

1 Title: Plasticity, repeatability, and phenotypic correlations of aerobic metabolic traits in a small
2 estuarine fish

3 Running title: Aerobic metabolism in an estuarine fish

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16 **SUMMARY STATEMENT**

17 Aerobic metabolism of an ecologically dominant estuarine fish is influenced by acclimation to
18 environmental changes without altering trait repeatability. Furthermore, specific metabolic traits
19 are phenotypically correlated.

20

21 **ABSTRACT**

22 Standard metabolic rate (SMR), maximum metabolic rate (MMR), absolute aerobic scope
23 (AAS), and critical oxygen tension (P_{crit}) were determined for the Gulf killifish, *Fundulus*
24 *grandis*, an ecologically dominant estuarine fish, acclimated to lowered salinity, elevated
25 temperature, and lowered oxygen concentration. Acclimation to low salinity resulted in a small,
26 but significant, elevation of P_{crit} ; acclimation to elevated temperature increased SMR, MMR,
27 AAS, and P_{crit} ; acclimation to low oxygen led to a small increase in SMR, but substantial
28 decreases in MMR, AAS, and P_{crit} . Variation in these metabolic traits among individuals was
29 consistent and repeatable when measured during multiple control exposures over seven months.
30 Trait repeatability was unaffected by acclimation condition suggesting that repeatability of these
31 traits is not context dependent. There were significant phenotypic correlations between specific
32 metabolic traits: SMR was positively correlated with MMR and P_{crit} ; MMR was positively
33 correlated with AAS; and AAS was negatively correlated with P_{crit} . In general, within-individual
34 variation contributed more than among-individual variation to these phenotypic correlations. The
35 effects of acclimation on these traits demonstrate that aerobic metabolism is plastic and
36 influenced by the conditions experienced by these fish in the dynamic habitats in which they
37 occur; however, the repeatability of these traits and the correlations among them suggest that
38 these traits change in ways that maintains the rank order of performance among individuals
39 across a range of environmental variation.

40

41 Keywords: acclimation, aerobic metabolism, hypoxia, individual variation, phenotypic
42 correlations, repeatability

43 List of symbols and abbreviations
44 a.s.: air saturation
45 AAS: Absolute aerobic scope
46 BC: Bayou Cumbest
47 BH: Bayou Heron
48 DO: dissolved oxygen
49 GBNERR: Grand Bay National Estuarine Research Reserve
50 M_b : body mass
51 MMR: Maximum metabolic rate
52 M_{O_2} : rate of oxygen consumption
53 P_{crit} : critical oxygen tension
54 RMR: routine metabolic rate
55 R_{adj} : adjusted repeatability
56 r_p : phenotypic correlation
57 r_e : within-individual correlation
58 r_{ind} : among-individual correlation
59 SMR: standard metabolic rate
60

61 INTRODUCTION

62 Elucidating the causes and consequences of variation in energy metabolism is a central goal of
63 animal physiology and ecology (Schmidt-Nielsen, 1997; Brown et al., 2004). In particular, there
64 is considerable interest in the effects of changes in the abiotic environment on the intensity of
65 energy metabolism, an interest that has been heightened by the rate and extent of contemporary
66 alterations in climate and other environmental variables brought about by human activities.

67 Estuaries are naturally dynamic habitats due to variable riverine input, diurnal and tidal cycles,
68 wind patterns, storms, and season. As a result, several abiotic factors, including salinity,
69 temperature, and dissolved oxygen (DO), vary broadly on time scales ranging from hours to
70 months. Anthropogenic changes in land use, hydrology, and climate can alter the mean value of
71 these abiotic variables (e.g., higher average temperature) or increase the frequency and duration
72 of extreme values (e.g., aquatic hypoxia). Understanding the response of energy metabolism in
73 estuarine organisms to natural environmental variation will provide insight into their resiliency
74 and potential responses to future habitat alteration.

75 The metabolic rate of an animal is an integrative measure of energy flow and includes
76 costs of ion transport, biosynthesis, locomotion, and myriad other processes. For organisms that
77 utilize oxidative phosphorylation for ATP synthesis, metabolic rate can be estimated as the rate
78 of oxygen consumption (M_{O_2}). In ectothermic animals, including fishes, the M_{O_2} in a post-
79 absorptive individual at rest is its standard metabolic rate (SMR), while maximum metabolic rate
80 (MMR) is the highest M_{O_2} , typically achieved during or immediately after intense exercise (Brett
81 and Groves, 1979; Norin and Clark, 2016). The difference between MMR and SMR is absolute
82 aerobic scope (AAS), which represents the animal's capacity to support energetically expensive
83 processes aerobically. At low levels of ambient oxygen (hypoxia) aerobic metabolism (SMR,
84 MMR, and AAS) becomes limited (Fry, 1947). The level of oxygen below which SMR can no
85 longer be sustained is the critical oxygen tension, or P_{crit} (Rogers et al., 2016; Claireaux and
86 Chabot, 2016; Reemeyer and Rees, 2019). Below P_{crit} , metabolism is increasingly supported by
87 anaerobic processes (e.g., glycolysis), which are not sustainable over the long term, or it is
88 reduced through metabolic suppression (Richards, 2009). Animals that can maintain SMR down
89 to lower levels of oxygen have lower P_{crit} ; therefore, P_{crit} has been proposed as an index of
90 hypoxia tolerance (Rogers et al., 2016).

91 In fishes, SMR, MMR, AAS, and P_{crit} , are influenced by changes in salinity, temperature,
92 and oxygen. Bony fish regulate the ion composition of plasma at about 1/3 that of sea water. At
93 salinities lower and higher than this, passive fluxes of ions out of or into the fish are balanced by
94 energy-dependent ion transport, which is predicted to increase SMR (Bœuf and Payan, 2001),
95 although this cost may be small (Ern et al., 2014). In addition, the “osmo-regulatory compromise”
96 posits that because passive ion fluxes occur across the same tissue responsible for respiratory gas
97 exchange (gill), changes that serve to limit one may also limit the other (Sardella and Brauner,
98 2007). Thus, a reduction of gill surface area, which could reduce passive ion flux, could
99 potentially limit MMR or increase P_{crit} . As temperature increases, SMR and MMR generally
100 increase; however, SMR increases exponentially, while MMR reaches a plateau or decreases at
101 high temperatures (Pörtner, 2010; Pörtner and Farrell, 2008). The effect of temperature on AAS,
102 therefore, is for it to increase at moderate temperatures, but then decline. At high temperatures,
103 AAS may fall to zero if SMR equals MMR (Pörtner, 2010; Pörtner and Farrell, 2008). P_{crit} is also
104 affected by temperature, increasing at higher temperatures (Rogers et al., 2016; McBryan et al
105 2016). Exposure to low oxygen limits aerobic metabolism, especially MMR and AAS, as the
106 oxygen available to support activities beyond SMR decreases. Acclimation of fishes to hypoxia,
107 however, may improve oxygen extraction, which has been documented as a decrease in P_{crit}
108 (Borowiec et al., 2015).

109 There is growing appreciation that traits related to aerobic metabolism are repeatable
110 when measured in the same individuals over time. As pointed out by Bennett (1987) and more
111 recently by others (Roche et al., 2016; Killen et al., 2016a), repeatable individual variation in
112 physiology arises from differences in genetics, development, and environment. When the
113 variation is due to genetic factors and is heritable, it represents the raw material upon which
114 natural selection can act. SMR, MMR, and AAS have been shown to be significantly repeatable
115 across multiple measures among individuals of several species (Killen et al., 2016a; Nespolo and
116 Franco, 2007; Norin and Malte, 2011; Norin et al., 2016; Virani and Rees, 2000). Although there
117 are fewer reports of the repeatability of P_{crit} , it, too, appears to be a repeatable trait (Pan et al.,
118 2018; Reemeyer and Rees, 2019). Currently, however, there is a lack of studies on whether the
119 repeatability of aerobic metabolism is altered by acclimation to changes in abiotic variables.
120 Norin et al. (2016) showed that acute exposure of Barramundi (*Lates calcarifer*) to low salinity,
121 elevated temperature, and low oxygen differentially affected individuals with low or high

122 metabolic rate. The result was that the rankings of individuals' SMR, MMR, and AAS were
123 different during acute exposure than during control conditions. Because that study examined
124 only short-term acute exposures, it remains to be seen how acclimation to these conditions
125 affects repeatability of these traits.

126 An experimental framework to assess repeatability of traits can also be valuable in
127 illuminating the relationships between traits. Traditionally, studies of the relationship between
128 traits measure each trait once each in a group of organisms. A repeated-measured design, where
129 each trait is measured multiple times in each individual, enables the determination of covariance
130 in the traits both among- and within-individuals (Dingemanse and Dotcherman, 2013; Careau
131 and Wilson, 2017). The phenotypic correlations (r_p) can then be partitioned into among-
132 individual correlations (r_{ind}) and within-individual correlations (r_e); with r_{ind} representing
133 linkages in traits due to a combination of genetic and fixed environmental factors, and r_e
134 representing linkages due to a combination of shared plasticity and correlated measurement error
135 (Brommer, 2013; Careau et al., 2014). High estimates of r_{ind} indicate that across individuals,
136 those with high values for one trait also have high values for another trait (and *vice versa*);
137 whereas, when r_e is high, the interpretation is that for a given individual measured at a specific
138 point in time, the values for the two traits are high, but when measured at another point in time,
139 both traits could be low.

