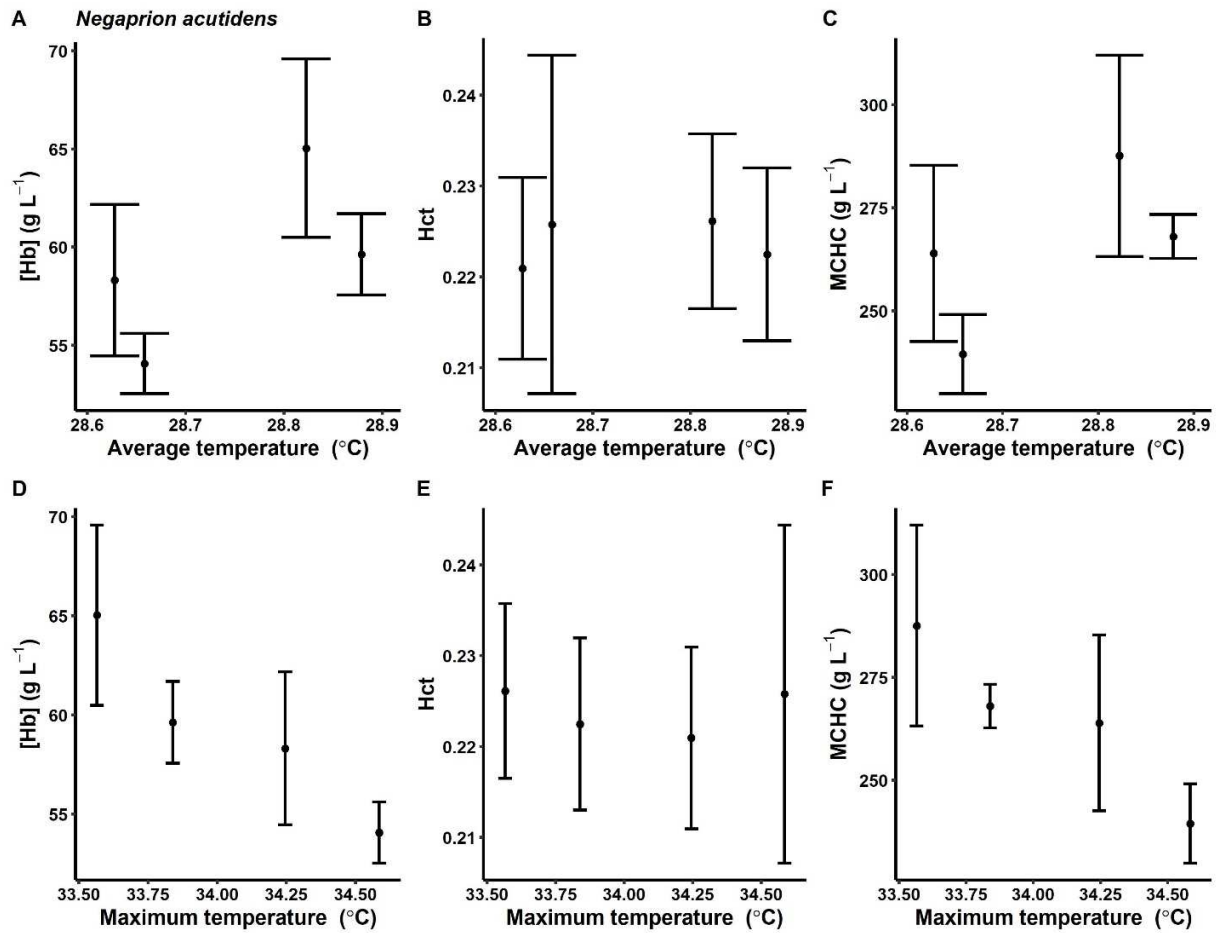
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723 **Fig. 1.** Associations between water temperature and dissolved oxygen at ten locations (A-J) around
724 Moorea, French Polynesia. Shading around regression lines represents 95% confidence intervals. The red
725 line represents 100% oxygen saturation;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



