OXFORD UNIVERSITY PRESS | Conservation Physiology

The impacts of warming and hypoxia on the performance of an obligate ram ventilator

SCHOLARONE™ Manuscripts

1 The impacts of warming and hypoxia on the performance of an obligate ram ventilator
2

3 Daniel P. Crear, Fisheries Science Department, Virginia Institute of Marine Science, College of
4 William & Mary, Gloucester Point, VA 23062, USA
5 Rich W. Brill, Fisheries Science Department, Virginia Institute of Marine Science, College of
6 William & Mary, Gloucester Point, VA 23062, USA
7 Peter G. Bushnell, Department of Biological Sciences, Indiana University South Bend, South
8 Bend, IN 46634, USA
9 Robert J. Latour, Fisheries Science Department, Virginia Institute of Marine Science, College of
10 William & Mary, Gloucester Point, VA 23062, USA
11 Gail D. Schwieterman, Fisheries Science Department, Virginia Institute of Marine Science,
12 College of William & Mary, Gloucester Point, VA 23062, USA
13 Rachel M. Steffen, Biology Department, Washington & Lee University, Lexington, VA 244150
14 Kevin C. Weng, Fisheries Science Department, Virginia Institute of Marine Science, College of
15 William & Mary, Gloucester Point, VA 23062, USA
16
17
18 Corresponding author: Daniel Crear
19 Phone Number: (804) 684-7819
20 Email: dcrear8@gmail.com

21
22
23
24
25 Lay Summary

26 We assessed the impact of warming and hypoxia on the metabolic rate and behavior of an
27 obligate ram-ventilating marine predator, the sandbar shark, that uses coastal habitat as a nursery
28 ground. Based on their thermal threshold and low hypoxia tolerance, we expect sandbar sharks to
29 be negatively impacted by climate change.

30
31
32
33
34
35
36
37
38
39
40 Total Word Count: 8934 (includes all sections)
41
42
43

44

45

Abstract

46

47   Climate change is causing the warming and deoxygenation of coastal habitats like

48 Chesapeake Bay that serve as important nursery habitats for many marine fish species. As

49 conditions continue to change, it is important to understand how these changes impact individual

50 species' behavioral and metabolic performance. The sandbar shark (*Carcharhinus plumbeus*) is

51 an obligate ram ventilating apex predator whose juveniles use Chesapeake Bay as a nursery

52 ground ~~for~~ up to 10 years of age. The objective of this study was to measure juvenile sandbar

53 shark metabolic and behavioral performance as a proxy for overall performance (i.e., fitness or

54 success) when exposed to warm and hypoxic water. Juvenile sandbar sharks (79.5 – 113.5 cm

55 TL) were collected from an estuary along the eastern shore of Virginia and returned to lab where

56 they were fitted with an accelerometer, placed in a respirometer, and exposed to varying

57 temperatures and oxygen levels. Juvenile sandbar shark overall performance declined

58 substantially at 32°C or when dissolved oxygen concentration was reduced below 3.5 mg l^{-1}

59 (51% oxygen saturation between 24 – 32°C). As the extent of warm hypoxic water increases in

60 Chesapeake Bay, we expect that the available sandbar shark nursery habitat will be reduced,

61 which may negatively impact the population of sandbar sharks in the western Atlantic, as well as

62 the overall health of the ecosystem within Chesapeake Bay.

63

64

65

66

67

68

69

70 **Introduction**

71   Climate change is warming coastal areas and estuaries worldwide. An increase in

72 anthropogenic nutrient input is likewise increasing the severity and extent of hypoxic episodes in

73 many of these areas (Conley *et al.*, 2007, Hagy *et al.*, 2004, Irby *et al.*, 2016, Kemp *et al.*, 2005,

74 Najjar *et al.*, 2010, Preston, 2004, Rabalais *et al.*, 2009). These conditions are expected to

75 worsen over the next 100 years as climate change impacts are exacerbated (Irby *et al.*, 2018,

76 Najjar, et al., 2010, Rabalais, et al., 2009).

77   Changing environmental conditions in coastal areas and estuaries are likely to impact

78 marine fish species that rely on these habitats as primary nursery grounds (Rijnsdorp *et al.*,

79 2009). For example, while in their nursery habitat, juvenile weakfish (*Cynoscion regalis*)

80 avoided waters in a tributary of Indian River Bay, DE, USA with dissolved oxygen below 2 mg l⁻

81 ¹ (Tyler and Targett, 2007). The prevalence of juvenile bull sharks (*Carcharhinus leucas*) has

82 actually increased in their nursery ground habitat within Pamlico Sound, NC, USA over the last

83 decade as a result of increased water temperatures (Bangley *et al.*, 2018). Shifts in species

84 distribution, similar to the examples above, can lead to changes in the timing of migration of

85 juveniles and adults (Nye *et al.*, 2009, Turner *et al.*, 2015), reproductive patterns of adults (Last

86 *et al.*, 2011), and abundance of all life stages (Last, et al., 2011, Lynch *et al.*, 2014); all of which

87 can influence population level success and the overall health of ecosystems (Morley *et al.*, 2018).

88   To understand these potential changes in fish ecology, it is important to assess the

89 relationship between environmental conditions and the performance of individual fish.

90 Performance is often assessed through measurement of aerobic scope (AS, i.e., the difference

91 between maximum and standard metabolic rates; Claireaux & Lefrançois 2007, Di Santo 2016) ,

92 which quantifies an individual's metabolic power (i.e., energy use per unit time) under which all

93 of life's processes beyond basic maintenance (e.g., growth, reproduction, digestion, movement)

94 must occur (Clark *et al.*, 2013). Consistent with the theory of oxygen- and capacity-limited

95 thermal tolerance (OCLTT), AS is a measure of fitness and performance in ectotherms.

96 According to OCLTT, AS follows a bell-shaped curve with temperature such that there is a

97 temperature where AS is optimized (Clark, et al., 2013, Fry, 1971). Therefore, it is expected that

98 long term warming will reduce AS and decrease the ability for many individuals to carry out

99 multiple life processes simultaneously unless individuals adjust their range and distribution

100 (Pörtner and Knust, 2007). However, the theory of OCLTT does not hold true for all species, as

101 some demonstrate an increase in aerobic scope with temperature until temperature approaches

102 lethal levels (Clark, et al., 2013, McKenzie *et al.*, 2016). This suggests that, for some species, the

103 temperature at which AS is optimized is not equivalent to the temperature at which performance

104 is maximized (Lefevre, 2016).

105   Increases in the extent and severity of hypoxic episodes in coastal areas are affecting the

106 physiology and thus ecology of coastal species (Diaz and Rosenberg, 1995, Ludsin *et al.*, 2009,

107 Tyler and Targett, 2007, Wannamaker and Rice, 2000, Zhang *et al.*, 2009). Hypoxia tolerance is

108 often quantified by measurement of critical oxygen levels or oxygen level at which individuals

109 can no longer maintain standard metabolic rate (SMR). The critical oxygen saturation (S_{crit})

110 should increase with temperature due to increases in SMR and decreases in oxygen solubility in

111 water. Individuals cannot occupy waters long term with an oxygen level below S_{crit} because,

112 under these conditions, at least some of the power needed to maintain homeostasis must be met

113 through anaerobic metabolism (Brill *et al.*, 2015, Fry and Hart, 1948, Schurmann and Steffensen,

114 1997). Similar to S_{crit}, the quantities C_{crit} and P_{crit} are the concentration and partial pressure of

115 oxygen, respectively, at which a fish can no longer maintain SMR. All of these measures of

116 critical oxygen, as well as AS, can be determined through intermittent-flow respirometry (Clark

117 *et al.*, 2011, Lapointe *et al.*, 2014).

118   Measuring metabolic performance (i.e., AS) of an obligate ram ventilator, like a tuna or

119 some shark species, is difficult because of the necessity of individuals to swim constantly and,

120 therefore, a need to measure activity simultaneously. This requires either a swim tunnel

121 respirometer where swimming speed can be controlled, or a respirometer large enough for fish to

122 swim independently coupled with a method to quantify activity. Since many species exhibit

123 difficulty swimming in a swim tunnel, a large circular tank can be used as the respirometer such

124 that fish are able to swim freely (Lear *et al.*, 2017). Because the swimming behavior and activity

125 of fish cannot be controlled in the large circular respirometer, accelerometers have been used to

126 measure activity during experimentation (Lear, et al., 2017). Activity measurements (i.e.,

127 behavioral performance), obtained through measured from accelerometers (Whitney *et al.*, 2007,

128 Whitney *et al.*, 2016), can be used as a performance metric to quantify mechanical work,

129 describe behavior (Gleiss *et al.*, 2011, Payne *et al.*, 2018), and be used as an indicator to

130 understand locomotor performance under different environmental regimes (Payne, et al., 2018,

131 Payne *et al.*, 2016). Activity is correlated with metabolic performance, which suggests that

132 accelerometers can be used to infer field metabolic rate when applied to free-ranging individuals

133 (Bouyoucos *et al.*, 2017, Lear, et al., 2017). However, the relationship between accelerometer

134 derived activity metrics and metabolic rate has not been assessed under high levels of

135 environmental stress.

136   The sandbar shark (*Carcharhinus plumbeus*) is an obligate ram ventilating species that

137 relies on coastal habitats as nursery grounds during younger life stages. In late spring, pupping

138 occurs in Chesapeake Bay and coastal estuaries along the mid-Atlantic, where young-of-year

139 (YOY) remain through summer (Conrath and Musick, 2007, Grubbs and Musick, 2007). After

140 moving south or offshore during winter, juveniles return to these nursery areas to forage and

141 avoid larger predators during summer for the following 4-10 years (Grubbs and Musick, 2007).

142 As a result of climate change and anthropogenic nutrient input, Chesapeake Bay is becoming

143 warmer and more hypoxic (Hagy, et al., 2004, Kemp, et al., 2005, Najjar, et al., 2010, Preston,

144 2004). Temperature and oxygen limitations are not well understood for juvenile sandbar sharks.

145 Therefore, the objective of this study was to measure juvenile sandbar shark metabolic and

146 behavioral performance as a proxy of overall performance (i.e., fitness or success) when exposed

147 to warm and hypoxic water.

148

149 **Methods**

150 *Shark Collection and Maintenance*

151   Thirteen juvenile sandbar sharks (79.5 – 113.5 cm TL; 2.6 – 7.8 kg) were collected along

152 the eastern shore of Virginia and brought back to the Virginia Institute of Marine Science

153 (VIMS) Eastern Shore Lab (ESL), Wachapreague, VA, USA during the summers of 2016 and

154 2017 (Table 1). Individuals were held in a fiberglass tank (6.1 m diameter, 45,000 l) with a flow-

155 through seawater system and acclimated to captivity for at least two weeks prior to

156 experimentation. Sharks were fed frozen menhaden (*Brevoortia tyrannus*) until satiation every 3-

157 5 days. All protocols for shark sampling, handling, and experimentation were approved by the

158   College of William and Mary Institutional Animal Care and Use Committee (protocol no.

159   IACUC-2017-05-26-12133-kcweng).

160

161   *Experimental Design*

162   Maximum metabolic rate (MMR), minimum routine metabolic rate (mRMR; a proxy for

163   SMR because sandbar sharks are obligate ram ventilators), AS, and S_{crit} were measured in 12

164   sharks at 24, 28, and 32°C using intermittent-flow respirometry to understand the effects of

165   warming and hypoxia on sandbar shark physiology (Brill, et al., 2015, Lapointe, et al., 2014).

166   Eleven sharks were tested at all three treatments (24, 28, and 32°C), while data collected at 24

167   and 28°C from one shark was combined with data collected at 32°C from a different shark to

168   represent one set of temperature treatments due to a mortality suffered in the holding tank

169   between experiments. Sharks were acclimated to treatment temperatures in their holding tank

170   through natural increases in water temperature throughout the summer (range: 18.0 - 33.8°C)

171   because we were unable to control temperature in our holding tank. However, by using this

172   approach we were able to maximize ecological relevance. When experimental temperatures

173   exceeded holding tank temperatures, individuals were transferred to a separate tank at the

174   experimental temperature for at least 24 - 48 h prior to being introduced into the respirometer.