140 In the present study, SMR, MMR, AAS, and P_{crit} were measured in the Gulf killifish,
141 *Fundulus grandis*, after acclimation to lowered salinity, elevated temperature, and lowered DO.
142 *Fundulus grandis* is a small-bodied, abundant species found throughout estuaries along the Gulf
143 of Mexico (Nordlie, 2006). Because they inhabit dynamic environments and tolerate large
144 fluctuations in abiotic variables, *F. grandis* and its sister species *F. heteroclitus* are excellent
145 model species for environmental biology research (Burnett et al., 2007). Fish used in this study
146 were collected at the Grand Bay National Estuarine Research Reserve (GBNERR), part of a
147 system of protected areas in coastal U.S.A. Specifically, *F. grandis* were collected at two sites
148 within the GBNERR, which long-term water quality records indicated were similar in salinity and
149 temperature, but differed in annual DO profiles. This allowed evaluation of local differences in
150 aerobic metabolism, as well as potential differences in the effects of acclimation to low oxygen.
151 The goals of this study were to address the following questions: (1) How are SMR, MMR, AAS,
152 and P_{crit} affected by acclimation to low salinity, high temperature, and low oxygen? (2) What are

153 the long-term repeatability estimates of SMR, MMR, AAS, and P_{crit} in *F. grandis*? (3) Does
154 acclimation to low salinity, elevated temperature, and low oxygen affect the repeatability of
155 SMR, MMR, AAS, and P_{crit} ? (4) Do individuals from different collection sites respond
156 differently to low salinity, elevated temperature, and low oxygen? (5) Are there phenotypic
157 correlations between these traits, and if so, do these arise from among-individual correlations,
158 within-individual correlations, or both.

159

160 MATERIALS AND METHODS

161 Field sites and fish collection

162 *Fundulus grandis* were collected at the GBNERR in August 2018. The GBNERR is a protected
163 wetland within the Mississippi Coastal Streams Basin (Fig. S1) and includes several sites with
164 long-term water quality data. Among these are Bayou Heron (BH, 30.4178° N, 88.4054° W) and
165 Bayou Cumbest (BC, 30.3836° N, 88.4364° W), which have comparable salinity and
166 temperature, but differ in DO profiles (Fig. 1). Bayou Heron experiences summer hypoxia due to
167 enhanced nutrient input from natural sources, while Bayou Cumbest remains normoxic
168 throughout the year. Approximately equal numbers of fish (50 each) were collected at the two
169 sites using baited minnow traps placed along the marsh edge within 1 km of permanently moored
170 water quality sensors, or sondes (Fig. S1).

171 To account for fine scale variation in water quality, temperature, salinity, and DO were
172 measured with a hand-held meter (YSI Pro2030, www.y si.com) at the exact times and locations
173 of trap deployment and retrieval (Table S1). These measurements showed that temperature was
174 essentially identical between collection sites, as well as between sondes and hand-held
175 measurements, although salinity was lower at the trap locations which were more affected by
176 freshwater runoff from recent rains. In support of long-term records, the average and range of
177 DO were lower at the BH sites than the BC sites, but this difference was not as pronounced as
178 the difference recorded over the same period by the sondes. This discrepancy was attributed to
179 the fact that sondes were located 1-2 m from the surface, and thus more influenced by vertical
180 stratification of the water column than the trap sites, which were all in the top 0.5 m of the water
181 column.

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183

184 **Fish husbandry**

185 Fish were held at the GBNERR for up to 2 days and then transported to the University of New
186 Orleans in aerated field-collected water. All individuals were treated prophylactically for
187 external parasites using API General Cure (www.apifishcare.com) according to the
188 manufacturer's instructions within 1 week of collection. Fish were maintained for at least 6
189 weeks in 38 l aquaria containing aerated, filtered, dechlorinated tap water adjusted to salinity ≈
190 10 using Instant Ocean Synthetic Sea Salt (www.instantocean.com). The photoperiod was 12:12
191 (light:dark) and temperature was approximately 25°C. During the initial 6-week period, fish were
192 fed twice daily to satiation with Tetramarine Large Saltwater Flakes (www.tetra-fish.com).
193 Thereafter, fish were fed 1-1.5% of the total fish mass once per day, except for the days prior to
194 and including respirometry (see below). After 6 weeks, fish were tagged with passive integrated
195 transponder (PIT) tags according to Reemeyer et al. (2019). Fish were maintained for a minimum
196 of 1 week after tagging before experiments. All fish maintenance and experimental procedures
197 were approved by the University of New Orleans Institutional Animal Care and Use Committee
198 (protocol no. 18-006).

199

200 **Acclimation regimes and morphological measurements**

201 One week prior to the experiment, two groups of 30 fish were moved into one of two 100 l tanks
202 and allowed to adjust to the new tanks. Fish were selected to achieve roughly equal numbers of
203 males and females from both BH and BC, and they ranged in size from 2 to 6 g. The experiment
204 consisted of serially acclimating fish over a period of approximately 7 months across a range of
205 salinity, temperature, and DO similar to those determined at the time of collection. Each
206 exposure interval lasted 4 weeks and followed the order (1) control conditions (T = 25°C,
207 salinity = 10, DO > 85% a.s., 6.6 mg l⁻¹, 17.5 kPa), (2) low salinity (salinity = 1), (3) control
208 conditions, (4) high temperature (T = 32°C), (5) control conditions, (6) low oxygen (DO = 30%
209 a.s., 2.3 mg l⁻¹, 6.2 kPa), and (7) control conditions. For each interval, water was adjusted to the
210 desired conditions on day 1 as described below. Fish were then held under these conditions for
211 14 d, respirometry was conducted over the next 12 d, and fish recovered 1 d prior to the next
212 change in conditions. Over the 12-d respirometry period, batches of 4 fish were randomly
213 selected for measurement (see below). Consequently, the acclimation period prior to
214 respirometry ranged from 15 to 26 d. Importantly, the results reported below include the effects

215 of acclimation as well as measurement under the same conditions. Water quality in the two
216 acclimation tanks did not differ, and the mean and range of temperature, salinity, and DO during
217 each interval are shown in Table S2.

218 For the low salinity acclimation, salinity was lowered from 10 to 1 over the course of 9 h
219 by gradual replacement of tank water with dechlorinated tap water. At the end of the low salinity
220 treatment, salinity was raised over the course of 9 h by adding artificial sea salt to achieve a final
221 salinity of 10. For the high temperature acclimation, temperature was increased from 25°C to
222 32°C over the course of 7 h at a rate of 1°C per h using a digital heater controller connected to a
223 titanium aquarium heater (www.Finnex.com). Temperature was maintained using the same
224 controller and heater over the course of the experiment. At the end of the high temperature
225 exposure, water temperature was lowered by turning down the heater and replacing part of the
226 tank water with water at 25°C. For the low oxygen acclimation, DO was lowered at
227 approximately 10% a.s. per h over 7 h by gassing tank water with nitrogen. DO was continuously
228 monitored by a galvanic oxygen sensor (www.atlas-scientific.com) connected to a raspberry pi
229 computer (www.raspberrypi.org). The computer was programmed to take input from the oxygen
230 sensor once per min and control the introduction of nitrogen from a gas cylinder via a solenoid
231 valve to achieve the desired DO level. At the end of hypoxic acclimation, nitrogen introduction
232 was halted, and water was aerated with aquarium air pumps to achieve an increase of
233 approximately 10% a.s. per h until DO exceeded 85% a.s.

234 At the conclusion of each interval, and before beginning the next exposure, all fish were
235 lightly anaesthetized in dechlorinated, salinity-adjusted water with 0.1 g l⁻¹ MS-222, gently
236 blotted, measured for mass (M) and standard length (SL). Fulton's (1904) condition factor (K)
237 was calculated as M SL⁻³. Daily specific growth rate (SGR) was calculated as 100 (e^G – 1) where
238 G = (ln(M₂) – ln(M₁))(t₂ – t₁)⁻¹, where M₁ and M₂ are masses determined at two times, t₁ and t₂
239 (Stierhoff et al., 2003). Over the course of the 7-month experiment, fish increased in M and SL
240 (Table S3). In general, SGR was low (0.03 – 0.07% body mass d⁻¹) but increased during
241 acclimation to 32°C (0.47 ± 0.20% d⁻¹) and the subsequent control interval (0.23 ± 0.18% d⁻¹).
242 Condition factor (K) remained relatively constant, and fish appeared to be in good health
243 throughout the experiment.