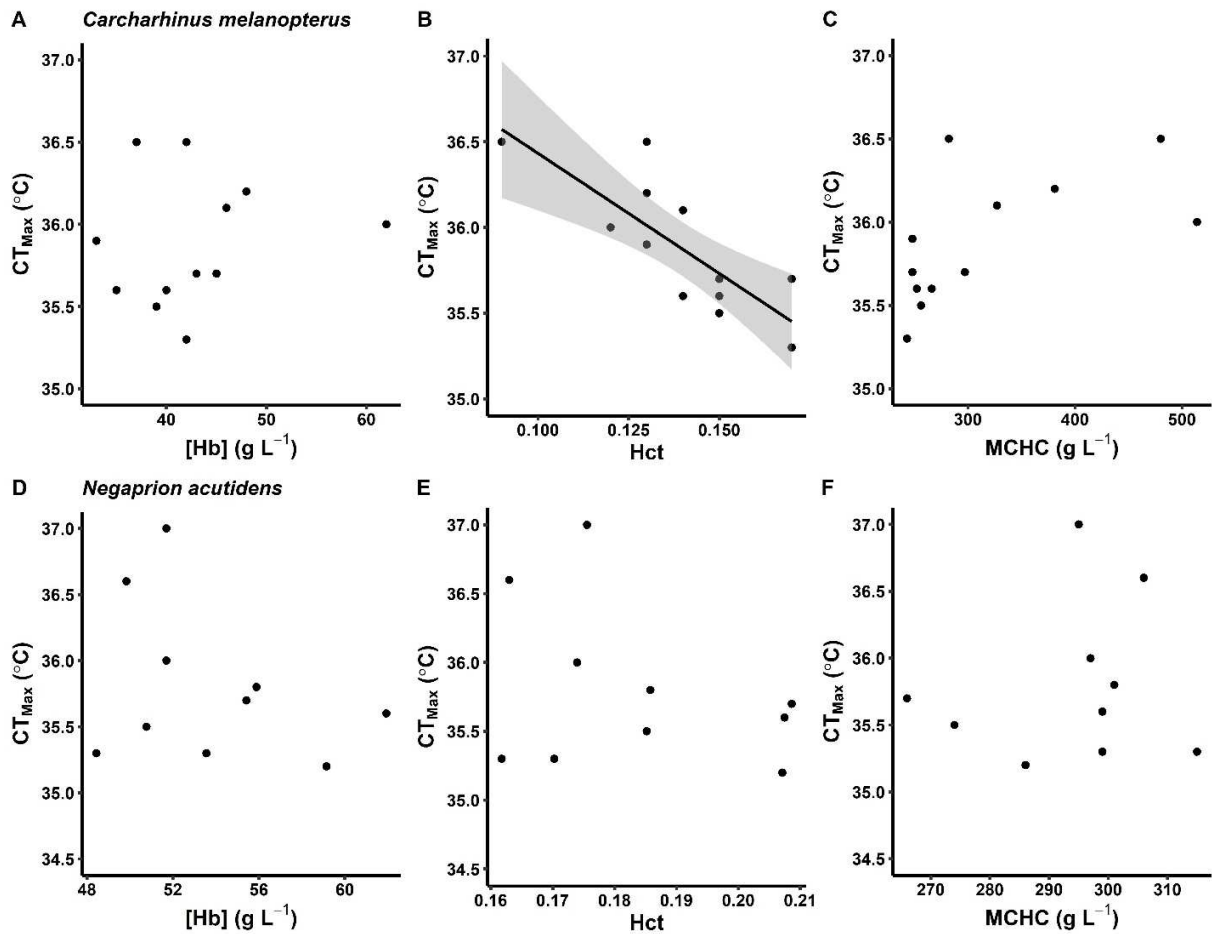
726

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),
 729 and mean corpuscular haemoglobin concentration (MCHC; C, F) are presented in response to either the
 730 average temperature (A-C) or maximum temperature (D-F) measured at eight sites. For panels A-C,
 731 individual observations represent the average temperature at a site from 2015-2019 and the average value
 732 of each haematological trait measured in sharks from 2014-2016 at the same sites ($n = 8$ sites). Error bars
 733 represent standard error of the mean. For panels D-F, individual observations represent the maximum
 734 habitat temperature at each site ($n = 8$ sites), calculated as the average from 2015-2019 plus three standard
 735 deviations, and the same data for sharks from panels A-C. Regression lines with 95% confidence intervals
 736 (i.e., shading) represent statistically significant associations between variables.