175   The respirometry system consisted of two fiberglass tanks (2.4 m diameter, 1500 l volume) such

176   that the first served as the respirometer and the second was a water reservoir used to flush the

177   respirometer. To reduce the volume of the respirometer and ensure each experimental shark

178   would swim in a circle, a negatively buoyant, smaller tank was placed in the center of the

179   respirometer to create a circular track. A clear plastic sheet was placed over the respirometer and

180   ~~synched~~ cinched to the side of the tank to prevent air-water gas exchange. Temperature was

181   controlled by a large chiller unit with the addition of a heater kit (Teco TK 6000 Aquarium

182   Chiller, Aquatic Solutions), while oxygen levels were controlled by bubbling air (normoxia) or

183   nitrogen gas (hypoxia) into the water. The two tanks were connected through a drain on the

184   bottom and PVC tubing over the top (Fig. 1A, B). Oxygen levels in the respirometer and

185   reservoir were monitored every second using a temperature compensated, two-channel FireSting

186   oxygen meter (Pyroscience) equipped with fiber optic oxygen probes that were fixed along the

187   side of the respirometer and reservoir. Output from the oxygen probe in the respirometer was

188   recorded in the FireStingO$_2$ software and relayed to a program designed in Dasylab 9.02

189   (National Instruments) specifically for repeatedly recording metabolic rate measurements in

190   intermittent flow respirometry (Brill, et al., 2015, Lapointe, et al., 2014). The program also

191   controlled the flow of nitrogen through a solenoid valve to maintain precise oxygen levels using

192   output from the oxygen probe in the reservoir.

193   During a trial, the respirometry system cycled between the flushing and measurement

194   period. During the flushing period (ranged from 30 – 45 min), oxygen and temperature

195   controlled water was pumped up and out of the reservoir, into the respirometer, and through a

196   diffuser at the bottom of the respirometer (Fig. 1B) to ensure thorough mixing. Water flowed

197   back into the reservoir through the bottom drain between the two tanks, allowing a continuous

198   exchange of water between the two tanks during the flushing period. During the 15 min

199   measurement period, the flush pump was turned off and the shark's oxygen consumption reduced

200   the oxygen in the respirometer. We assumed the shark's swimming motion adequately mixed the

201   water in the respirometer during trials. The measurement period consisted of a three min

202   equilibration interval (to ensure oxygen mixing in the respirometer) followed by 12 min of data

203 recording to measure the rate of oxygen decline. The slope of a linear regression model fitted to

204 the oxygen measurements was used to calculate metabolic rate using the equation:

205 $$MO_2 = b \; x \; V \; x \; W^{-1}$$

206 where MO_2 = metabolic rate (mg O_2 kg^{-1} h^{-1}), b = rate of change of oxygen content (estimated

207 slope of linear regression) over the 12 min recording period (s^{-1}), V = respirometer volume (l)

208 corrected for the volume of the shark, and W = weight of the fish (kg). Metabolic rate

209 measurements where the regression R^2 value was below 0.8 were eliminated and assumed to be

210 compromised due to either poorly mixed water in the respirometer or contact between the shark

211 and oxygen probe (which did occur occasionally).

212   To account for microbial respiration, oxygen consumption measurements were made in

213 the absence of an experimental shark for at least 3 h prior to, and after, each trial. Based on those

214 oxygen consumptions, a linear regression was used to estimate the rate of oxygen decline due to

215 microbial respiration during the trial. The estimated oxygen consumptions were then subtracted

216 from the measured rates of oxygen decline when the shark was present (Brill, et al., 2015,

217 Svendsen *et al.*, 2016).

218

219 *Behavior and Activity Metrics*

220   The behavior and activity of a eight individuals (sharks from 2017) were measured while

221 in the respirometer to understand how activity was affected by temperature and hypoxia. To

222 quantify activity, individuals were fitted with the X16-4 mini accelerometer (Gulf Coast Data

223 Concepts) attached to the first dorsal fin, which recorded triaxial acceleration at 25 Hz. The

224 accelerometer weighed 27 g, which represented 0.35-1.1% of the body mass of the experimental

225 sharks. Accelerometers were removed at the completion of each trial. We received a full

226   acceleration data set for each 2017 trial except for three trials at 24°C where data were

227   unreliable.

228   The static component (effect of gravity) in the x, y, and z-axes were extracted from the

229   raw acceleration values using a 3 s smoothing window, a time frame often used for the size of

230   sharks in this study and a tailbeat cycle of approximately 1 Hz (Shepard *et al.*, 2008, Whitney, et

231   al., 2016). The dynamic components in the x, y, and z-axes were determined by subtracting the

232   static component from the raw acceleration values. A wavelet analysis was then used to extract

233   multiple activity metrics (Bouyoucos, et al., 2017, Whitney, et al., 2016): tailbeat frequency

234   (TBF, number of tailbeats per second extracted from the dynamic component of the z-axis),

235   tailbeat acceleration amplitude (TBAA, measure of amplitude of the acceleration wave also

236   taken from the dynamic component of the z-axis), and overall dynamic body acceleration

237   (ODBA, overall activity defined as sum of the dynamic components of all three axes). TBF,

238   TBAA, and ODBA were then averaged for each corresponding measurement period.

239

240   *Maximum and Minimum Routine Metabolic Rate*

241   Before the start of each trial, an individual was transferred out of the holding tank, fitted

242   with an accelerometer (for some individuals, described above), placed in the respirometer, and

243   allowed 30 mins to acclimate. Each experimental shark was exercised using our chase protocol,

244   which consisted of 10 min of prodding to induce the animal to reach MMR (Killen *et al.*, 2017,

245   Lapointe, et al., 2014). This time period was selected because after chasing the first shark of the

246   experiments for 10 min, it stopped swimming. During another trial a shark stopped swimming

247   after being chased for 6.5 mins. Further, Marshall et al. 2015 found that in waters cooler than our

248   experimental treatments (15-21°C) post release mortality of sandbar sharks was 29%. Based on

249   these accounts, to avoid mortalities (for a species that is considered to be overfished; SEDAR

250   2017) and for ethical reasons, we ~~aired~~ erred on the side of caution and deemed 10 mins to be a

251   sufficient chase time. It is important to note this may have led to a slight underestimation of

252   MMR of some individuals. Immediately following, the respirometer was sealed and the first

253   metabolic rate measurement was initiated. Metabolic rate measurements were then made for

254   approximately 20 h to determine MMR and to allow individuals to recover (indicated by the

255   metabolic rate leveling out) and reach mRMR.

256

Critical Oxygen Saturation

258   Once mRMR had been established following the procedures described above, the

259   hypoxia part of the trial was initiated, where metabolic rate measurements were taken as oxygen

260   content was decreased in a stepwise fashion until the shark was no longer able to maintain its

261   mRMR. S_{crit} was determined as the oxygen level at which the metabolic rate declined.~~a decline~~

262   ~~in metabolic rate was evident.~~ After measuring at least three metabolic rates below S_{crit}, the trial

263   was terminated, and the oxygen was brought back to 90% saturation before the shark was

264   transferred back into the holding tank. Fig. 2 displays an example of a full trial.

265

Data Analysis

267   MMR was calculated by taking the mean of 10% of the highest metabolic rate

268   measurements (3-4 measurements) during the entire normoxia period. This method was selected

269   instead of using the common approach of taking the highest metabolic rate measurement because

270   we wanted to ensure that MMR was not represented by an outlier measurement that may not

271   have been the result of our chase protocol. In addition, unlike many non-obligate ram ventilators,

272 we noticed that peak metabolic rate measurements often did not occur until further into the trial.

273 This could be due to serological changes induced through the chase protocol, which limit oxygen

274 delivery mechanisms as further discussed below (Discussion). as a result of the chase protocol,

275 may have been due to the shark's oxygen delivery being compromised. We calculated mRMR

276 from the mean of 10% of the lowest metabolic rate measurements during the normoxic period to

277 account for the varying numberamount of measurements among individuals during that period of

278 the trial (Norin *et al.*, 2014). AS was calculated by computing the difference between MMR and

279 mRMR. To determine S_{crit}, we found the first value where the shark's metabolic rate dropped

280 below mRMR and where the remaining metabolic rates were also below mRMR. Those

281 metabolic rates were isolated, and linear regression was applied to those values and the oxygen

282 content associated with each value (ranged from $2 - 11$ values). The oxygen content where the

283 regression line and mRMR intersected was defined as C_{crit} (Schurmann and Steffensen, 1997).

284 S_{crit} was calculated by dividing C_{crit} by the oxygen concentration at 100% saturation at the tested

285 temperature. Lastly, C_{crit} was converted into critical oxygen partial pressure (P_{crit}) by calculating

286 the partial pressure of oxygen at 1 atmosphere for the start day of the trial in Wachapreague, VA,

287 USA and multiplying by S_{crit}.

288   A multivariate repeated-measures mixed effects model was developed in SAS 9.4 (SAS

289 Institute) using the MIXED procedure to understand the effect of temperature on MMR, SMR,

290 AS, and P_{crit} (Lapointe, et al., 2014). The responses were MMR, SMR, AS, and P_{crit} for each

291 trial, the covariate was temperature, and the random effect was individual fish. To maintain the

292 assumption of normality, the four responses were multiplied by a constant so they would be on

293 the same scale as MMR prior to being put in the model. We modeled the heterogeneity in

294 responses among temperature treatments and specified the Kenward-Roger method for

295   calculating the degrees of freedom (Kenward and Roger, 1997). Compound Symmetry, AR1, and

296   Toeplitz correlation structures were fitted to the data and the Bayesian Information Criterion

297   (BIC) was used to identify the model with the most supported correlation structure (Littell *et al.*,

298   2006). Our model chosen for inference included a correlation structure of AR1. Lastly, a priori

299   contrast statements of least-square means were generated using the LSMestimate statement in

300   SAS to assess the effects of temperature on the four response variables. At the completion of the

301   analysis, all model estimates were converted back to scale. All statistics were evaluated at

302   significance levels of $\alpha = 0.05$.

303   To determine how temperature, hypoxia, and shark activity together impacted metabolic

304   rate, linear mixed effects models were fitted to data from sharks equipped with accelerometers.

305   Separate models were applied to data obtained from the normoxic and hypoxic parts of the trials,

306   with the former corresponding to the part of the trial when the oxygen saturation was 80% or

307   higher, and the latter when the oxygen saturation was below 80%. Potential covariates for the

308   normoxic model included temperature, TBF, TBAA, ODBA, shark total length (TL), and the

309   time since start of trial. The full model was used to assess the need to model heterogeneity and

310   correlation structure. Based on BIC, modeling the heterogeneity in responses among temperature

311   treatments and using a correlation structure of AR1 was supported. A series of models was then

312   developed using different combinations of the covariates mentioned above, and the model

313   chosen for inference was selected using BIC. Potential covariates for the hypoxic model included

314   temperature, oxygen content, TBF, TBAA, ODBA, TL, as well as a calculated binary variable

315   denoted as the crash metric. Since it was assumed that metabolic rate will differ before and after

316   C_{crit} is reached, the crash metric consisted of a 1 for metabolic rate values during the hypoxic part

317   of the trial that occurred prior to the shark reaching C_{crit}, and a 0 for the metabolic rates that

318   occurred after C_{crit} was reached. Similar to the normoxic model, the decision to model

319   heterogeneity in responses among temperatures and with an AR1 correlation structure was based

320   on the full hypoxic model and ultimately supported through BIC. Multiple models were

321   developed with various covariate combinations and BIC was used for model selection. Predicted

322   metabolic rates for the selected normoxic and hypoxic models were generated using estimated

323   marginal means (Searle *et al.*, 1980). Estimates of uncertainty were generated from 1000

324   bootstrapped samples (Efron and Tibshirani, 1993). Predicted P_{crit} and associated uncertainty was

325   converted back to C_{crit} and S_{crit}. All linear mixed effects models were fitted using the nlme

326   package in R v.3.4.3 (Pinheiro *et al.*, 2013).

327

328   **Results**

329   *Maximum metabolic rate, minimum routine metabolic rate, aerobic scope, & critical oxygen*
330   *partial pressure*
331
332   The multivariate repeated measures mixed effects model was successfully fitted and

333   evaluation of diagnostics (e.g., plots of residuals for each response variable) showed reasonable

334   goodness-of-fit. Although there was high variability in measured MMR, mRMR, and AS among

335   individuals (Fig. 3), model results indicated that differences were evident for these three metrics

336   among temperatures. Statistically significant differences were detected over the three

337   experimental temperatures, primarily between 24 and 28°C and between 24 and 32°C (Fig. 4,

338   Table 2). Specifically, MMR increased by 21% and 42% from 24 to 28°C and 32°C, respectively

339   (Fig. 4A; Table 2), while mRMR increased by 18% from 24 to 28°C, and 45% from 24 to 32°C

340   (Fig. 4B; Table 2). Similar to MMR, AS increased considerably from 24 to 28°C (26%) and 24

341   to 32°C (39%; Fig. 4C; Table 2). In contrast to the other metrics analyzed, mean P_{crit} only

342   increased by 18% and 19% when comparing 24 and 28 to 32°C, respectively and no significant

343   differences were detected (Fig. 5A; Table 2). Plots of the raw data for MMR, mRMR, AS, S_{crit},

344   and C_{crit}, are presented in the supplementary data.