244

245

246 **Respirometry**

247 Intermittent-flow respirometry was used to measure oxygen consumption rates (M_{O_2}) as
248 described by Svendsen et al. (2016) and Reemeyer et al. (2019). The respirometry system
249 consisted of four respirometers, each having a cylindrical glass chamber of either 118 ml or 245
250 ml, chosen to maintain a ratio of chamber volume to fish mass between 20 and 50 (Svendsen et
251 al., 2016). Each chamber was fitted with two sets of tubing. One set of tubing formed a loop with
252 a water pump that continuously circulated water from the chamber past an optical oxygen sensor
253 and back to the chamber. The oxygen sensor was connected to a Witrox-4 oxygen meter and the
254 oxygen saturation was measured once per second using AutoResp software (Loligo Systems;
255 www.loligosystems.com). The second set of tubing was connected to a second pump that
256 intermittently flushed the chamber with water from a surrounding reservoir (ca. 10 l). Water was
257 shared among the four reservoirs (one for each respirometer) and was the same salinity,
258 temperature, and oxygen level as the acclimation condition. This water was continuously
259 circulated through a UV-sterilizer and heat exchanger, which, along with small aquarium heaters
260 in each reservoir, maintained water temperature within 0.1°C of the target temperature (either 25
261 or 32°C). The flushing water pumps and heaters were connected to a DAQ-M relay system
262 (Loligo Systems; www.loligosystems.com) and controlled by AutoResp software. During
263 measurements at low oxygen, the DO in reservoir water was controlled with an apparatus
264 identical to the one controlling DO during low oxygen acclimation.

265 Fish were fasted 24 h prior to respirometry. For each trial, MMR, SMR, and P_{crit} were
266 determined sequentially over approximately 20 h. Between 15:00-16:00 fish were weighed (to
267 the nearest 0.01 g) and placed into a circular arena (diameter = 55 cm) filled with approximately
268 8 l of water and chased by hand for 3 min to induce exhaustion (see pilot studies below).
269 Immediately following the chase protocol fish were placed into the respirometer, and a cycle of
270 60 s flush, 30 s wait, and 120 s M_{O_2} measurement was started. After 1 h, the cycle was adjusted
271 to 300 s flush, 60 s wait, and 240 s M_{O_2} measurement, which was continued for approximately
272 17 h. Throughout the combined ~18 h period, P_{O_2} was maintained at > 80% a.s., except for
273 measurements during acclimation to low oxygen, when it was ~30% a.s.. At 10:00 the following
274 morning, the flush pumps were turned off, thereby creating a closed system (respirometry
275 chamber, tubing, recirculating pump, and oxygen sensor), after which the P_{O_2} declined due to
276 M_{O_2} by the fish. During this closed period, M_{O_2} was measured over consecutive 60 s intervals

277 until there were at least five M_{O_2} measurements below that individual's SMR. The closed period
278 generally lasted about 60 min, after which the flush pumps were turned on to reoxygenate the
279 chambers. All fish were given at least 10 min to recover, after which they were returned to their
280 holding tank.

281 Background microbial respiration in each chamber of the respirometry system was
282 measured before and after each trial using the following settings: 300 s flush, 60 s wait, and 1200
283 s M_{O_2} measurement. Two M_{O_2} measurements immediately before each trial were averaged, and
284 two M_{O_2} measurements immediately after each trial were averaged. Then, a time-corrected value
285 for background respiration was subtracted from fish M_{O_2} , assuming a linear increase in microbial
286 respiration over the duration of the measurement period (Reemeyer et al., 2019; Rosewarne et
287 al., 2016). When microbial respiration exceeded $0.1 \mu\text{mol min}^{-1}$, or about 25% of the mean SMR
288 value, the entire respirometry system was drained and sanitized with dilute bleach. This
289 corresponded to two trials under all acclimation conditions except for high temperature where
290 microbial respiration increased more quickly and the respirometry system was sanitized after
291 every trial. The oxygen sensors were calibrated every 2 weeks using vigorously aerated water
292 (100% a.s.) and water deoxygenated by the addition of sodium sulfite (0% a.s.) at the salinity and
293 temperature of the given experimental interval.

294 Pilot studies validated the method to determine MMR. No significant difference in MMR
295 was found between chasing fish for a 3 min period (above) compared to a chase of 5 min or until
296 the fish stopped responding to a tail pinch (Brennan et al., 2016; Healy and Schulte, 2012). In
297 addition, MMR was not higher if fish were held in air for 60 s after the chase protocol, a
298 treatment shown to yield higher MMR in other species (Norin and Clark, 2016; Roche et al.,
299 2013). Furthermore, MMR was taken as the single highest M_{O_2} estimate during the entire
300 respirometry trial. Typically, this is assumed to occur within the first few minutes of the chase
301 protocol (Clark et al., 2013). This was true for many trials in the present study; however, in
302 numerous trials, the highest M_{O_2} occurred several hours after the chase protocol, frequently
303 coinciding with the room lights turning off (20:00) or on (08:00). In the present study (including
304 over 300 respirometry trials), the median time to MMR was 4 h after chasing, and the M_{O_2}
305 measured immediately after chasing would have seriously underestimated MMR.

306 SMR was calculated as the 20% quantile of 60 M_{O_2} measurements made during the dark
307 phase of the photoperiod (between 20:00-06:00). This method has been advocated by others

308 (Chabot et al., 2016) and produces reliable estimates of SMR for *F. grandis* (Reemeyer and
309 Rees, 2019). Absolute aerobic scope (AAS) was calculated as the difference between MMR and
310 SMR. P_{crit} was determined during the period of closed respirometry (10:00 – 11:00). Linear
311 regression was fit to values of M_{O_2} after it dropped below and remained below SMR. Based upon
312 this relationship, P_{crit} was determined as the oxygen level at which M_{O_2} equalled that fish's SMR,
313 determined in the immediately preceding overnight intermittent-flow respirometry trial
314 (Claireaux and Chabot, 2016; Reemeyer and Rees, 2019).

315

316 **Statistical analyses**

317 To address the goals of this study, it was important to measure aerobic metabolism during the
318 maximum number experimental intervals. Out of a total of 60 fish used here, 36 were measured
319 at all experimental intervals and an additional 7 fish were measured in 6 of the 7 intervals.
320 Hence, data from these 43 fish were included in these analyses. All statistical calculations were
321 performed in R v3.3.3 (R Core Team, 2017).

322 Univariate linear mixed models (LMMs) were fit using the lme4 package in R (Bates et
323 al., 2015). Response variables (SMR, MMR, AAS, and P_{crit}) were \log_{10} -transformed and then z-
324 transformed to a mean of 0 and standard deviation of 1. Models included salinity, temperature,
325 DO, interval number, sex, collection site, and \log_{10} mass as fixed factors, and individual ID as a
326 random (intercept) factor. Acclimation treatments (salinity, temperature, and DO) were included
327 as categorical variables, whereas interval number was included as a continuous variable to
328 account for any time-dependent change in response variables over the duration of the experiment
329 (Biro and Stamps, 2015). Initially, all factors and two-way interactions were included in the
330 models and then removed in a stepwise fashion when doing so improved model fit [judged by a
331 decrease in the Akaike information criterion (AIC) greater than 2]. Based on this criterion, all
332 interaction terms were removed, and the minimum adequate model and AIC are presented for
333 each response variable.

334 Pearson's correlation coefficient, r , was used to compare mass-corrected residuals of
335 SMR, MMR, AAS, and P_{crit} between all possible pairs of intervals. Adjusted repeatabilities (R_{adj})
336 were determined to estimate the repeatability of metabolic traits over the entire experiment
337 (Stoffel et al., 2017). This approach uses an LMM framework that includes the effects of fixed

338 factors, uses parametric bootstrapping to determine confidence intervals, and calculates statistical
339 significance by likelihood ratio tests. Bootstrapping of 10,000 simulations was used in this study.

340 Phenotypic correlations (r_p) were calculated and partitioned into among-individual (r_{ind})
341 and within-individual (r_e) correlations as outlined in Roche et al. (2016) and Houslay and Wilson
342 (2017). Briefly, \log_{10} , z-transformed response variables were fit with bivariate mixed models
343 using the MCMCglmm package in R (Hadfield 2010) with mass, salinity, temperature, DO, and
344 interval as fixed factors, and individual as a random factor. The settings for the model fitting
345 were: nitt = 390,000, burnin = 9000, and thin = 100. The covariance coefficients were then
346 extracted from the models and used to calculate r_p , r_{ind} , and r_e using equations adapted from
347 Dingemanse et al. (2012) as outlined in Careau and Wilson (2017). The highest posterior
348 distribution (HPD) interval was calculated for each estimate as a measure of credibility,
349 analogous to the 95% confidence interval used in frequentist statistics.