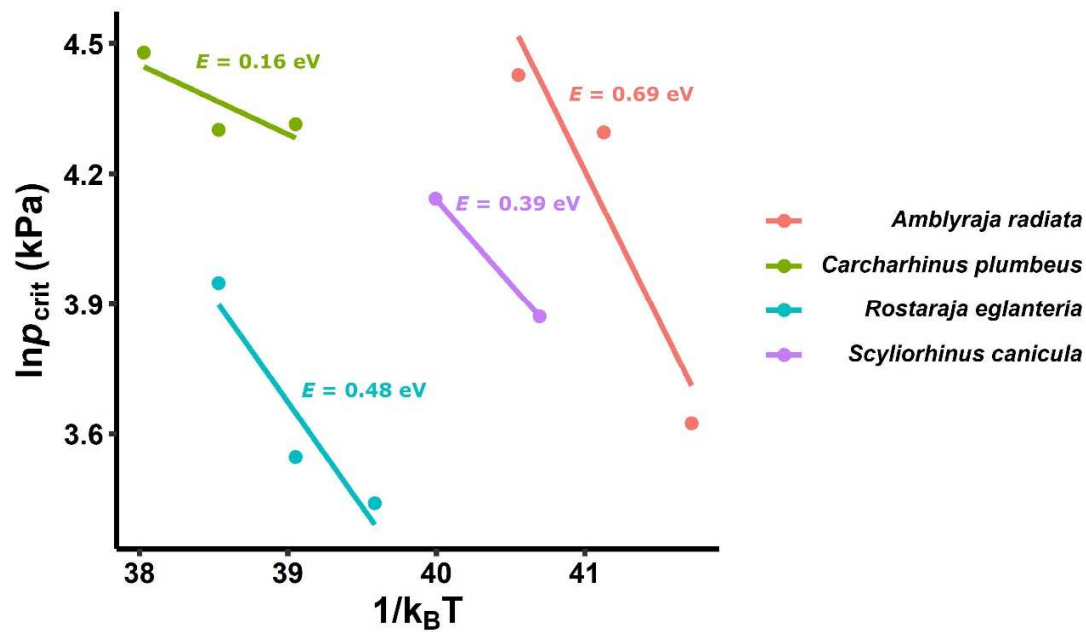
737

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),
 740 and mean corpuscular haemoglobin concentration (MCHC; C, F) are presented in response to either the
 741 average temperature (A-C) or maximum temperature (D-F) measured at eight sites. For panels A-C,
 742 individual observations represent the average temperature at a site from 2015-2019 and the average value
 743 of each haematological trait measured in sharks from 2014-2016 at the same sites ($n = 4$ sites). Error bars
 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.



748

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



755

756 **Fig. 5.** Temperature sensitivity of critical oxygen tension (p_{crit}) of four elasmobranch species. The natural
 757 logarithm of p_{crit} (kPa) is plotted against the inverse of temperature ($^{\circ}$ Kelvin) multiplied by the Boltzmann
 758 constant. The slope of this line is the temperature sensitivity (E , in eV). Available data from the literature
 759 were collected for the thorny skate, *Amblyraja radiata* (Schwieterman et al., 2019b), sandbar shark,
 760 *Carcharhinus plumbeus* (Crear et al., 2019), clearnose skate, *Rostaraja eglantera* (Schwieterman et al.,
 761 2019b), and small-spotted catshark, *Scyliorhinus canicula* (Butler and Taylor, 1975).