345

346   *Normoxia*

347   The normoxic model with the most empirical support included ODBA, TBAA, time since

348   chase, and an interaction between TBF and temperature. We concluded that each of these

349   covariates was important in explaining variation in the metabolic rate data (see ΔBIC table in

350   Supplementary Table 1). Predicted metabolic rate increased by 47 ± 6 mg O_2 kg^{-1}h^{-1} for every

351   0.1 unit increase in ODBA (Fig. 6A), whereas metabolic rate only increased by 5 mg O_2 kg^{-1}h^{-1}

352   for every unit increase in TBAA (Fig. 6B). For every hour increase since the animal was chased,

353   the metabolic rate dropped approximately 3 mg O_2 kg^{-1}h^{-1}. The estimated effect in metabolic rate

354   over TBF differed among the levels of temperature considered. That is, for every unit increase in

355   TBF, metabolic rate increased 63 and 142 mg O_2 kg^{-1}h^{-1} for 24 and 28°C, respectively. However,

356   at 32°C metabolic rate actually decreased by 20 mg O_2 kg^{-1}h^{-1} for every unit increase in TBF

357   (Fig. 6C). Lastly, it is important to note that during three trials at 32°C during normoxia, we

358   ~~anecdotally~~ documented periods of time when sharks intermittently stopped swimming, ranging

359   from a few seconds to over an hour.

360

361   *Hypoxia*

362   The model that provided the most parsimonious description of sandbar shark metabolic

363   rate under hypoxic conditions included TBF, temperature, and an interaction between ODBA and

364   oxygen content (see ΔBIC table in Supplementary Table 2). Predicted metabolic rate increased

365   by 55 ± 20 mg O_2 kg^{-1}h^{-1} for every unit increase in TBF. In hypoxic water, metabolic rate

366   differed among temperatures such that as temperature increased from 24 to 28 to 32°C,

367   metabolic rate increased from 149 ± 9 to 183 ± 6 to 217 ± 13 mg O_2 kg^{-1}h^{-1}, respectively. Lastly,

368   as ODBA and oxygen content increased, particularly at higher oxygen concentrations (>3.5 mg l^{-1}

369   ^{1}), predicted metabolic rate also increased. However, as oxygen decreased, particularly at lower

370   oxygen concentrations (<3.5 mg l^{-1}) and ODBA increased, predicted metabolic rate decreased

371   (Fig. 7). Often during hypoxic conditions (<80% oxygen saturation), sharks displayed a banking

372   behavior where they would swim along the edge of the respirometer with its ventral side facing

373   the side. We also observed during hypoxia, two trials at 24°C, three trials at 28°C, and four trials

374   at 32°C periods of time when sharks either stopped swimming completely (in this case the trial

375   was terminated) or intermittently stopped swimming when oxygen was lower in the

376   respirometer.

377

378   **Discussion**

379   To the best of our knowledge this is the first study to measure AS and P_{crit} in an obligate

380   ram-ventilating elasmobranch. In addition, the size of our respirometer allowed us to measure the

381   metabolic rate and activity of juvenile sandbar sharks across a wide range of sizes (79.5 – 113.5

382   cm TL). We were also able to use the various activity measurements to better understand

383   environmental thresholds of sandbar sharks. Our findings lead us to suggest that caution should

384   be exercised when using activity to estimate field metabolic rate because, as demonstrated in this

385   study, the correlative relationship between activity and metabolic rate breaks down at high

386   temperatures and low oxygen levels.

387

388   *Maximum metabolic rate, minimum routine metabolic rate, aerobic scope, & normoxia*

389   Referenced model response variables (e.g. mRMR, AS, metabolic rate, etc…) below

390   represent model predictions, while raw values are indicated as such. As expected, mRMR values

391   in the present study were higher than the SMR measurements reported by Dowd et al. (2006) for

392   immobile individuals (91 ± 4 mg O_2 kg^{-1}h^{-1} at 24°C; 125 ± 7 mg O_2 kg^{-1}h^{-1} at 28°C). When

393   compared to other obligate ram-ventilators, such as the tuna species (Korsmeyer and Dewar,

394   2001), sandbar shark AS was substantially lower. Contributing to this trend may be the

395   significantly smaller gill surface area observed in sandbar sharks compared to other obligate ram

396   ventilating teleosts and elasmobranchs (Emery and Szczepanski, 1986). In addition, with a diet

397   consisting of mostly benthic crustaceans and fishes (Ellis and Musick, 2007), juvenile sandbar

398   sharks may not require a high AS.

399   The variation in raw AS among individual juvenile sandbar sharks over the experimental

400   temperature regime suggests that the potential underlying mechanisms describing AS may differ

401   from individual to individual. For example, some individuals displayed a bell-shaped curve in

402   AS over temperature (e.g. SB28, SB31, SB34), some showed an increase in AS with temperature

403   (e.g. SB03, SB12, SB33), and others displayed very little difference in AS over temperature (e.g.

404   SB02, SB04, SB29) (Fig. 3). Due to this high variability, particularly at 32°C, it is difficult to

405   broadly determine if our data support the OCLTT theory (i.e., maximum AS occurs at the

406   optimal temperature); or if sandbar shark AS increases until lethal temperature limits. In

407   individuals that displayed a bell-shaped curve, maximum AS and thus optimal performance

408   occurred at 28°C ($24°C < T_{optAS} < 32°C$). Whereas, for those individuals that maximized AS at

409   32°C, we assume AS would decline above 32°C followed by mortality shortly thereafter. This

410   would suggest that performance or fitness is not optimized at AS for those individual sharks.

411   Clark, et al. (2013) suggested that some species display multiple performances – multiple optima

412   (MPMO), where there are separate optimal temperatures for different physiological processes

413   (e.g. growth, reproduction, and digestion). However, it is difficult to determine if our results

414   support this theory because the optimal temperatures for those processes are unknown for

415   sandbar sharks. The variability in the data may be the result of differences in activity, physical

416   fitness, adaptation, and physiological processes among individuals. Variation in AS may have

417   also resulted from a potential slight underestimation of MMR in some individuals in an effort to

418   avoid mortalities during or following the chase protocol. Further, because temperature could not

419   be controlled in the holding tank and all individuals were tested in the same order of increasing

420   temperature treatments, the relationships of the measured metrics could potentially be affected.

421   For example, sharks may be more acclimated to the chase protocol or the respirometer for

422   subsequent experiments, which could impact MMR and mRMR (Clark, et al., 2013). Lastly,

423   variation in acclimation time for individuals in particular when exposed to 32°C could have

424   affected metabolic rate metrics. Although we were unable to control these aforementioned

425   conditions, it is possible that they contributedattributed to the individual variation in these

426   metrics observed here.

427   Clearer trends were evident when both metabolic rate and activity were considered.

428   Under normoxic conditions, the expected correlation between metabolic rate and activity

429   (Bouyoucos, et al., 2017, Lear, et al., 2017) broke down at 32°C, where the metabolic rate of

430   sandbar sharks actually slightly declined as activity increased (Fig. 6). At high temperatures,

431   sandbar sharks have to delicately balance the increase in oxygen received from increasing their

432   TBF to improve ram ventilation againstand the energy expenditure needed for locomotion. This

433   is evident when some sharks actually intermittently stopped swimming for periods of time under

434   normoxic conditions at 32°C. A decline in shark performance at 32°C may be due to the

435 physiological stress incurred from exercise, acute stress, increased demand for oxygen, and/or

436 decreased oxygen in the water. These stressors are known to cause acidosis and hyperkalemia in

437 fishes (Brill *et al.*, 2008, Cliff and Thurman, 1984, Skomal and Mandelman, 2012). It is well

438 known that acidosis and hyperkalemia impact muscle performance, impairing cardiovascular

439 function and potentially reducing stroke volume at a time when a shark may actually need to

440 increase stroke volume to increase oxygen delivery (Farrell *et al.*, 2009, Norin, et al., 2014).

441

442 *Critical oxygen partial pressure & hypoxia*

443 Based on the P_{crit} values, juvenile sandbar sharks are not hypoxia tolerant. Regardless of

444 temperature, P_{crit} was above those reported for other teleost and elasmobranch species. For

445 example, striped bass (*Morone saxatilis*), a common fish species in Chesapeake Bay, has an S_{crit}

446 of $35 \pm 2\%$ and C_{crit} of 2.5 ± 0.2 mg l^{-1}, at 28°C (Lapointe, et al., 2014), both substantially lower

447 than the S_{crit} ($51 \pm 2\%$) and C_{crit} (3.3 ± 0.2 mg l^{-1}) of juvenile sandbar sharks at the same

448 temperature (Figs. 4B & 4C). When compared to elasmobranch fishes where P_{crit} was measured,

449 such as the epaulette shark (*Hemiscyllium ocellatum*, 38 ± 3 mmHg) and shovelnose ray

450 (*Aptychotrema rostrate*, 54 ± 3 mmHg), the juvenile sandbar shark's P_{crit} values are likewise

451 substantially higher (Speers-Roesch *et al.*, 2012). When compared to another obligate ram-

452 ventilating species, such as the yellowfin tuna at 25°C (hypoxia tolerance of 3.7 mg l^{-1}; Bushnell

453 & Brill 1991, Bernal et al. 2017), sandbar sharks have a similar hypoxia tolerance. The high P_{crit}

454 values in juvenile sandbar sharks are likely the result of this species also being (like many

455 carcharhinid species) an obligate ram ventilator (Carlson *et al.*, 2004, Dowd, et al., 2006). In

456 hypoxia, obligate ram ventilator species rely on increasing their swimming speed or mouth gape

457 to counteract a decline in oxygen in the water (Bushnell and Brill, 1991, Carlson and Parsons,

458 2001, Dizon, 1977); however, once P_{crit} is reached, obligate ram-ventilating fishes will not be

459 able to maintain minimum swimming speeds to ~~adequately~~ ventilate their gills adequately or to

460 maintain hydrostatic equilibrium (Carlson and Parsons, 2001). Although, P_{crit} did not differ

461 substantially among temperatures, the mean P_{crit} at 32°C was highest when compared to means at

462 24 and 28°C, which was expected because as temperature increases, the demand for oxygen

463 increases and the solubility of oxygen in seawater decreases (Schurmann and Steffensen, 1997).

464 The lack of a substantial difference was the result of a high amount variability in P_{crit} values,

465 particularly at 32°C (Fig. 5A). Sandbar shark metabolic rate did increase as temperature

466 increased in hypoxic waters (<80% oxygen saturation), suggesting that at warmer temperatures

467 (~32°C), the demand for oxygen is higher while in less oxygenated water, which can lead to

468 increased stress and a higher risk of mortality. An alternative hypothesis is that juvenile sandbar

469 sharks are adapted to large tidal fluctuations in temperature (4.6°C; D. Kelley unpublished data);

470 therefore, the ability to deliver oxygen to the tissues is not compromised due to increased

471 temperatures.

472   In hypoxic waters, sandbar shark metabolic rate was highly dependent on oxygen level

473 and the shark's activity. The synergistic effect of oxygen and activity on metabolic rate led

474 ~~todisplayed~~ different trends above and below approximately 3.5 mg l^{-1}, which was similar to the

475 mean (± SE) C_{crit} of sandbar sharks between 24 - 32°C (3.4 ± 0.1 mg l^{-1}). The positive correlation

476 of metabolic rate and activity, as oxygen concentrations increased from 3.5 mg l^{-1}, followed

477 trends similar to those when sharks were under normoxic conditions (>80% oxygen saturation).

478 When oxygen dropped below 3.5 mg l^{-1}, however, an inverse relationship occurred between

479 metabolic rate and activity, a trend that was also evident at 32°C under normoxic conditions. The

480 breakdown in the positive relationship between metabolic rate and activity suggests that at these

481 lower oxygen levels sandbar sharks employ anaerobic metabolism to power swimming, which ~~in~~

482 ~~turn, again,~~ can lead to acidosis and hyperkalemia. It is clear this behavior cannot be sustained,

483 based on the many periods of time when sharks stopped swimming either completely or

484 intermittently during hypoxia. According to our data~~,~~ the oxygen concentration threshold for

485 juvenile sandbar sharks is approximately 3.5 mg l^{-1} and ~~that~~ this threshold is positively correlated

486 with temperature.