350 Data from this study will be made freely available on figshare.com upon acceptance of
351 this manuscript.

352

353 RESULTS

354 Mass effects on aerobic metabolism

355 SMR, MMR, and AAS were positively related to body mass at all control and acclimation
356 intervals (Table 1; Figs. S2-S5). When expressed as the relationship, $M_{O_2} = aM^b$, values for the
357 scaling coefficient, b, ranged from 0.73 to 1.03 for SMR, from 0.98 to 1.34 for MMR, and from
358 0.98 to 1.49 for AAS. On the other hand, P_{crit} was negatively related to mass (Table 1; Fig. S5),
359 with scaling coefficients from -0.18 to -0.41.

360

361 Acclimation effects on aerobic metabolism

362 SMR, MMR, AAS, and P_{crit} were determined for *F. grandis* after acclimation to low salinity,
363 high temperature, or low oxygen. Because these metabolic traits are affected by body mass, and
364 because body mass increased over the course of the experiment (Table S3), mass-adjusted values
365 for each variable were determined from log-log relationships with body mass and presented for a
366 fish of average mass (4.39 g) for visualization purposes (Table 2; Fig. 2). Linear mixed models
367 assessed the effects of fixed factors (sex, collection site, acclimation condition, and experimental

368 interval), while accounting for body mass (\log_{10} transformed) and individual (as a random factor)
369 (Table 3).

370 SMR of *F. grandis* was not affected by acclimation to low salinity, but it was
371 significantly elevated after acclimation to high temperature or low oxygen (Fig. 2A, Tables 2, 3).
372 At 32°C, SMR was 33% higher than the average value recorded under control conditions (25°C),
373 while at 30% a.s., SMR was about 14% higher than normoxic controls (Table 2). MMR was
374 similarly unaffected by acclimation to low salinity and increased significantly after acclimation
375 to high temperature (by 15% over control conditions); however, unlike SMR, MMR was
376 dramatically suppressed (by 37%) at low oxygen (Fig. 2B, Tables 2, 3). Changes in AAS,
377 mirrored those of MMR, being unaffected by acclimation to low salinity, increasing after
378 acclimation to high temperature (but only by 8%), and decreasing after acclimation to low
379 oxygen (by 55%) (Fig. 2C, Tables 2, 3). Changes in P_{crit} were generally small (Fig. 2D, Table 2)
380 but significant (Table 3). P_{crit} was modestly elevated after acclimation to low salinity or high
381 temperature, while it decreased after acclimation to low oxygen. The effects of high temperature
382 and low oxygen on P_{crit} appeared to persist through the control interval that followed the
383 respective acclimation period (Fig. 2D).

384

385 **Lack of collection site, sex, and interval effects**

386 Although the two collection sites differed in DO profiles, both annually (Fig. 1) and at the time
387 of fish collection (Table S1), collection site failed to explain significant variation in any of the
388 measured traits related to aerobic metabolism. Moreover, the interaction between collection site
389 and low oxygen treatment did not explain significant variation in any variable, indicating that
390 fish from these two sites responded similarly to low oxygen acclimation. Additionally, there was
391 no difference between sexes for any trait related to aerobic metabolism measured here. Finally,
392 experimental interval did not explain significant variation in any variable, demonstrating that
393 there was no temporal effect on traits related to aerobic metabolism over the 7-month laboratory
394 experiment.

395

396 **Repeatability of aerobic metabolism**

397 Pearson's product moment correlation coefficients (r) were calculated to compare each response
398 variable measured among all individuals between all possible pairs of intervals. In general,

399 values of r were positive, but varied in magnitude and statistical significance among metabolic
400 traits (Table S5). Thirteen of 21 pairwise comparisons between trials of SMR were significant;
401 16 were significant for MMR; and 11 were significant for AAS. Only three out of 15
402 comparisons were significant for P_{crit} . There were no obvious patterns indicating that certain
403 acclimation conditions were more or less likely to be correlated with each other or with control
404 intervals.

405 The adjusted repeatability (R_{adj}) evaluates trait consistency across the entire experiment,
406 rather than between pairs of intervals, and accounts for main effects on trait values. To determine
407 whether acclimation influenced the repeatability of the metabolic traits in question, R_{adj} was
408 calculated two ways: first, R_{adj} was determined using data collected only during the control
409 intervals, and second, R_{adj} was calculated over all intervals including acclimation treatments
410 (Table 4). For control conditions, R_{adj} varied from 0.11 for P_{crit} to 0.36 for MMR. When
411 including acclimation intervals, R_{adj} varied from 0.16 for P_{crit} to 0.37 for SMR. All values of R_{adj}
412 were significantly different from zero, indicating that all traits were repeatable over the 7-month
413 experiment, although there was a trend of lower R_{adj} for P_{crit} compared to the other variables.
414 This result is consistent with the lower number of significant pairwise correlations for P_{crit} than
415 for SMR, MMR, and AAS (Table S5). Moreover, values of R_{adj} were not materially affected
416 when acclimation intervals were included in its calculation, suggesting that the repeatability of
417 these traits is not influenced by acclimation to different conditions.

418

419 **Phenotypic correlations between aerobic metabolic traits**

420 Phenotypic correlations between pairs of metabolic variables were determined and partitioned
421 into among-individual and within-individual correlations (Table 5, Fig. 3). There was a positive
422 phenotypic correlation between SMR and MMR. This correlation arose from significant among-
423 individual and within-individual correlations. Likewise, there was a positive phenotypic
424 correlation between MMR and AAS, that was attributed to significant among-individual and
425 within-individual correlations. On the other hand, there was no correlation between SMR and
426 AAS at any level. The relationship of AAS with MMR, but not SMR, shows that the major factor
427 determining AAS is MMR. There was a positive phenotypic correlation between SMR and P_{crit} .
428 While the magnitudes of the among-individual and within-individual correlations were similar,
429 only the within-individual correlation was significant. Thus, for a given individual at a given

430 time point, when SMR was high, so was P_{crit} , and *vice versa*. This stands to reason because the
431 determination of P_{crit} depends upon SMR (see Materials and Methods). Finally, there was a
432 negative phenotypic correlation between AAS and P_{crit} , which was attributed to a significant,
433 negative within-individual correlation. Thus, in a trial when a given individual had a high P_{crit} , it
434 had a relatively low AAS.

435

436 DISCUSSION

437 Mass effects on aerobic metabolism

438 In this study, all metabolic variables were significantly influenced by body mass. For SMR, the
439 scaling coefficients were similar to those found previously in this species ($b=0.79$; Reemeyer et
440 al., 2019) and other teleosts (reviewed in Jerde et al., 2019). In a recent meta-analysis of the
441 relationship between SMR and body mass in fishes, Jerde et al (2019) provided strong evidence
442 of an intra-specific mass scaling exponent near 0.89, aligning closely with the values reported
443 here (Table 1). In the present study, the scaling coefficients for MMR and AAS were slightly
444 higher than those calculated for SMR (Table 1), supporting the suggestion that MMR often
445 scales isometrically with mass (reviewed in Glazier, 2009). On the other hand, the relationship
446 between body mass and P_{crit} was negative. If P_{crit} is an index of hypoxia tolerance (Speers-Roach
447 et al., 2013; Rogers et al., 2016; Regan et al., 2019; Wood, 2018), this result suggests that larger
448 individuals are more tolerant of hypoxia than smaller individuals. Over a 3-fold range of body
449 masses, and using an average value of $b = -0.32$, the largest individual would have a P_{crit} 30%
450 lower than the smallest fish. In other words, the SMR of the larger fish would not be limited until
451 oxygen dropped to values considerably lower than those that limit SMR of the smaller fish. A
452 previous study in *F. grandis* found a similar result (Everett and Crawford, 2009), however other
453 studies have found no effect of body mass on P_{crit} in *F. grandis* (Virani and Rees, 2000) or in *F.*
454 *heteroclitus* (Borowiec et al., 2015; McBryan et al., 2016). Among other fishes, the relationship
455 between P_{crit} and body mass is extremely variable, ranging from being positively related (Pan et
456 al., 2016), to unrelated (Nilsson and Östlund-Nilsson, 2008; Timmerman and Chapman, 2004;
457 Verheyen et al., 1994), to negatively related (Sloman et al., 2006; Perna and Fernandes, 1996;
458 current study). While this diversity in scaling of P_{crit} may reflect real differences among species,
459 it might also arise from different experimental and analytical methods used to determine P_{crit} ,

460 highlighting the need for standardization in this area (Reemeyer and Rees, 2019; Regan et al.,
461 2019; Wood, 2018).

