1   Positive feedback promotes terrestrial emergence behaviour in an amphibious fish

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Abstract

Major ecological transitions such as the invasion of land by aquatic vertebrates have been hypothesised to be facilitated by positive feedback between habitat choice and phenotypic plasticity. We tested whether aquatic hypoxia, emergence behaviour, and plastic changes in gill surface area could create such a positive feedback loop and drive an amphibious fish to spend increasing amounts of time out of water. We found that terrestrially acclimated amphibious mangrove rivulus *Kryptolebias marmoratus* were more sensitive to, and less tolerant of, aquatic hypoxia relative to water-acclimated fish, which are necessary trade-offs for positive feedback to occur. Next, we acclimated fish to normoxic or hypoxic water with the opportunity to emerge for 7d to test the predictions that fish in hypoxic conditions should regularly leave water, reduce gill surface area, and become less hypoxia tolerant. Consistent with these predictions, fish in severe hypoxia spent almost 50% of the time out of water and coverage of the gill lamellae by an inter-lamellar cell mass almost doubled. Hypoxia acclimated fish were also more sensitive to acute aquatic hypoxia (emergence at higher oxygen levels), and lost equilibrium faster in hypoxic water compared to control fish. Thus, we show that a positive feedback loop develops in amphibious fish where emergence behaviour begets further emergence behaviour, driven by gill remodelling which reduces aquatic respiratory function. Such a scenario may explain how amphibious behaviour has repeatedly evolved in fishes that occupy hypoxic aquatic habitats despite the associated challenges of life on land.

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Introduction

51

52   Spatial and temporal environmental heterogeneity creates a fundamental

53 phenotype-environment mismatching problem for animals, as phenotypes well-suited to

54 one environment are often poorly suited to different environments (Agrawal, 2020). To

55 minimize these functional trade-offs, animals that inhabit variable environments either

56 adopt generalist "jack-of-all-trades" phenotypes or are responsive to environmental

57 conditions (Kassen, 2002; West-Eberhard, 2003). One form of responsiveness is

58 phenotypic plasticity, the ability of an animal to modify its phenotype to match prevailing

59 conditions (Pfennig, 2021). Another form of environmental responsiveness occurs when

60 animals respond to variability by choosing to occupy the most favourable subset of

61 available habitats (Edelaar et al., 2008). However there is little empirical data about how

62 plasticity and habitat choice interact to determine phenotypes in heterogeneous

63 environments (Edelaar and Bolnick, 2019).

64

65   Phenotypic plasticity and habitat choice can theoretically negate or increase the

66 strength of the other process. For an organism that can always select an optimal

67 habitat, there is no need for plasticity to improve the environment-phenotype match

68 (Scheiner, 2016; Edelaar et al., 2017). Similarly, phenotypically plastic animals are often

69 habitat generalists, as phenotypes can easily be adjusted to suit various conditions

70 (Asbury and Adolph, 2007; Manenti et al., 2013). However, plasticity and habitat choice

71 can also reinforce one another via positive feedback, although empirical examples are

72 rare (Dingemanse and Wolf, 2010; Miner et al., 2005; West-Eberhard, 1989). In

73 stream-living salamanders, individuals choose to inhabit fast-flowing riffles or slow-

74 moving pools depending on morphological traits linked to swimming ability; phenotypic

75 plasticity then reinforces these morphological differences between habitats (Lowe and

76 Addis, 2019). Similarly, predatory ambush bugs choose to perch on flowers that are a

77 similarly coloured to their own bodies, and then this match is improved via plastic

78 changes to body colouration (Boyle and Start, 2020). These examples of habitat choice-

79 plasticity feedbacks focus on spatially heterogeneous but temporally consistent

80 environments. In many cases, however, environmental conditions vary in both space

81 and time, but to date there has been little consideration of how plasticity and habitat

82 choice interact in these scenarios.

83

84   In the face of temporal environmental change, animals must evaluate whether to

85 remain and use plasticity to mitigate the phenotype-environment mismatch or maintain

86 their morphological phenotype and move elsewhere (Hendry, 2016; Scheiner 2016). If

87 environmental changes are gradual, plastic responses that produce a more "tolerant"

88 phenotype may be expected given that dispersal often has fitness costs (Edelaar et al.,

89 2008). However, for many environmental variables, there are probably thresholds of

90 change that cannot be managed via plasticity and therefore require avoidance

91 behaviour. In these situations, previous acclimation to the changing environment often

92 results in the expression of a specialist phenotype which may be poorly suited to

93 alternative environmental conditions due to functional trade-offs. Plastic expression of

94 an alternative phenotype in the new environment is thus expected, and as the

95 phenotype-environment match improves, a return to the previously occupied habitat

96 should become less likely. In this way, the existence of habitat choice-plasticity

97 feedback may reduce the occurrence of habitat transitions but also increase their

98 success.