487

488 *Climate Change Impacts*

489 It is critical to understand environmental thresholds for marine species amidst climate

490 change. Although sandbar sharks are not expected to encounter 32°C waters often, the potential

491 to encounter these temperatures will increase as climate change continues to alter marine

492 environments, particularly within coastal habitats. With an increase in temperature we also

493 expect to see an increase in the extent and severity of hypoxic waters which, may have a large

494 impact on the obligate ram ventilating sandbar shark. For example, along the eastern shore of

495 VA, where known sandbar shark nursery habitat exists, water temperatures during the months of

496 July and August during 2016 and 2017 exceeded 32°C at times (range: 21.3 – 32.8°C; mean:

497 27.4°C; D. Kelley unpublished data) and oxygen levels ranged from 2.9 – 8.2 mg l^{-1}. By the mid-

498 21st century, Chesapeake Bay, which is the largest sandbar shark nursery habitat in the United

499 States, is expected to increase 1.75°C relative to the mid-1990s (Muhling *et al.*, 2018). In

500 addition, by the end of the century, heat waves are also projected to increase by over two

501 standard deviations along the Mid-Atlantic (Najjar, et al., 2010). Also by 2050, Chesapeake Bay

502 is predicted to have the largest increase in cumulative hypoxic volume (72 - 202 km^3 days) at

503 oxygen concentrations between 2 - 5 mg l^{-1} (Irby et al. 2018), a range of oxygen concentrations

504   that include the P_{crit} of juvenile sandbar sharks. This volume of hypoxic water is expected to

505   occur earlier in the summer as well (Irby, et al., 2018). These environmental changes will likely

506   significantly diminish the suitability of Chesapeake Bay and adjacent coastal areas to serve as

507   nursery habitat for sandbar sharks in the western Atlantic. As climate change impacts worsen,

508   juvenile sandbar sharks, which can spend up to 10 years in these nursery habitats, may see

509   available habitat reduced and be forced to seek out novel nursery habitats or risk increases in

510   juvenile mortality. This may, in turn, affect the overall abundance of an already overfished

511   sandbar shark population (SEDAR, 2017). At an ecological level, juvenile sandbar sharks are top

512   predators within coastal habitats and may control the populations of other fish species (Ellis and

513   Musick, 2007). Shifts in juvenile sandbar shark distribution could, therefore, also have negative

514   effects on the population of lower trophic species within these habitats.

515

Conclusions

516

517   This study was able to identify the temperature and oxygen thresholds of juvenile sandbar

518   sharks. We found that their performance substantially declines at 32°C (even in normoxia) and at

519   oxygen concentrations below 3.5 mg l^{-1}, and that activity becomes inversely correlated with

520   oxygen consumption rates under high stress conditions. These impacts suggest that in the face of

521   climate change, areas of Chesapeake Bay may become less suitable nursery habitat for sandbar

522   sharks. It is critical for future studies to use environmental thresholds like those identified in this

523   study to predict species distribution under climate change scenarios to understand potential

524   habitat shifts.

525

526

527

528

529

530

531

532

533

534

535   Tables

536   Table 1. Size and treatment summary of the juvenile sandbar sharks in the study.

537

Animal ID	Year Tested	Animal Mass (kg)	Total Length (cm)	Treatments (°C)	Accelerometer
SB02	2016	7.30	109	24, 28, 32	No
SB03	2016	2.66	80	24, 28, 32	No
SB04	2016	5.09	99	24, 28, 32	No
SB05	2016	5.46	98	24, 28, 32	No
SB12	2016	5.69	103.5	24, 28, 32	No
SB28	2017	7.76	113.5	24, 28, 32	Yes
SB29	2017	3.31	91	24, 28, 32	Yes
SB30	2017	4.22	94	24, 28, 32	Yes
SB31	2017	3.98	91	24, 28, 32	Yes
SB32	2017	2.56	81	24, 28	Yes
SB33	2017	6.81	109	24, 28, 32	Yes
SB34	2017	6.84	107	24, 28, 32	Yes
SB37	2017	2.60	79.5	32	Yes

538

539
540
541
542
543
544
545

1
2
3   546
4   547
5   548
6   549
7   550
8   551
9   552
10   553
11   554
12   555
13   556
14   557
15   558
16   559
17   560
18   561

22   562   Table 2. T statistics from the a priori contrast statements of least-square means generated from
23   563   the multivariate repeated measures mixed effects model to assess the impact of temperature (24,
24   564   28, and 32°C) on MMR, mRMR, AS and P_{crit}. The degrees of freedom are in parentheses. The
25   565   (*) signifies a significant difference between the two temperatures.
26   566

Metric	24°C x 28°C	24°C x 32°C	28°C x 32°C
MMR	$t_{(57.34)} = -2.33*$	$t_{(47.03)} = -3.12*$	$t_{(56.82)} = -1.43$
mRMR	$t_{(57.34)} = -1.90$	$t_{(47.03)} = -3.25*$	$(t_{(56.11)} = -1.81$
AS	$t_{(57.34)} = -2.81*$	$t_{(47.03)} = -2.90*$	$t_{(56.11)} = -0.93$
P_{crit}	$t_{(56.82)} = 0.14$	$t_{(46.93)} = -1.29$	$t_{(59.02)} = -1.25$

567
568
569
570
571
572
573
574
575
576

1
2
3   577
4
5   578
6
7
8   579
9
10   580
11
12   581
13
14
15   582
16
17   583
18
19   584
20
21
22   585
23
24   586   Figures
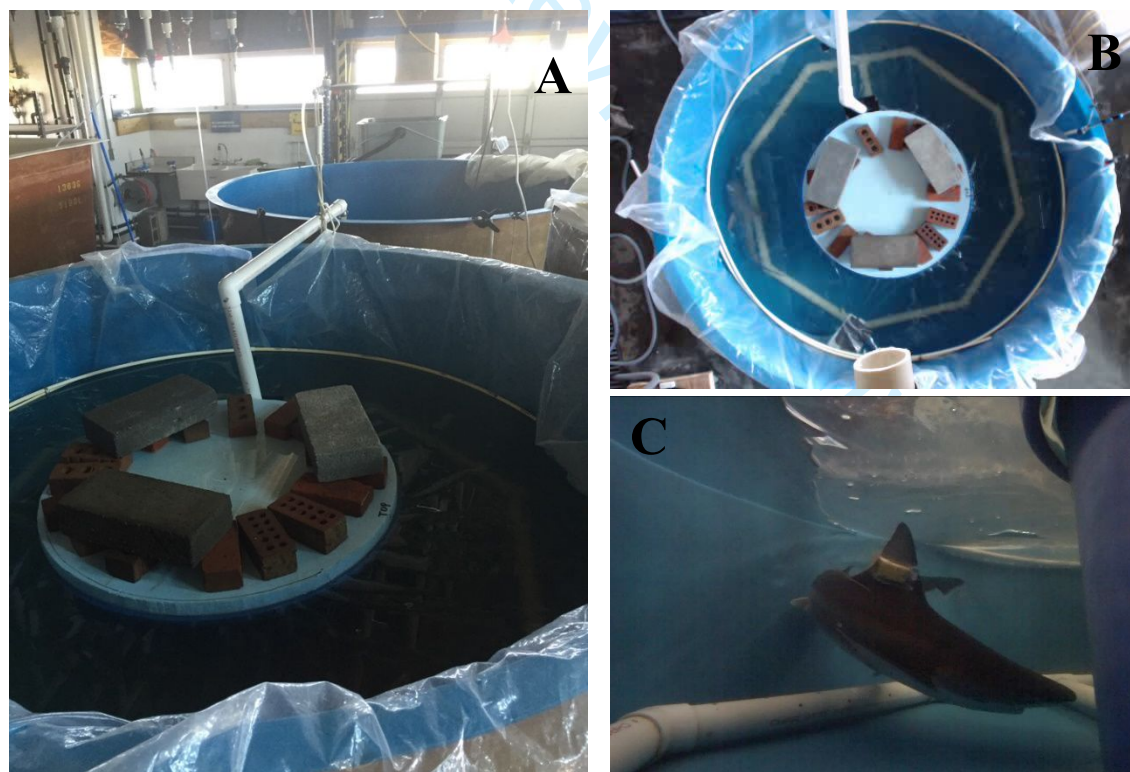
Figure 1. (A) The sandbar shark respirometer system with the respirometer in the foreground and the reservoir in the background. A PVC pipe connects the flush pump to the diffuser in the bottom of the respirometer. (B) An overhead view of the respirometer with a shark present. The

608 PVC octagon is the diffuser that ensures mixing during the flush cycle. (C) A shark in the
609 respirometer with an accelerometer fitted to its dorsal fin.
610
611
612
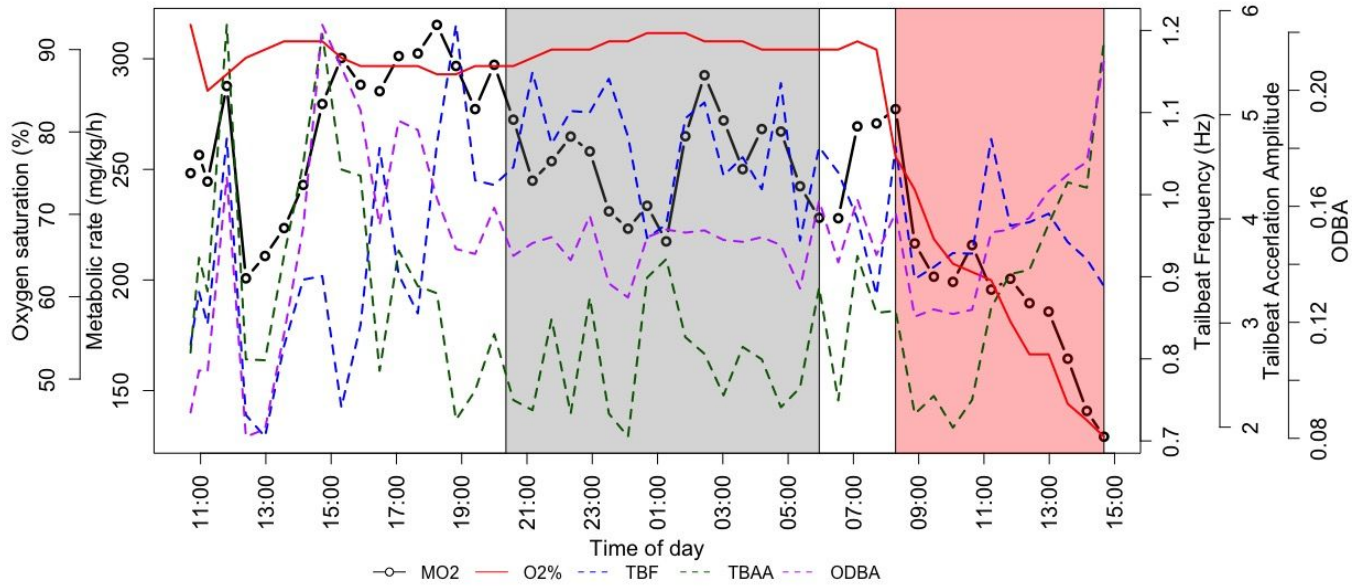
613
614 Figure 2. An example of a full trial from SB33 tested at 28°C. Each point corresponds to a
615 calculated metabolic rate (MO_2) along with the associated oxygen saturation ($O_2\%$), tailbeat
616 frequency (TBF), tailbeat acceleration amplitude (TBAA), and ODBA values. The grey shaded
617 area represents night hours created from sunrise/sunset time. The red shaded area represents the
618 hypoxia period ($O_2\% < 80\%$). S_{crit} for this individual at 28°C was 58% oxygen saturation.
619
620
621
622
623
624
625
626
627
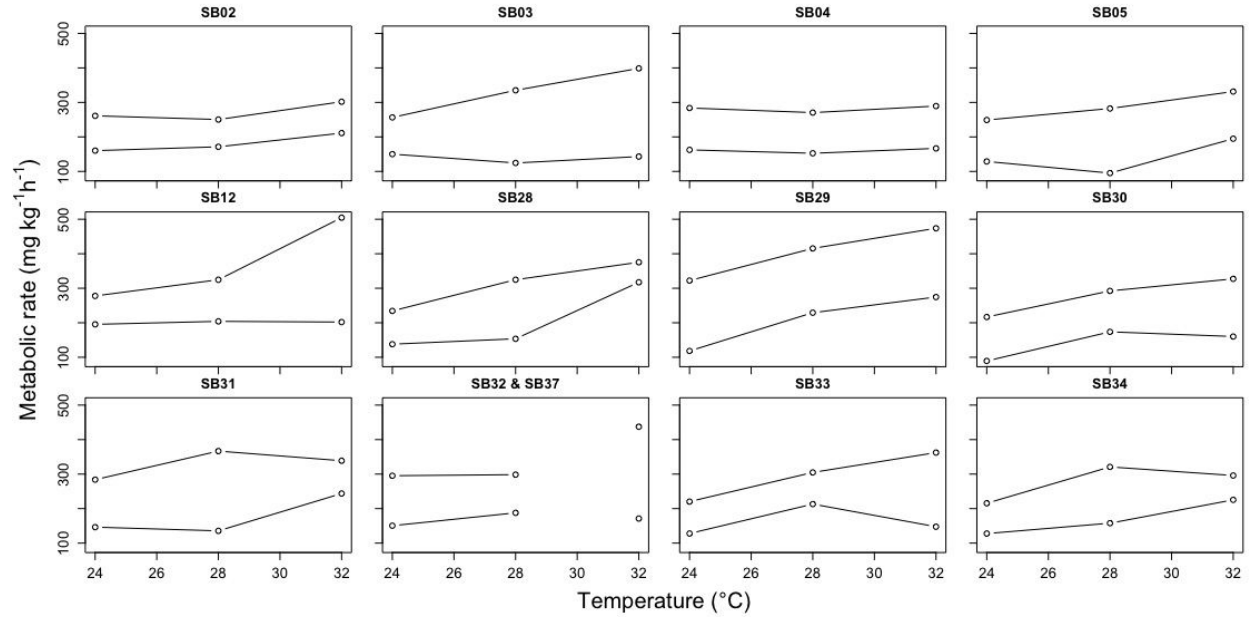
628
629 Figure 3. Measured maximum metabolic rate (MMR; top line in each plot) and minimum routine
630 metabolic rate (mRMR; bottom line in each plot) for each shark tested in this study. The
631 metabolic rate was measured at 24, 28, and 32°C for each shark except for SB32 which was only
632 measured at 24 and 28°C (line connected those two data points). SB37 replaced SB32 at 32°C
633 (single points).