462

463 **Acclimation effects on aerobic metabolism**

464 Acclimation of *F. grandis* to low salinity brought about a small, but significant, increase in P_{crit} ,
465 consistent with predictions based upon the osmoregulatory compromise (Sardella and Brauner,
466 2007). Giacomin et al. (2019) recently found higher P_{crit} , lower gill surface area, and larger
467 interlamellar cell masses in *F. heteroclitus* acclimated to freshwater (0 salinity) versus 11 and 35
468 salinity. Acclimation of *F. grandis* to low salinity, however, did not alter SMR, MMR, or AAS. In
469 contrast, acclimation of *F. heteroclitus* to fresh water (0.3 salinity) significantly decreased
470 factorial aerobic scope (FAS), calculated as MMR divided by routine metabolic rate (RMR)
471 (Brennan et al., 2016). This effect was due to a trend toward lower in MMR of *F. heteroclitus* at
472 low salinity ($p = 0.06$). This difference in response to low salinity between closely related
473 species might be due to the use of FAS by Brennan et al. (2016) compared to AAS here. While
474 both have their merits, AAS reflects the actual energy available to the organism to support
475 activities beyond maintenance and, as such, has been argued to be more ecologically relevant
476 (Clark et al., 2013). Results of the present study suggest that low salinity may hinder the ability
477 to extract oxygen as DO drops (i.e., increase P_{crit}), without altering the rate of oxygen uptake
478 when oxygen is plentiful (SMR, MMR, and AAS were all determined at normoxia).

479 Acclimation of *F. grandis* to elevated temperature resulted in significant increases in all
480 aerobic metabolic variables measured here. Based upon average values determined under control
481 conditions (25°C), the increases in SMR, MMR, and AAS at 32°C correspond to Q_{10} values of
482 1.5, 1.2, and 1.1, respectively. While these values are lower than the range of 2 to 3 generally
483 seen for aerobic metabolism of other fishes (Clarke and Johnston, 1999), they are consistent with
484 the low temperature sensitivity of aerobic metabolism reported for *F. heteroclitus* acclimated to
485 similar temperatures (Targett, 1978; Healy and Schulte, 2012). Specifically, Healy and Schulte
486 (2012) showed that (RMR), MMR, and AAS sharply increased with an increase in acclimation
487 temperature from 5 to 25°C, but then plateaued or decreased at higher acclimation temperatures
488 (30 and 33°C). Taken together, these observations suggest that aerobic metabolism in these
489 species is only moderately affected by temperature over the range studied here: The “oxygen and
490 capacity limited thermal tolerance” theory suggests that MMR is more limited by temperature

491 than SMR, leading to a diminution of AAS (Pörtner 2010). The above Q_{10} values suggest that
492 SMR is more temperature-dependent than MMR, although this comparison belies the fact that
493 the absolute increase in MMR was approximately twice that of SMR. In absolute terms, AAS
494 increased at 32°C, albeit with a low Q_{10} . Across this temperature range, therefore, aerobic
495 metabolism by *F. grandis* does not appear to be limited. This conclusion is supported by the
496 observation that growth rates were highest during acclimation to high temperature (Table S3), as
497 well as with observations made in the field, where these fish were active at temperatures at or
498 above 32°C.

499 P_{crit} values were also higher following acclimation of *F. grandis* to high temperature,
500 suggesting that fish may be less tolerant to low oxygen as temperatures increase. Previous work
501 showed that acute warming from of *F. heteroclitus* from 15 to 30°C led to a decrease in hypoxia
502 tolerance when measured as time to loss of equilibrium (LOE) during exposure to severe hypoxia
503 (2% a.s.; McBryan et al., 2016). Interestingly, acclimation to warm temperature partially
504 reversed the negative effects of acute warming on LOE, a response that was correlated with an
505 increase in gill surface area in warm-acclimated fish. Despite differences in experimental design
506 (acclimation vs. acute exposure, P_{crit} vs. LOE), the current study and McBryan et al. (2016) both
507 point to a decrease in hypoxia tolerance of *F. grandis* and *F. heteroclitus*, respectively, at higher
508 temperatures. These results are consistent with observations and theoretical arguments made for
509 other ectothermic species (reviewed in McBryan et al., 2013).

510 Acclimation of *F. grandis* to low oxygen led to decreases in MMR and AAS. This was
511 expected due to the restriction of MMR, and hence AAS, at low DO, even at levels above P_{crit}
512 (Richards, 2009; Rogers et al., 2016). Moreover, P_{crit} was also lowered, which supports previous
513 work in *F. heteroclitus* (Borowiec et al., 2015) and in other fishes (reviewed in Rogers et al.,
514 2016). This indicates that acclimation to low oxygen increases hypoxia tolerance, presumably
515 through a variety of morphological, physiological, and biochemical adjustments (Richards, 2009;
516 Sollid et al., 2003). Surprisingly, acclimation to hypoxia resulted in a slight increase in SMR.
517 This result contrasts with Borowiec et al. (2015), who showed that acclimation of *F. heteroclitus*
518 for 28 d to ~24 % a.s. did not affect RMR measured in normoxia. The current observation of
519 higher SMR under hypoxia might be explained, in part, by an experimental design in which
520 MMR was measured prior to overnight determination of SMR. To induce MMR, fish were
521 chased to exhaustion, a protocol that elicits anaerobic metabolism and lactate accumulation

522 (Rees et al., 2009). Lactate clearance after exercise, either by oxidation or gluconeogenesis,
523 results in an increase in oxygen-consumption, the well-described “excess post-exercise oxygen
524 consumption” (Hill and Lupton, 1923; Scarabello et al., 1991; Wood, 1991). After a similar
525 exercise protocol, blood lactate decreased to control values within 3 h under normoxia (Rees et
526 al., 2009). This decrease would have occurred prior to the beginning of SMR measurements in
527 normoxic exposures. Under hypoxia, though, EPOC probably lasts longer (Svendsen et al.,
528 2012), and may have contributed to an elevation of SMR. An increased duration of increased
529 oxygen consumption, after either exercise or the ingestion of food (specific dynamic action,
530 Jobling, 1981; Chabot et al., 2016), could have important ecological implications, because it
531 would contribute to a decrease in AAS and the energy available to perform other tasks. Indeed,
532 AAS at low oxygen was dramatically reduced in the current study, being less than half the value
533 measured during normoxic controls.

534

535 **Lack of collection site and sex effects on aerobic metabolism**

536 Fish in this study were sampled from two sites within the GBNERR that differed in seasonal DO
537 profiles, where one site (BH) experiences a higher frequency of hypoxia than the other (BC). It
538 was hypothesized that due to these differences in DO, fish from BH may show fixed
539 developmental or evolved differences in these metabolic variables (e.g. lower SMR and P_{crit}) or
540 the degree to which these variables responded to low oxygen acclimation. Previous work in *F.*
541 *grandis* suggested that populations differed in M_{O_2} at severe hypoxia (ca. 9% a.s.) but found no
542 differences under normoxia nor differences in P_{crit} (Everett and Crawford, 2009). In sailfin
543 mollies (*Poecilia latipinna*), a species in the same order (Cyprinodontiformes) and occurring in
544 similar habitats as *F. grandis*, fish from a periodically hypoxic salt marsh have significantly
545 lower P_{crit} and higher gill surface area than fish from a normoxic river site (Timmerman and
546 Chapman, 2004). In the present study none of the response variables differed by collection site,
547 nor did fish from the two sites differ in their response to acclimation low oxygen. There are at
548 least three reasons why site-dependent differences were not observed in the present study. First,
549 it is possible that there is no significant genetic differentiation between collection sites. These
550 sites were separated by about 10 km, a distance much greater than the expected home range of
551 this species (Nelson et al., 2014), but substantially less than that in studies where population
552 differences were found [up to 650 km in Everett and Crawford (2009) and 78 km in Timmerman

553 and Chapman (2004)]. Although migration of individual fish between sites probably does not
554 occur, low rates of gene flow over several generations may be sufficient to overwhelm selection
555 to local conditions (Slatkin, 1987). Second, it is possible that fish behaviourally avoid hypoxia
556 and exploit microenvironments that have higher DO than those reflected in long-term data and
557 point samples. For example, several species, including *F. grandis*, use aquatic surface respiration
558 to improve oxygen uptake at low DO by ventilating their gills with surface waters that have
559 higher levels of oxygen due to diffusion from the atmosphere (McKenzie and Chapman, 2009;
560 Love and Rees, 2001; Rees and Matute, 2018). Third, the DO level chosen for acclimation might
561 not have been low enough to elicit site-dependent differences. The DO level during acclimation
562 (30% a.s.) was based upon data from field sites; however, this is above the P_{crit} of this species
563 (Virani and Rees, 2000; present study). Acclimation to more severe hypoxia would be expected
564 to recruit additional behavioural and physiological responses that might reveal site-dependent
565 differences.

566 No metabolic trait measured here differed between male and female fish. This lack of sex
567 effects contrasts the view that sex differences in reproductive investment and behaviour lead to
568 differences in energy expenditure (Biro and Stamps, 2010). The lack of sex effects in this study
569 might be attributed to the age of the fish used, which were probably young-of-the-year (Greeley
570 and McGregor, 1983) and not reproductively active at the time of capture. In addition, fish in the
571 current study were held at relatively high densities (~60 fish per m^3), which have been shown to
572 reduce egg production in *F. grandis* (Chesser et al., 2019). On the other hand, over the course of
573 laboratory maintenance, fish grew significantly and developed dimorphic coloration typical for
574 sexually mature individuals of this species. Moreover, previous research on larger individuals of
575 this species found no difference in SMR among males and females (Reemeyer et al., 2019).
576 Thus, sex effects on aerobic metabolism in this species, if any, are small in magnitude.