634
635
636
637
638
639
640
641
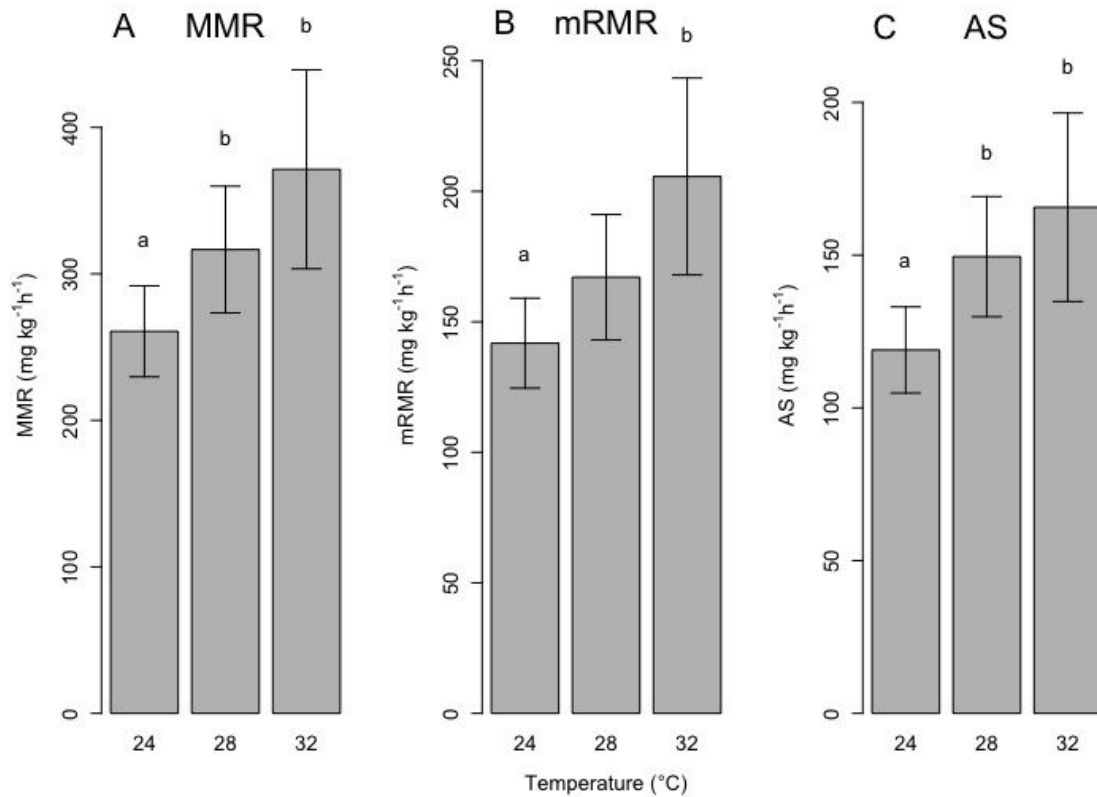
642

643 Figure 4. (A) Maximum metabolic rate (MMR), (B) minimum routine metabolic rate (mRMR),
644 and (C) aerobic scope (AS) at 24, 28, and 32°C of sandbar sharks when in normoxic waters. Data
645 are model estimates of the mean ± 95% CI. Different lower case letters indicate a significant
646 difference between two temperatures, whereas the lack of a letter indicates no significance.
647
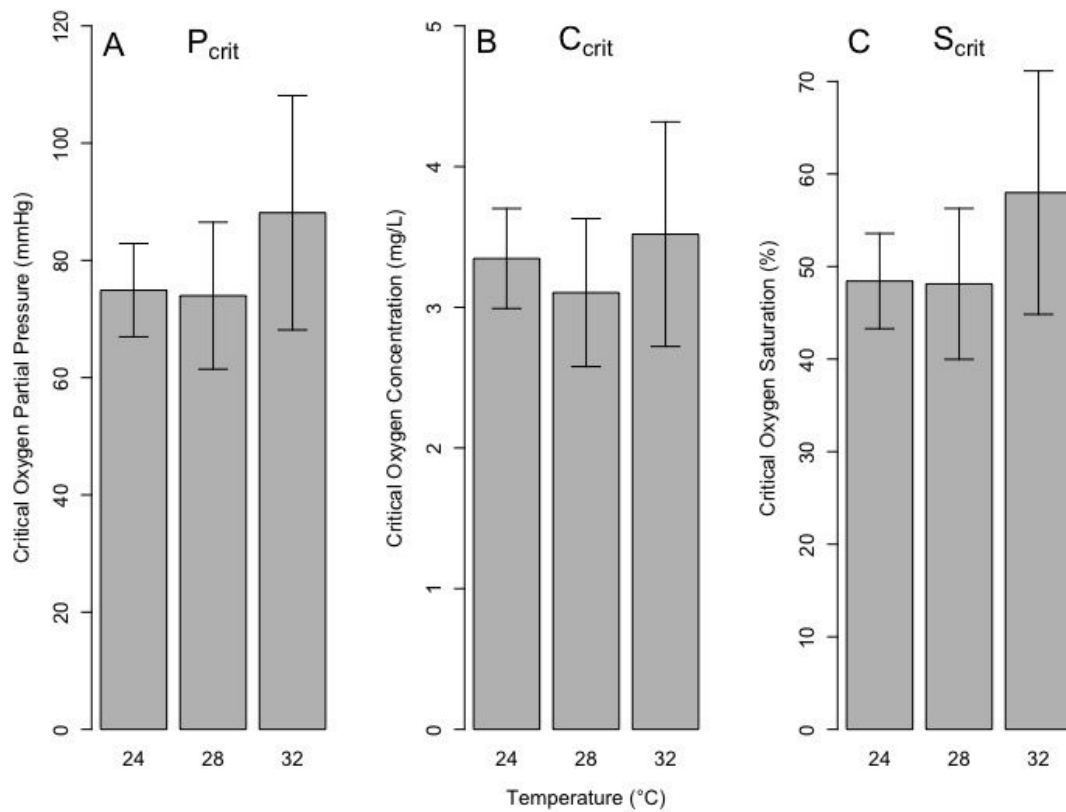
648
649   Figure 5. (A) Critical oxygen partial pressure (P_{crit}), (B) critical oxygen concentration (C_{crit}), and
650   (C) critical oxygen saturation (S_{crit}) at 24, 28, and 32°C of sandbar sharks when in normoxic
651   waters. P_{crit} data are model estimates of the mean ± 95% CI. C_{crit} and S_{crit} were calculated from
652   P_{crit} model estimates. The lack of a letter indicates no significance.
653
654
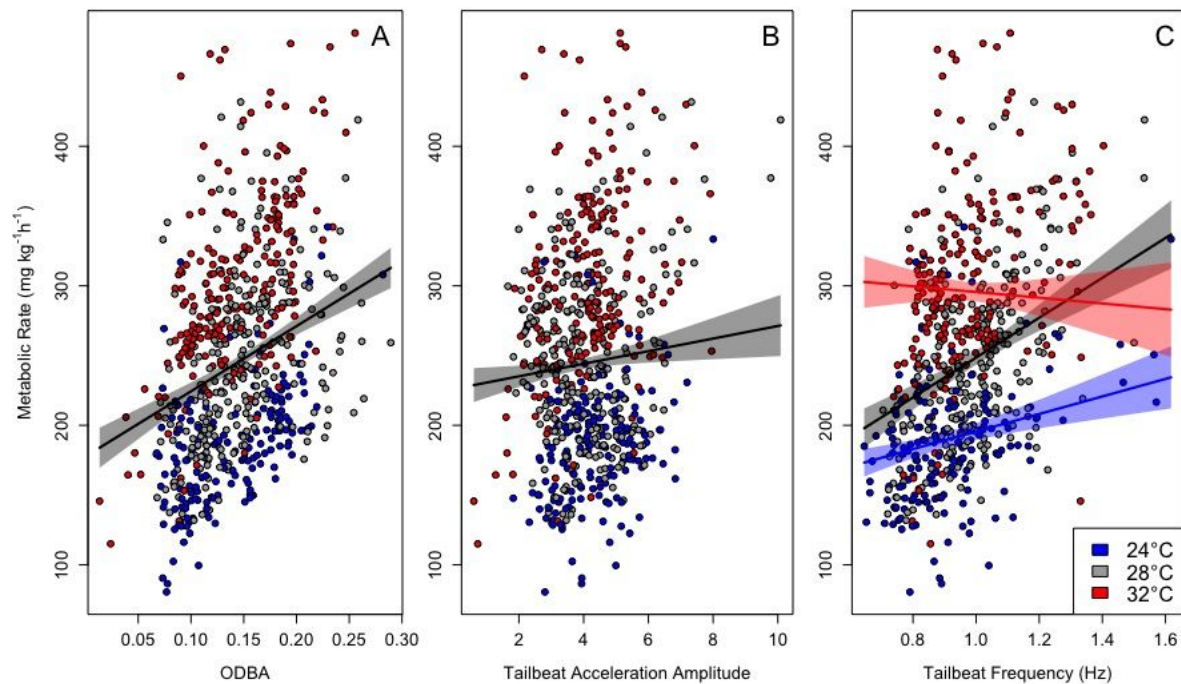
655
656 Figure 6. Metabolic rate values by ODBA (A), tailbeat acceleration amplitude (B), and tailbeat
657 frequency (C) when sandbar sharks are exposed to normoxic waters. The colored circles
658 represent the observed metabolic rate values for a given temperature. The line and associated
659 shaded region represent the estimated metabolic rate values and the 95% CI, respectively. An
660 interaction did not occur between temperature and ODBA (A) or tailbeat acceleration amplitude
661 (B); therefore, temperature was combined when estimating metabolic rate for those metrics.
662 However, because there was a significant interaction between temperature and tailbeat frequency
663 there are separate estimated metabolic rates for each temperature which are represented by
664 different colored lines and shaded regions in C.