577

578 **Repeatability of aerobic metabolism**

579 All variables measured exhibited moderate repeatability over the course of the experiment.
580 Values of R_{adj} ranged from 0.27 – 0.37 for SMR, MMR, and AAS, and fall within the range of
581 values reported for aerobic metabolism (Nespolo and Franco, 2007; Norin and Malte, 2011;
582 White et al., 2013). R_{adj} for P_{crit} was lower (0.11 – 0.16), suggesting that within-individual
583 variation relative to among-individual variation was greater for this metric than for SMR, MMR,

584 and AAS. Importantly, R_{adj} values reported here were determined over 7 months. Previous
585 measurements of the repeatability of M_{O_2} by *F. grandis* determined over shorter intervals have
586 produced higher estimates of repeatability. Reemeyer et al. (2019) reported Pearson's r ranging
587 from 0.35 – 0.76 and an R_{adj} of 0.56 for SMR of *F. grandis* measured five times over six weeks.
588 Virani and Rees (2000) reported a similarly high Pearson's r for RMR (0.68) when measured
589 twice within 6 weeks. Previous measurements of P_{crit} in *F. grandis* measured twice over 2 weeks
590 resulted in a Pearson's r of 0.74 (Reemeyer and Rees, 2019). The lower R_{adj} estimates found in
591 the present study support the trend that repeatability of metabolic variables decreases over time
592 as reported for other species (Norin and Malte, 2011; White et al., 2013). Nevertheless, the
593 values R_{adj} reported here were all statistically greater than zero and suggest some degree of
594 consistency among individuals with respect to aerobic metabolism.

595 While acclimation to altered salinity, temperature, and oxygen had significant effects on
596 the magnitude of all metabolic variables measured here, R_{adj} estimates were virtually identical
597 when determined on only control intervals and when calculated over the entire experiment,
598 including acclimation intervals. Furthermore, pairwise correlations calculated between control
599 intervals were similar to those calculated between control and acclimation intervals (Table S5).
600 These observations suggest that the repeatability of these metabolic traits in *F. grandis* is not
601 context dependent across this range of salinity, temperature, and DO. Auer et al. (2018) assessed
602 the effect of temperature acclimation on repeatability of SMR, MMR, and AAS in juvenile
603 brown trout (*Salmo trutta*) after serial acclimation to 10, 13, and 16°C. Although R_{adj} values
604 were similar to those seen here (0.32 for SMR, 0.43 for MMR, and 0.42 for AAS), the R_{adj} of
605 MMR and AAS, but not SMR, decreased with warming. For MMR and AAS, therefore, Auer et
606 al (2018) provide some support for context-dependency of repeatability. In that study, fish were
607 measured once at each temperature in the same order without a common control treatment
608 between or after temperature acclimation; thus, it is possible that the lower R_{adj} determined at
609 high temperatures were due, in part, by a time-dependent decrease in repeatability.

610

611 **Phenotypic correlations between aerobic metabolic traits**

612 Because SMR, MMR, AAS, and P_{crit} were measured in a given respirometric trial and trials were
613 repeated over time, it was possible to calculate phenotypic correlations between pairs of traits
614 and partitioning these correlations into among-individual and within-individual correlations

615 (Dingemanse and Dotchermann, 2012). The aerobic capacity model predicts a positive
616 correlation between SMR and MMR (Bennett and Ruben, 1979; Hayes and Garland, 1995),
617 however support for this relationship is mixed. A recent meta-analysis confirmed a positive
618 relationship between SMR and MMR when comparing across species but failed to support a
619 relationship between SMR and MMR within species (Auer et al., 2017). Similarly, Killen et al.
620 (2016b) found that among species of teleost fishes, the correlation between RMR and MMR was
621 strongly positive, but within species, the same correlation varied greatly. The present study
622 provides strong evidence for a positive phenotypic correlation between SMR and MMR within a
623 species. Moreover, this correlation can be attributed to significant correlations both among
624 individuals and among repeated measures on the same individual. There was also a positive
625 phenotypic correlation between MMR and AAS, which was due to significant among- and
626 within-individual correlations. On the other hand, SMR and AAS were not correlated. Because
627 AAS is calculated as the difference between MMR and SMR, the strong correlation between
628 MMR and AAS highlights that variation in MMR is quantitatively more important in
629 determining AAS than variation in SMR.

630 There was a positive phenotypic correlation between SMR and P_{crit} . Although the among-
631 and within-individual correlations were of similar magnitude, only the latter was statistically
632 significant. Thus, during a given trial on a given individual, if SMR was elevated, P_{crit} was also
633 elevated. While this result indicates that these variables may be linked by shared phenotypic
634 plasticity, it would also arise from the method of determining P_{crit} , which is directly dependent
635 upon the magnitude of SMR (Reemeyer and Rees, 2019). Finally, there was a significant
636 negative phenotypic correlation between P_{crit} and AAS, which was similarly attributed to a
637 significant within-individual correlation. This would be predicted based upon the ways in which
638 SMR is used to calculate P_{crit} and AAS. A lower SMR generally yields a lower P_{crit} , but a higher
639 AAS (depending upon variation in MMR, of course). Although this relationship can be explained
640 by how these variables are calculated, it might have biological relevance. In particular, this result
641 suggests that when a fish has a low SMR it simultaneously has a greater hypoxia tolerance
642 (lower P_{crit}) and a higher scope for activity (higher AAS).

643 These last relationships, which are driven primarily by within-individual variation,
644 highlight the importance of repeated measures when examining the relationship between traits.
645 Had these metabolic traits been measured only once among a group of individuals, significant

646 correlations could have been attributed to among-individual differences, rather than covariation
647 of these traits within an individual (Careau and Wilson, 2017). That conclusion would lead to
648 experiments examining genetic or developmental factors that produced these correlations rather
649 than an investigation of why those traits covary when measured multiple times for a given
650 individual, perhaps due to shared plasticity or factors related to experimental design.

651

652 **Perspectives**

653 Acclimation of SMR, MMR, AAS, and P_{crit} in *F. grandis* indicate plasticity of aerobic
654 metabolism and may contribute to the broad environmental tolerances of this ecologically
655 dominant estuarine species. It is possible that these tolerances, however, will be exceeded by
656 changes in salinity, temperature, and DO that are more extreme, longer lasting, or
657 contemporaneous. Predictions of future characteristics of estuaries are strongly influenced by
658 local conditions (Wong et al., 2014). In the Southeast U.S.A., increased precipitation will likely
659 decrease salinity, while simultaneously increasing nutrient input, eutrophication, and the
660 incidence of aquatic hypoxia. Average and maximum water temperatures are projected to
661 increase due to climate change (Pörtner et al., 2014). Thus, *F. grandis* will be challenged by
662 increased oxygen demands at the same time as oxygen availability, directly or indirectly due to
663 the osmoregulatory compromise, will limit their ability to meet those demands. Future studies on
664 the effects of simultaneous variation in multiple abiotic factors will help elucidate the limits of
665 resiliency of this and other estuarine organisms.

666

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672

673 **Competing interests**

674 No competing interests declared.

675

676

677 **Author contributions**

678 J.E.R. and B.B.R conceived and designed the methodology. J.E.R collected and analysed the
679 data. J.E.R and B.B.R. interpreted the data. J.E.R drafted the manuscript. B.B.R edited the
680 manuscript. Both authors contributed critically to the drafts and gave final approval for
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682

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935

936 **Table 1:** Scaling relationships for aerobic metabolic traits of *F. grandis* during laboratory acclimation. Coefficients were calculated
 937 for $y = aM^b$, where y is the response variable, M is mass in g, and a and b are constants, shown with 95% confidence intervals in
 938 brackets. r^2 values are for log-log regressions of mass versus the respective response variable.