665
666
667
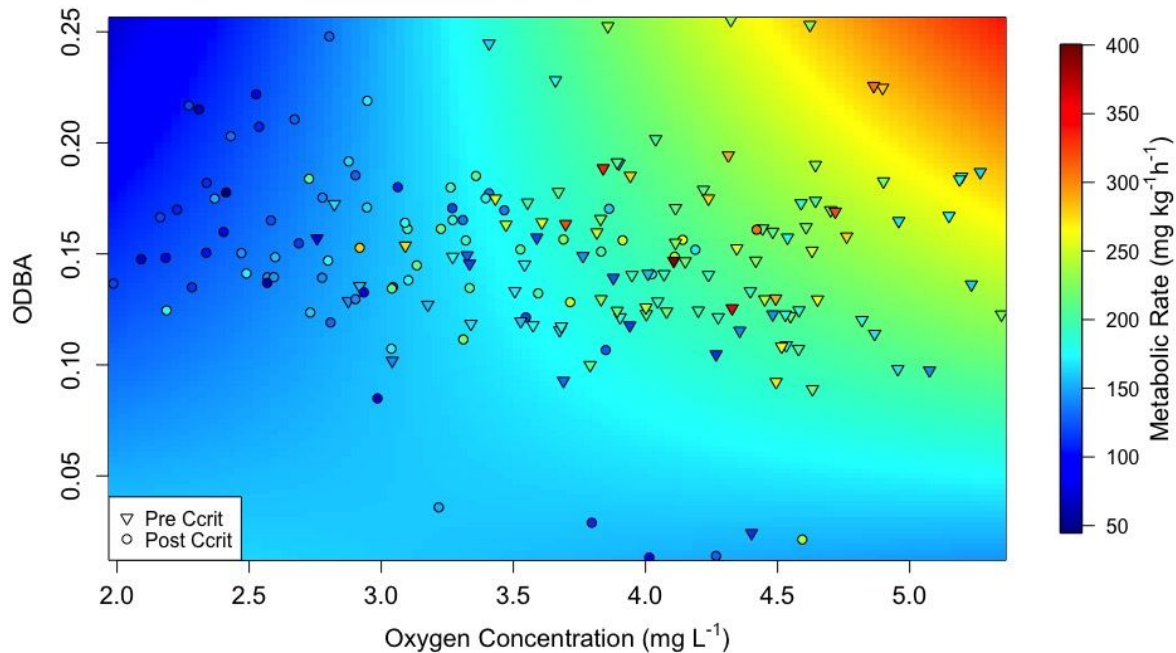
Figure 7. Metabolic rate in relation to ODBA and oxygen concentration during hypoxia (< 80% oxygen saturation). Symbols represent observed data, where ∇ are before C_{crit} was reached and O are after C_{crit} was reached. The colors on the symbols represent the observed metabolic rate. The surface behind the symbols represents the predicted metabolic rate estimated from the model.

696

697 **Supplementary Data**

698

699 Supplementary Table 1. ΔBIC table of potential models to describe metabolic rate under
700 normoxic conditions. All of these models are already assumed to include heterogeneity and a
701 correlation structure of AR1.

Model #	Models describing metabolic rate	DF	ΔBIC
1	(1|Animal_ID) + ODBA + (TBF * Temp) + TBA + Trial_Time	14	0
2	(1|Animal_ID) + (ODBA * Temp) + (TBF * Temp) + TBA + Trial_Time	16	11.1
3	(1|Animal_ID) + ODBA + TBF + Temp + Trial_Time	11	23.1
4	(1|Animal_ID) + (ODBA * Temp) + (TBF * Temp) + (TBA * Temp) + Trial_Time	18	23.4
5	(1|Animal_ID) + (ODBA * Temp) + TBF + TBA + Trial_Time	14	24.4
6	(1|Animal_ID) + ODBA + TBF + TBA + Temp + Trial_Time	12	26.6
7	(1|Animal_ID) + ODBA + TBF + Temp + TL + Trial_Time	12	29.3
8	(1|Animal_ID) + ODBA + TBF + TBA + Temp + TL + Trial_Time	13	32.9
9	(1|Animal_ID) + ODBA + TBF + Temp + Trial_Time	10	68.2

702

703 Supplementary Table 2. ΔBIC table of potential models to describe metabolic rate under hypoxic
704 conditions. All of these models are already assumed to include heterogeneity and a correlation
705 structure of AR1.

Model #	Models describing metabolic rate	DF	ΔBIC
1	(1|Animal_ID) + (ODBA * O2) + TBF + Temp	12	0
2	(1|Animal_ID) + (ODBA * O2) + TBF + TBA + Temp	13	2.2
3	(1|Animal_ID) + (ODBA * O2) + (TBF * O2) + Temp	13	3.9
4	(1|Animal_ID) + (ODBA * O2) + TBF + TL + Temp	13	4.4
5	(1|Animal_ID) + (ODBA * O2) + TBF + TBA + TL + Temp	14	6.6
6	(1|Animal_ID) + (ODBA * O2) + (TBF * O2) + TBA + TL + Temp	15	9.6
7	(1|Animal_ID) + (ODBA * O2) + (TBF * O2) + (ODBA * Temp)	15	12.4
8	(1|Animal_ID) + (ODBA * O2) + (TBF * O2) + (ODBA * Temp) + TL	16	16.8
9	(1|Animal_ID) + (ODBA * O2) + (TBF * O2) + (ODBA * Temp) + TBA + TL	17	18.5
10	(1|Animal_ID) + ODBA + TBF + TBA + Temp + O2 + TL	13	21.9
11	(1|Animal_ID) + ODBA + TBF + TBA + Temp + Crash_metric + TL	13	76.4

706

1
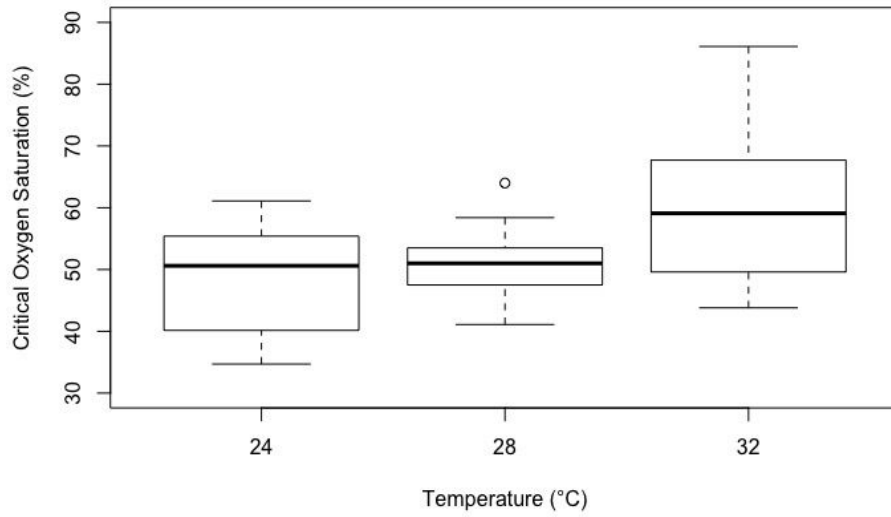2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
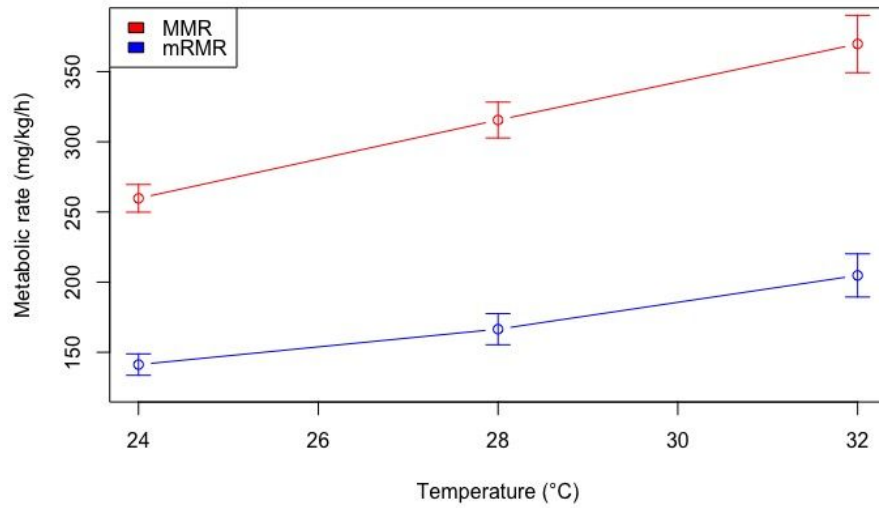
707
708   Supplementary Figure 1. Raw means (± SE) of maximum metabolic rate (MMR) and minimum
709   routine metabolic rate (mRMR) of sandbar sharks tested at 24, 28, and 32°C.
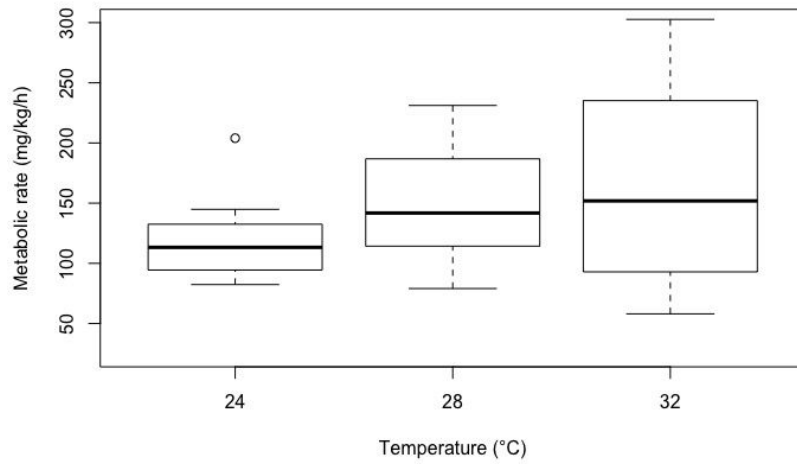
710
711   Supplementary Figure 2. Raw values of aerobic scope of sandbar sharks tested at 24, 28, and
712   32°C.
713

714
715   Supplementary Figure 3. Raw values of the critical oxygen saturation (S_{crit}) of sandbar sharks
716   tested at 24, 28, and 32°C.
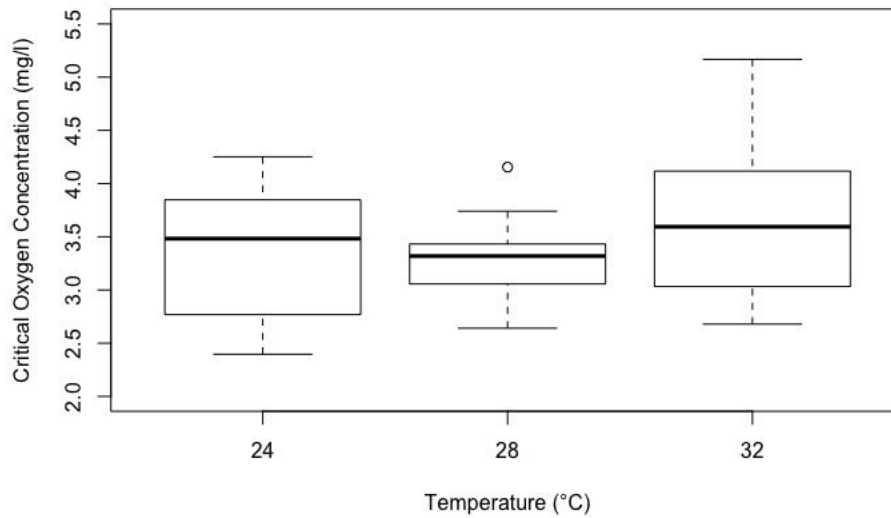
717
718   Supplementary Figure 4. Raw values of the critical oxygen concentration (C_{crit}) of sandbar sharks
719   tested at 24, 28, and 32°C.
720
721
722
723
724

725

References

Bangley CW, Paramore L, Shiffman DS, Rulifson RA (2018) Increased Abundance and Nursery Habitat Use of the Bull Shark (*Carcharhinus leucas*) in Response to a Changing Environment in a Warm-Temperate Estuary. *Scientific Reports* 8: 6018

Bernal D, Brill RW, Dickson KA, Shiels HAJRiFB, Fisheries (2017) Sharing the water column: physiological mechanisms underlying species-specific habitat use in tunas 27: 843-880

Bouyoucos IA, Montgomery DW, Brownscombe JW, Cooke SJ, Suski CD, Mandelman JW, Brooks EJ (2017) Swimming speeds and metabolic rates of semi-captive juvenile lemon sharks (*Negaprion brevirostris*, Poey) estimated with acceleration biologgers. *Journal of Experimental Marine Biology and Ecology* 486: 245-254

Brill R, Bushnell P, Schroff S, Seifert R, Galvin M (2008) Effects of anaerobic exercise accompanying catch-and-release fishing on blood-oxygen affinity of the sandbar shark (*Carcharhinus plumbeus*, Nardo). *Journal of Experimental Marine Biology and Ecology* 354: 132-143

Brill RW, Bushnell PG, Elton TA, Small HJ (2015) The ability of blue crab (*Callinectes sapidus*, Rathbun 1886) to sustain aerobic metabolism during hypoxia. *Journal of Experimental Marine Biology and Ecology* 471: 126-136