939

Interval	n	SMR ($\mu\text{mol min}^{-1}$)			MMR ($\mu\text{mol min}^{-1}$)			AAS ($\mu\text{mol min}^{-1}$)			P _{crit} (% as)		
		a	b	r^2	a	b	r^2	a	b	r^2	a	b	r^2
Control 1	41	0.07 [0.05-0.09]	1.03 [0.80-1.25]	0.68**	0.29 [0.24-0.36]	0.99 [0.84-1.14]	0.81**	0.22 [0.17-0.28]	0.98 [0.80-1.16]	0.75**	n.d. ^a	n.d. ^a	n.d.
Low Salinity	40	0.10 [0.08-0.14]	0.73 [0.49-0.97]	0.49**	0.29 [0.21-0.39]	0.98 [0.74-1.21]	0.65**	0.19 [0.13-0.28]	1.06 [0.77-1.34]	0.60**	29.25 [16.57-51.67]	-0.36 [-0.78-0.07]	0.07
Control 2	43	0.08 [0.06-0.11]	0.87 [0.68-1.05]	0.68**	0.26 [0.19-0.34]	1.05 [0.84-1.26]	0.71**	0.18 [0.13-0.25]	1.11 [0.85-1.37]	0.64**	25.06 [16.48-38.12]	-0.38 [-0.69- -0.07]	0.13*
High Temperature	42	0.12 [0.08-0.17]	0.89 [0.64-1.13]	0.57**	0.28 [0.21-0.38]	1.09 [0.89-1.30]	0.74**	0.17 [0.11-0.26]	1.18 [0.90-1.47]	0.63**	24.64 [17.91-33.90]	-0.19 [-0.41-0.03]	0.07
Control 3	43	0.11 [0.08-0.15]	0.77 [0.58-0.97]	0.60**	0.25 [0.18-0.35]	1.09 [0.88-1.30]	0.73**	0.14 [0.09-0.23]	1.23 [0.94-1.52]	0.64**	32.14 [19.70-52.42]	-0.41 [-0.72- -0.09]	0.14*
Low Oxygen	42	0.08 [0.06-0.12]	1.00 [0.75-1.24]	0.63**	0.15 [0.12-0.19]	1.09 [0.95-1.23]	0.86**	0.07 [0.04-0.11]	1.20 [0.92-1.48]	0.65**	17.68 [10.66-29.33]	-0.18 [-0.50-0.14]	0.03
Control 4	43	0.08 [0.05-0.12]	0.96 [0.69-1.21]	0.56**	0.16 [0.11-0.22]	1.34 [1.13-1.56]	0.80**	0.09 [0.06-0.14]	1.49 [1.23-1.75]	0.76**	26.18 [15.55-44.11]	-0.40 [-0.73- -0.08]	0.13*

940

941 ^a P_{crit} was not determined (n.d.) during Control 1.

942 * $P \leq 0.05$

943 ** $P \leq 0.001$

944

945 **Table 2:** Mass-corrected aerobic metabolic traits (mean \pm S.D.) of *F. grandis* during laboratory
946 acclimation to changes in salinity, temperature, and dissolved oxygen. Values of SMR, MMR,
947 AAS, and P_{crit} were determined for a fish of 4.39 g (average ass) based upon log-log relationship
948 of each variable and body mass. The range is shown in parentheses.

Interval	n	SMR ($\mu\text{mol min}^{-1}$)	MMR ($\mu\text{mol min}^{-1}$)	AAS ($\mu\text{mol min}^{-1}$)	P_{crit} (% as)
Control 1	41	0.30 ± 0.05 ($0.22 - 0.40$)	1.21 ± 0.13 ($0.92 - 1.47$)	0.91 ± 0.11 ($0.62 - 1.12$)	n.d. ^a
Low Salinity	40	0.31 ± 0.06 ($0.21 - 0.43$)	1.19 ± 0.20 ($0.73 - 1.75$)	0.88 ± 0.19 ($0.50 - 1.41$)	$18.2 \pm 6.0^*$ ($10.8 - 32.3$)
Control 2	43	0.30 ± 0.04 ($0.23 - 0.40$)	1.19 ± 0.20 ($0.88 - 1.60$)	0.89 ± 0.18 ($0.61 - 1.35$)	15.0 ± 4.6 ($11.1 - 33.3$)
High Temperature	42	$0.42 \pm 0.07^*$ ($0.30 - 0.60$)	$1.40 \pm 0.21^*$ ($1.03 - 1.89$)	$0.97 \pm 0.21^*$ ($0.65 - 1.38$)	$19.0 \pm 3.0^*$ ($13.4 - 26.3$)
Control 3	43	0.35 ± 0.05 ($0.26 - 0.48$)	1.25 ± 0.18 ($0.84 - 1.53$)	0.90 ± 0.18 ($0.50 - 1.18$)	18.3 ± 4.4 ($12.4 - 32.3$)
Low Oxygen	42	$0.36 \pm 0.06^*$ ($0.23 - 0.56$)	$0.76 \pm 0.08^*$ ($0.56 - 0.92$)	$0.40 \pm 0.08^*$ ($0.20 - 0.56$)	$14.4 \pm 4.7^*$ ($9.5 - 36.9$)
Control 4	43	0.31 ± 0.06 ($0.20 - 0.47$)	1.19 ± 0.20 ($0.75 - 1.84$)	0.89 ± 0.19 ($0.45 - 1.54$)	15.0 ± 4.2 ($10.6 - 31.0$)

949

950 ^a P_{crit} was not determined (n.d.) during Control 1.

951 * Variable was significantly affected by acclimation condition (LMM, Table 3).

952

953 **Table 3:** Factors influencing aerobic metabolic variables of *F. grandis* during laboratory
954 acclimation to changes in salinity, temperature, and dissolved oxygen. Univariate LMMs were fit
955 for SMR, MMR, AAS, and P_{crit} with collection site, sex, mass, salinity, temperature, dissolved
956 oxygen, and experimental interval as fixed factors and individual ID as a random (intercept)
957 factor. Response variables and body mass were \log_{10} transformed and z-transformed. The
958 minimum adequate model for each response variable is presented with corresponding AIC
959 (Akaike Information Criterion).

960

Variable	Factor	Estimate	SE	AIC
SMR	Mass	0.830	0.045	443.3
	Temperature	0.937	0.074	
	Dissolved Oxygen	-0.374	0.079	
MMR	Mass	0.780	0.036	320.5
	Temperature	0.446	0.061	
	Dissolved Oxygen	1.414	0.064	
AAS	Mass	0.623	0.037	381.2
	Temperature	0.200	0.007	
	Dissolved Oxygen	1.950	0.072	
P_{crit}	Mass	-0.217	0.072	664.8
	Salinity	-0.467	0.153	
	Temperature	0.687	0.144	
	Dissolved Oxygen	0.436	0.146	

961

962

963 **Table 4:** Adjusted repeatabilities (R_{adj}) of SMR, MMR, AAS, and P_{crit} of *F. grandis* measured
964 during long-term laboratory maintenance. Values were determined for control intervals only, as
965 well as across all intervals including acclimation to changes in salinity, temperature, and
966 dissolved oxygen.

967

Control Intervals All Intervals

Variable	R_{adj}	SE	95% CI	p	R_{adj}	SE	95% CI	p
SMR	0.35	0.09	0.18-0.51	<0.001	0.37	0.07	0.23-0.51	<0.001
MMR	0.36	0.08	0.18-0.51	<0.001	0.35	0.07	0.21-0.48	<0.001
AAS	0.32	0.09	0.14-0.47	<0.001	0.27	0.07	0.14-0.40	<0.001
P_{crit}	0.11	0.06	0.01-0.23	0.007	0.16	0.07	0.05-0.30	<0.001