Bushnell PG, Brill RW (1991) Responses of swimming skipjack (*Katsuwonus pelamis*) and yellowfin (*Thunnus albacares*) tunas to acute hypoxia, and a model of their cardiorespiratory function. *Physiological Zoology* 64: 787-811

Carlson JK, Goldman KJ, Lowe CG (2004) Metabolism, energetic demand, and endothermy. In Carrier J, Musick J, Heithaus M eds, Biology of sharks and their relatives Vol. 10, pp 269-286

Carlson JK, Parsons GR (2001) The effects of hypoxia on three sympatric shark species: physiological and behavioral responses. *Environmental Biology of Fishes* 61: 427-433

Claireaux G, Lefrançois C (2007) Linking environmental variability and fish performance: integration through the concept of scope for activity. *Philosophical Transactions of the Royal Society B: Biological Sciences* 362: 2031-2041

Clark TD, Jeffries KM, Hinch SG, Farrell AP (2011) Exceptional aerobic scope and cardiovascular performance of pink salmon (*Oncorhynchus gorbuscha*) may underlie resilience in a warming climate. *The Journal of Experimental Biology* 214: 3074-3081

Clark TD, Sandblom E, Jutfelt F (2013) Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. *Journal of Experimental Biology* 216: 2771-2782

Cliff G, Thurman G (1984) Pathological and physiological effects of stress during capture and transport in the juvenile dusky shark, Carcharhinus obscurus. *Comparative Biochemistry and Physiology Part A: Physiology* 78: 167-173

Conley DJ, Carstensen J, Ærtebjerg G, Christensen PB, Dalsgaard T, Hansen JL, Josefson AB (2007) Long-term changes and impacts of hypoxia in Danish coastal waters. *Ecological Applications* 17: S165-S184

Conrath CL, Musick JA (2007) The sandbar shark summer nursery within bays and lagoons of the eastern shore of Virginia. *Transactions of the American Fisheries Society* 136: 999-1007

Di Santo V (2016) Intraspecific variation in physiological performance of a benthic elasmobranch challenged by ocean acidification and warming. *Journal of Experimental Biology* 219: 1725-1733

Diaz RJ, Rosenberg R (1995) Marine benthic hypoxia: a review of its ecological effects and the behavioural responses of benthic macrofauna. In Ansell A, Gibson R, Barnes M eds, Oceanography and Marine Biology. An Annual Review Vol. 33, London, UK: University College London Press, pp 245-303

Dizon A (1977) Effect of dissolved oxygen concentrations and salinity on swimming speed of two species of tuna. *Fisheries Bulletin* 75: 649-653

Dowd WW, Brill RW, Bushnell PG, Musick JA (2006) Standard and routine metabolic rates of juvenile sandbar sharks (*Carcharhinus plumbeus*), including the effects of body mass and acute temperature change. *Fishery Bulletin* 104: 323-331

Efron B, Tibshirani RJ (1993) An introduction to the bootstrap. CRC press, New York, NY.

Ellis JK, Musick JA (2007) Ontogenetic changes in the diet of the sandbar shark, *Carcharhinus plumbeus*, in lower Chesapeake Bay and Virginia (USA) coastal waters. *Environmental Biology of Fishes* 80: 51-67

Emery SH, Szczepanski A (1986) Gill dimensions in pelagic elasmobranch fishes. *The Biological Bulletin* 171: 441-449

Farrell A, Eliason E, Sandblom E, Clark T (2009) Fish cardiorespiratory physiology in an era of climate change. *Canadian Journal of Zoology* 87: 835-851

Fry FEJ (1971) The effect of environmental factors on the physiology of fish. *Fish Physiology* 6: 1-98

Fry FEJ, Hart J (1948) The relation of temperature to oxygen consumption in the goldfish. *The Biological Bulletin* 94: 66-77

Gleiss AC, Wilson RP, Shepard EL (2011) Making overall dynamic body acceleration work: on the theory of acceleration as a proxy for energy expenditure. *Methods in Ecology and Evolution* 2: 23-33

Grubbs RD, Musick J (2007). Spatial delineation of summer nursery areas for juvenile sandbar sharks in Chesapeake Bay, Virginia, American Fisheries Society Symposium Vol. 50, American Fisheries Society, pp 63-86

Hagy JD, Boynton WR, Keefe CW, Wood KV (2004) Hypoxia in Chesapeake Bay, 1950–2001: long-term change in relation to nutrient loading and river flow. *Estuaries* 27: 634-658

Irby ID, Friedrichs MA, Da F, Hinson KE (2018) The competing impacts of climate change and nutrient reductions on dissolved oxygen in Chesapeake Bay. *Biogeosciences* 15: 2649-2668

Irby ID, Friedrichs MA, Friedrichs CT, Bever AJ, Hood RR, Lanerolle LW, Scully ME (2016) Challenges associated with modeling low-oxygen waters in Chesapeake Bay: a multiple model comparison. *Biogeosciences* 13: 2011-2028

Kemp WM, Boynton WR, Adolf JE, Boesch DF, Boicourt WC, Brush G, Cornwell JC, Fisher TR, Glibert PM, Hagy JD (2005) Eutrophication of Chesapeake Bay: historical trends and ecological interactions. *Marine Ecology Progress Series* 303: 1-29

Kenward MG, Roger JH (1997) Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics*: 983-997

Killen SS, Norin T, Halsey LG (2017) Do method and species lifestyle affect measures of maximum metabolic rate in fishes? *Journal of Fish Biology* 90: 1037-1046

Korsmeyer K, Dewar H (2001) Tuna metabolism and energetics. Tuna physiology, ecology and evolution. In Block B, Stevens E eds, Fish Physiology Vol. 19, San Diego, CA: Academic Press, pp 35-78

Lapointe D, Vogelbein WK, Fabrizio MC, Gauthier DT, Brill RW (2014) Temperature, hypoxia, and mycobacteriosis: effects on adult striped bass Morone saxatilis metabolic performance. *Dis. Aquat. Org* 108: 113-127

Last PR, White WT, Gledhill DC, Hobday AJ, Brown R, Edgar GJ, Pecl G (2011) Long-term shifts in abundance and distribution of a temperate fish fauna: a response to climate change and fishing practices. *Global Ecology and Biogeography* 20: 58-72

Lear KO, Whitney NM, Brewster LR, Morris JJ, Hueter RE, Gleiss AC (2017) Correlations of metabolic rate and body acceleration in three species of coastal sharks under contrasting temperature regimes. *Journal of Experimental Biology* 220: 397-407

Lefevre S (2016) Are global warming and ocean acidification conspiring against marine ectotherms? A meta-analysis of the respiratory effects of elevated temperature, high CO2 and their interaction. *Conservation Physiology* 4: 1-31

Littell RC, Milliken GA, Stroup WW, Wolfinger RD, Schabenberger O (2006) SAS for mixed models, 2nd edn, SAS Inst., Cary, NC.

Ludsin SA, Zhang X, Brandt SB, Roman MR, Boicourt WC, Mason DM, Costantini M (2009) Hypoxia-avoidance by planktivorous fish in Chesapeake Bay: implications for food web interactions and fish recruitment. *Journal of Experimental Marine Biology and Ecology* 381: S121-S131

Lynch PD, Nye JA, Hare JA, Stock CA, Alexander MA, Scott JD, Curti KL, Drew K (2014) Projected ocean warming creates a conservation challenge for river herring populations. *ICES Journal of Marine Science* 72: 374-387

McKenzie DJ, Axelsson M, Chabot D, Claireaux G, Cooke SJ, Corner RA, De Boeck G, Domenici P, Guerreiro PM, Hamer B (2016) Conservation physiology of marine fishes: state of the art and prospects for policy. *Conservation Physiology* 4: 1-20

Morley JW, Selden RL, Latour RJ, Frölicher TL, Seagraves RJ, Pinsky ML (2018) Projecting shifts in thermal habitat for 686 species on the North American continental shelf. *PloS one* 13: 1-28

Muhling BA, Gaitán CF, Stock CA, Saba VS, Tommasi D, Dixon KW (2018) Potential salinity and temperature futures for the Chesapeake Bay using a statistical downscaling spatial disaggregation framework. *Estuaries and Coasts* 41: 349-372

Najjar RG, Pyke CR, Adams MB, Breitburg D, Hershner C, Kemp M, Howarth R, Mulholland MR, Paolisso M, Secor D (2010) Potential climate-change impacts on the Chesapeake Bay. *Estuarine, Coastal and Shelf Science* 86: 1-20

Norin T, Malte H, Clark TD (2014) Aerobic scope does not predict the performance of a tropical eurythermal fish at elevated temperatures. *Journal of Experimental Biology* 217: 244-251

Nye JA, Link JS, Hare JA, Overholtz WJ (2009) Changing spatial distribution of fish stocks in relation to climate and population size on the Northeast United States continental shelf. *Marine Ecology Progress Series* 393: 111-129

Payne NL, Meyer CG, Smith JA, Houghton JD, Barnett A, Holmes BJ, Nakamura I, Papastamatiou YP, Royer MA, Coffey DM (2018) Combining abundance and performance data reveals how temperature regulates coastal occurrences and activity of a roaming apex predator. *Global Change Biology*: 1-10

Payne NL, Smith JA, Meulen DE, Taylor MD, Watanabe YY, Takahashi A, Marzullo TA, Gray CA, Cadiou G, Suthers IM (2016) Temperature dependence of fish performance in the wild: links with species biogeography and physiological thermal tolerance. *Functional Ecology* 30: 903-912

Pinheiro J, Bates D, DebRoy S, Sarkar D (2013) nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-111 <http://CRAN.R-project.org/package=nlme>

Pörtner HO, Knust R (2007) Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science* 315: 95-97

Preston BL (2004) Observed winter warming of the Chesapeake Bay estuary (1949–2002): implications for ecosystem management. *Environmental Management* 34: 125-139

Rabalais NN, Turner RE, Díaz RJ, Justić D (2009) Global change and eutrophication of coastal waters. *ICES Journal of Marine Science: Journal du Conseil* 66: 1528-1537

Rijnsdorp AD, Peck MA, Engelhard GH, Möllmann C, Pinnegar JK (2009) Resolving the effect of climate change on fish populations. *ICES Journal of Marine Science: Journal du Conseil*: 1-14

Schurmann H, Steffensen J (1997) Effects of temperature, hypoxia and activity on the metabolism of juvenile Atlantic cod. *Journal of Fish Biology* 50: 1166-1180

Searle SR, Speed FM, Milliken GAJTAS (1980) Population marginal means in the linear model: an alternative to least squares means 34: 216-221

SEDAR (2017). SEDAR 54 stock assessment report: HMS Sandbar Shark, North Charleston, SC

Shepard EL, Wilson RP, Quintana F, Laich AG, Liebsch N, Albareda DA, Halsey LG, Gleiss A, Morgan DT, Myers AE (2008) Identification of animal movement patterns using tri-axial accelerometry. *Endangered Species Research* 10: 47-60

Skomal GB, Mandelman JW (2012) The physiological response to anthropogenic stressors in marine elasmobranch fishes: a review with a focus on the secondary response. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 162: 146-155

Speers-Roesch B, Richards JG, Brauner CJ, Farrell AP, Hickey AJ, Wang YS, Renshaw GM (2012) Hypoxia tolerance in elasmobranchs. I. Critical oxygen tension as a measure of blood oxygen transport during hypoxia exposure. *Journal of Experimental Biology* 215: 93-102

Svendsen M, Bushnell P, Steffensen J (2016) Design and setup of intermittent-flow respirometry system for aquatic organisms. *Journal of Fish Biology* 88: 26-50

Turner SM, Manderson JP, Richardson DE, Hoey JJ, Hare JA (2015) Using habitat association models to predict Alewife and Blueback Herring marine distributions and overlap with Atlantic Herring and Atlantic Mackerel: can incidental catches be reduced? *ICES Journal of Marine Science* 73: 1912-1924

Tyler RM, Targett TE (2007) Juvenile weakfish, *Cynoscion regalis,* distribution in relation to diel-cycling dissolved oxygen in an estuarine tributary. *Marine Ecology Progress Series* 333: 257-269

Wannamaker CM, Rice JA (2000) Effects of hypoxia on movements and behavior of selected estuarine organisms from the southeastern United States. *Journal of Experimental Marine Biology and Ecology* 249: 145-163

Whitney NM, Papastamatiou YP, Holland KN, Lowe CG (2007) Use of an acceleration data logger to measure diel activity patterns in captive whitetip reef sharks, *Triaenodon obesus.* *Aquatic Living Resources* 20: 299-305