968

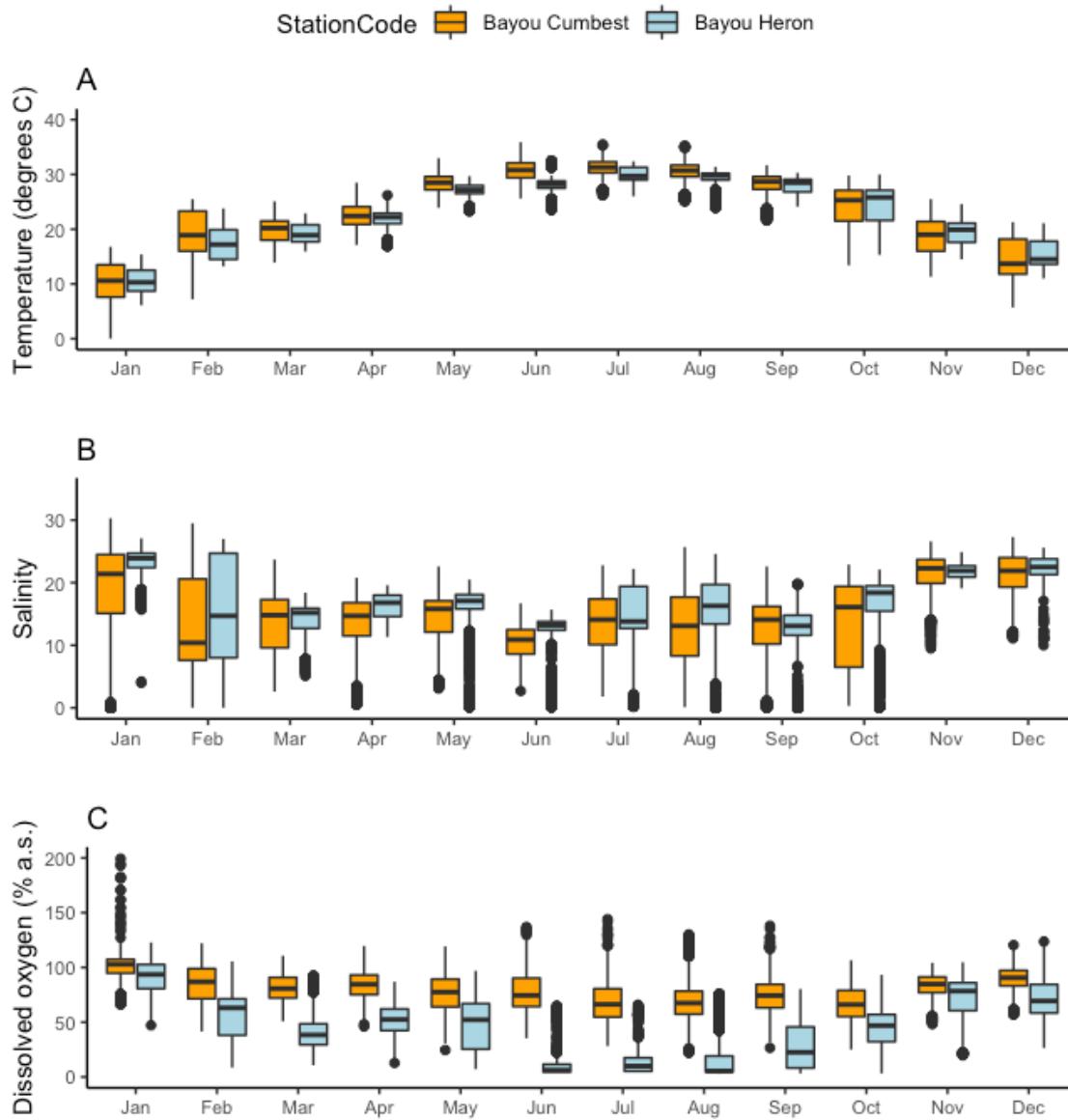
969

970 **Table 5:** Phenotypic (r_p), among-individual (r_{ind}), and within-individual (r_e) correlations between
971 aerobic metabolic traits of *F. grandis*. The highest posterior distribution (HPD) interval was
972 calculated for each estimate as a measure of credibility. When the HPD does not overlap zero,
973 the correlation is significant (bold type).

974

Correlation	r_p	HPD Interval	r_{ind}	HPD Interval	r_e	HPD Interval
SMR vs MMR	0.24	0.17 – 0.32	0.68	0.37 – 0.88	0.28	0.17 – 0.40
SMR vs AAS	-0.04	-0.12 – 0.05	0.14	-0.20 – 0.49	-0.09	-0.20 – 0.04
SMR vs P_{crit}	0.31	0.19 – 0.40	0.32	-0.03 – 0.66	0.36	0.24 – 0.48
MMR vs AAS	0.57	0.52 – 0.66	0.64	0.43 – 0.82	0.87	0.84 – 0.90
MMR vs P_{crit}	0.02	-0.10 – 0.11	-0.13	-0.24 – 0.50	-0.01	-0.17 – 0.11
AAS vs P_{crit}	-0.11	-0.22 – -0.01	0.09	-0.34 – 0.41	-0.20	-0.32 – -0.06

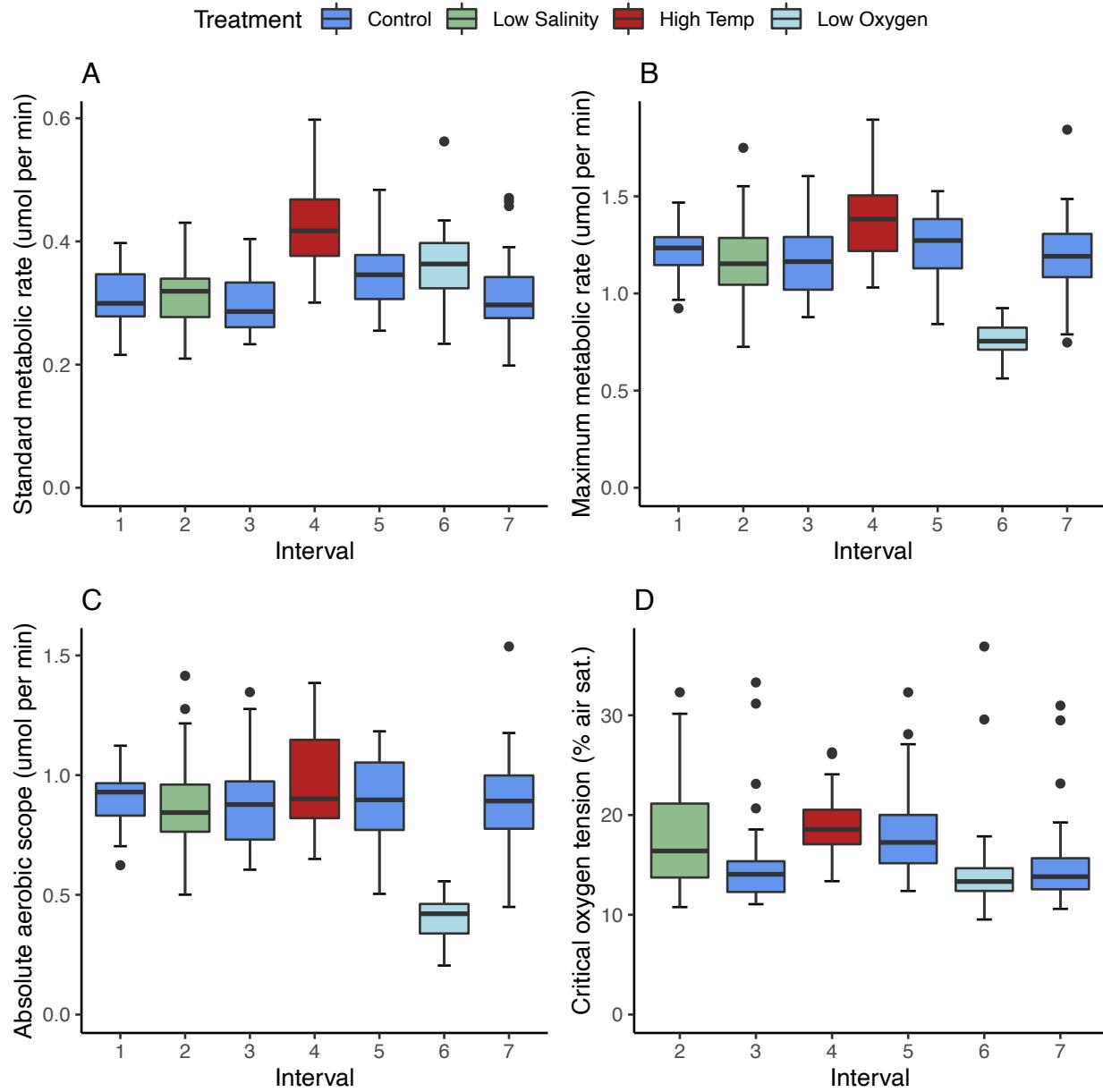
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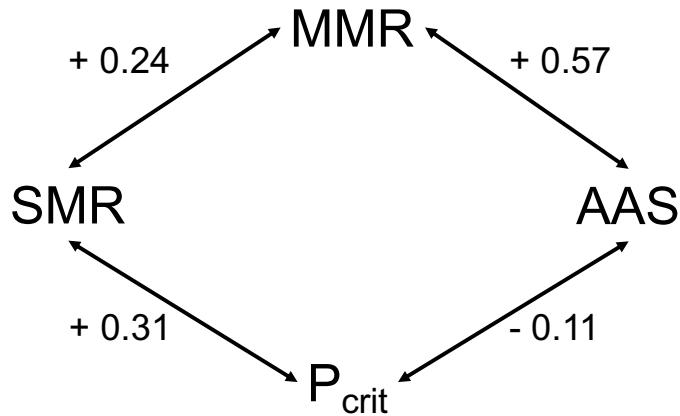
978 **Figure 1:** Annual variation in water temperature (A), salinity (B), and DO (C) at the Bayou
979 Cumbest (orange) and Bayou Heron (blue) monitoring sondes. Data were collected every 15 min
980 and compiled for 1 August 2017 to 31 September 2018. Box and whisker graphs show medians
981 (center line), upper and lower quartiles (box), and total data range (whiskers) after removing
982 outliers (black dots).

983



984

985 **Figure 2:** Aerobic metabolic traits of *F. grandis* during laboratory acclimation to low salinity
 986 (green), high temperature (red), and low oxygen (light blue). Measurements were made under
 987 control conditions (blue) before and after every acclimation interval. SMR (A); MMR (B); AAS
 988 (C); P_{crit} (D). All variables have been standardized for a fish of 4.39 g (the overall average mass).
 989 Box and whisker graphs show medians (centre line), upper and lower quartiles (box), and total
 990 data range (whiskers) after removing outliers (black dots).



991

992 **Figure 3:** Phenotypic correlations (r_p) between aerobic metabolic traits (SMR, MMR, AAS, and
993 P_{crit}) in *F. grandis*. For among-individual (r_{ind}), and within-individual (r_e) correlations and
994 credibility statistics, see Table 5.