Whitney NM, White CF, Gleiss AC, Schwieterman GD, Anderson P, Hueter RE, Skomal GB (2016) A novel method for determining post-release mortality, behavior, and recovery period using acceleration data loggers. *Fisheries Research* 183: 210-221

Zhang H, Ludsin SA, Mason DM, Adamack AT, Brandt SB, Zhang X, Kimmel DG, Roman MR, Boicourt WC (2009) Hypoxia-driven changes in the behavior and spatial distribution of pelagic fish and mesozooplankton in the northern Gulf of Mexico. *Journal of Experimental Marine Biology and Ecology* 381: S80-S91
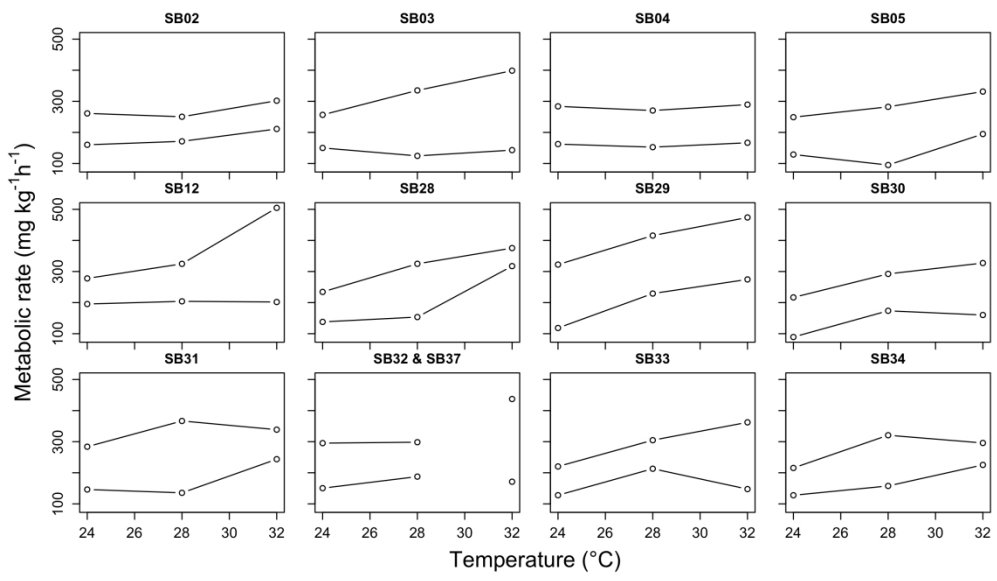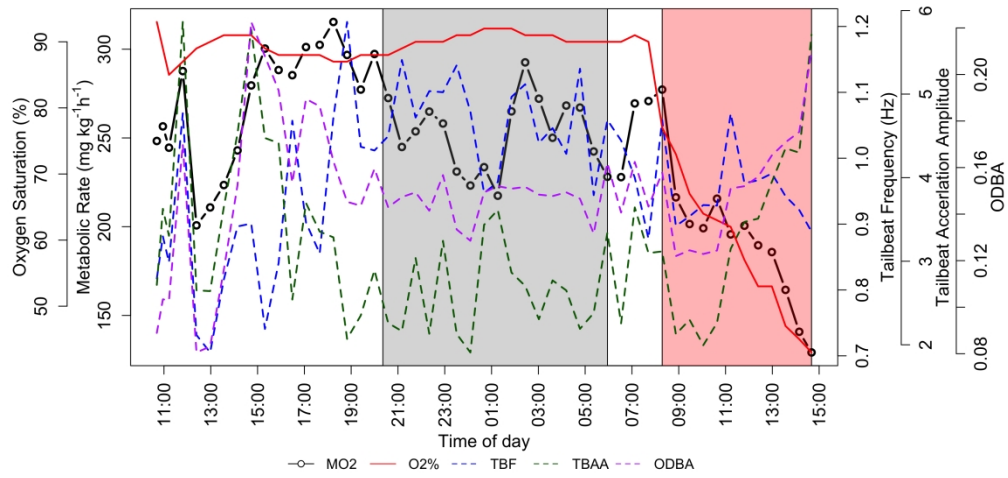
Figure 3

999x583mm (72 x 72 DPI)

Fig 2

1249x624mm (72 x 72 DPI)

Fig 4

708x583mm (72 x 72 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

fig 5

708x583mm (72 x 72 DPI)

916x624mm (72 x 72 DPI)

1
2
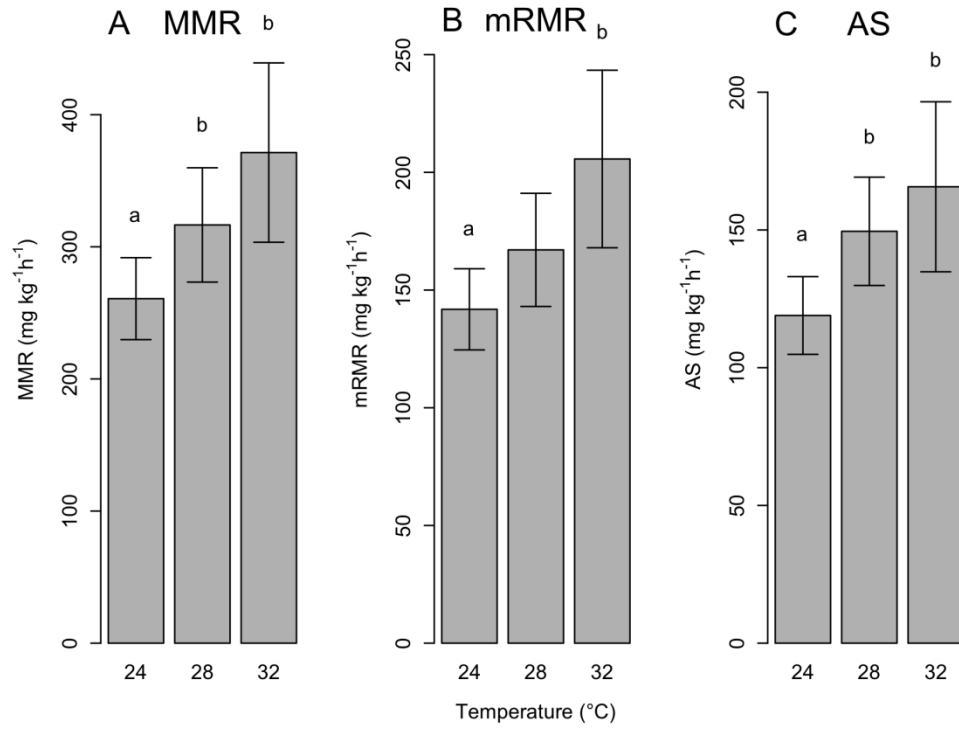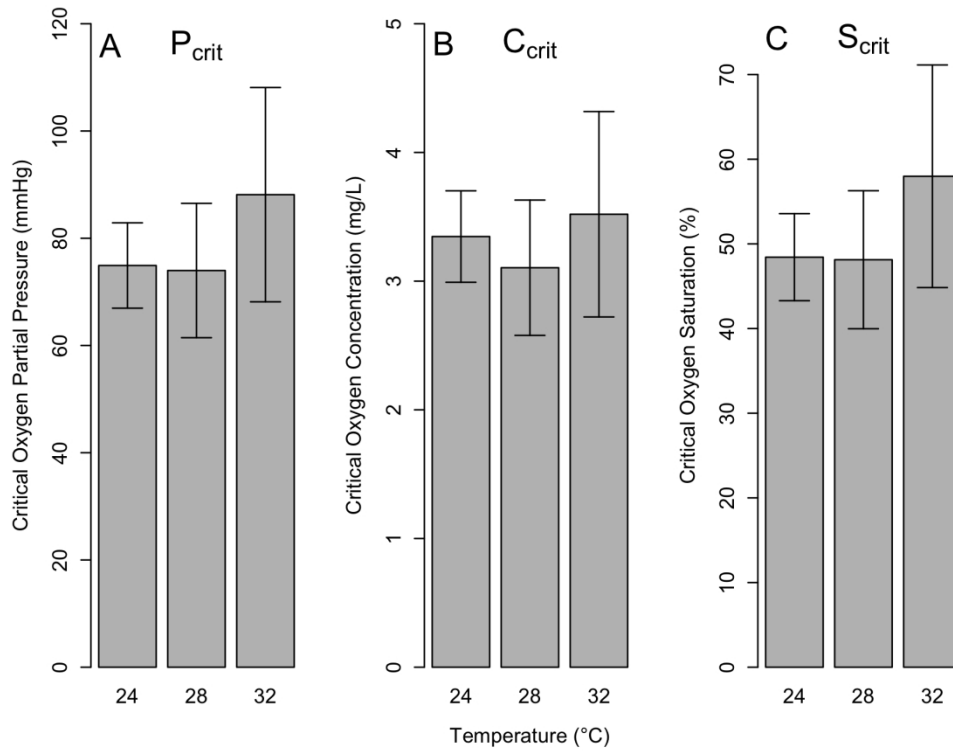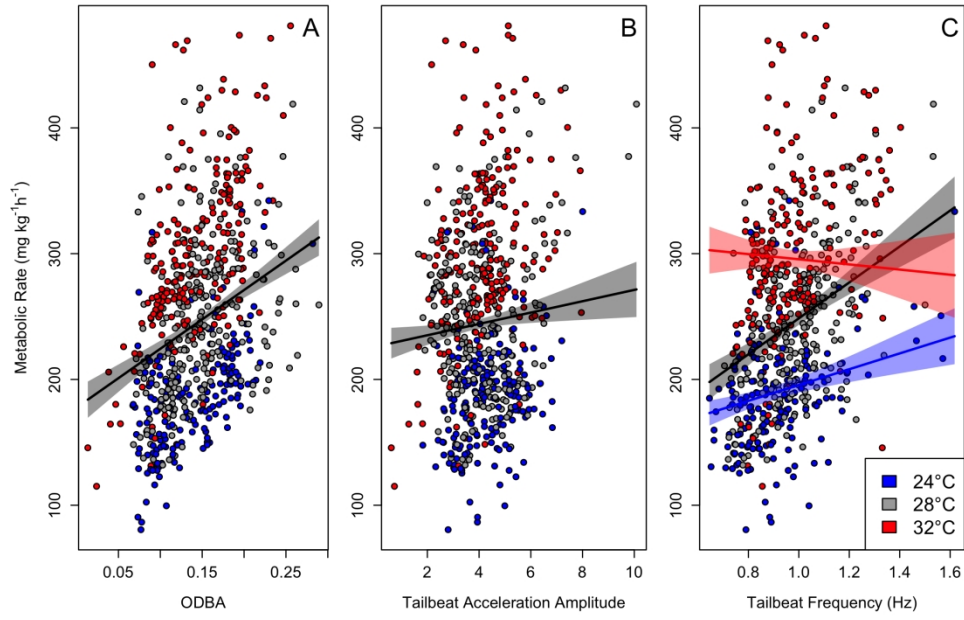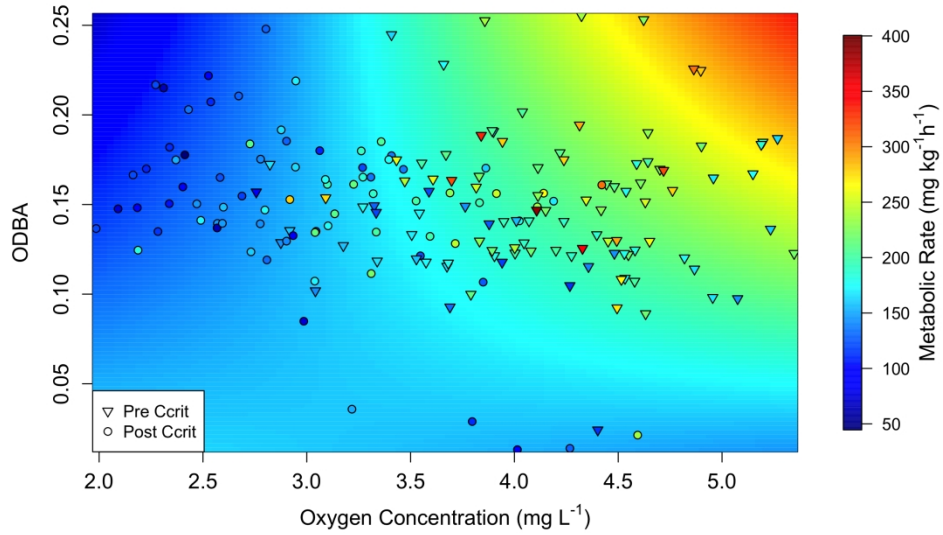3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1041x624mm (72 x 72 DPI)