99

100   One of the most dramatic environmental transitions experienced by any animal

101 occurs when amphibious fishes move between water and land (Ord and Cooke, 2016;

102 Damsgaard et al., 2019; Turko et al., 2021). The fundamental physical differences

103 between aquatic and terrestrial habitats result in a suite of functional trade-offs (Dejours,

104 1989). For example, fishes in water benefit from a large gill surface area that enhances

105 oxygen uptake and improves tolerance to hypoxia, but in terrestrial conditions large gills

106 may be a liability because of the potential for damage and evaporative water loss

107 (Nilsson et al., 2012). This respiratory trade-off is especially problematic for amphibious

108 fishes, as many species live in hypoxic aquatic habitats (Graham, 1997) and severe

109 hypoxia is a common proximate reason why amphibious fishes leave water (e.g.

110 Livingston et al., 2018; Mandic et al., 2009; Urbina et al., 2011; reviewed by Sayer,

4

111 2005). Once out of water, many amphibious fishes use alternative respiratory surfaces

112 and gills become largely nonfunctional (Randall, et al., 1981). Some amphibious fishes,

113 such as the mangrove rivulus *Kryptolebias marmoratus* (Ong et al., 2007) and bichir

114 *Polypterus senegalus* (Turko et al., 2019a), modulate this gill surface area trade-off

115 when moving between water and land by altering the size of the interlamellar cell mass

116 (ILCM). In air, these fishes reversibly increase the ILCM, reducing overall gill surface

117 area. However, upon the return to water, several days are required to decrease the

118 ILCM and during this lag-time period respiratory function in water is impaired (Turko et

119 al., 2012). Thus, by moving to land to escape aquatic hypoxia, and then developing a

120 slowly reversed terrestrial gill morphology that limits oxygen uptake in water, a feedback

121 loop may emerge that promotes terrestrial habitat choice (Turko et al., 2018).

122

123   We experimentally tested the hypothesis that habitat choice (behavioural hypoxia

124 avoidance) and morphological plasticity (gill remodelling) reinforce one another in a

125 positive feedback loop using the amphibious mangrove rivulus as a model. These fish

126 readily move between water and land, survive prolonged episodes out of water, and

127 experience severely hypoxic aquatic habitats in the wild (Taylor, 2012; Wright, 2012,

128 Rossi et al., 2019). We first tested the critical predictions that terrestrial acclimation

129 should decrease aquatic hypoxia tolerance and increase the sensitivity of the hypoxic

130 emergence response. Next, we tested the prediction that hypoxia acclimation should

131 cause emergence behaviour to become more frequent, and subsequent hypoxia

132 tolerance should decrease (due to gill remodelling on land). In this hypoxia acclimation

133 experiment, we observed that some fish remained in water despite extremely low

134 oxygen saturation. To understand how these fish were able to obtain sufficient oxygen

135 under these conditions, we quantified the usage of aquatic surface respiration (ASR) by

136 mangrove rivulus across a range of dissolved oxygen concentrations.

137

138 **Methods**

139 *Experimental Animals*

140 Adult *K. marmoratus* hermaphrodites (0.09 g ± 0.02 g) originating from Belize (strain

141 50.91; Tatarenkov et al., 2010) and maintained in a breeding colony at the University of

142   Guelph were used in these experiments. We kept fish individually in 120 ml clear plastic

143   containers filled with 60 ml of brackish water (15‰, 25°C, 12-hour light:dark cycle), and

144   fed them *Artemia* nauplii three times a week (Frick and Wright, 2002). Air exposure (7d)

145   was achieved by placing fish on a brackish water-saturated filter paper substrate in

146   standard housing containers (Ong et al., 2007). Control fish were held in identical

147   containers filled with water and were fasted for the 7d acclimation period because

148   mangrove rivulus are unable to feed while on land (Turko et al., 2019b; Wells et al.,

149   2015).

150

151   *Experimental Protocol*

152

153   *Series 1 - Plasticity of hypoxia sensitivity and tolerance*

154   To test how hypoxia sensitivity varied between air- and water-acclimated fish, we

155   measured the acute emergence response of each acclimation group (water n=10, air

156   n=10) as described previously (Regan et al., 2011, Livingston et al 2018). In brief, we

157   placed individual fish into a two-part container, comprised of a lower section filled with

158   water at 25°C, and an air-exposed platform that allowed fish to leave water. The fish

159   were left in the lower section for 30 minutes to allow for recovery from handling stress, a

160   time period determined to be sufficient in preliminary experiments. Atmospheric air was

161   bubbled for the final 15 minutes of this acclimation period through an airline attached to

162   the bottom of the water-filled container to acclimate the fish to gently bubbling water.

163   We then remotely switched the air line to nitrogen gas and measured water oxygen

164   saturation using a fibre optic oxygen probe (Witrox-4, Loligo Systems, Copenhagen,

165   Denmark). We decreased the oxygen from 21 kPa to 0 kPa over 20 minutes and

166   recorded the oxygen level at which the fish emerged.

167   To assess hypoxia tolerance, we measured time until loss of equilibrium (LOE)

168   under severe hypoxia using standard methods (Regan et al., 2017). Briefly, we placed

169   fish (water n=6, air n=6) into small, mesh sided chambers, and left them submerged in

170   brackish water for 15 minutes to recover from handling stress. These chambers were

171   then gently submerged into a 5-gallon glass aquarium to prevent the fish from reaching

172   the air-water interface. The aquarium was covered with plastic film to prevent gas

173   exchange with atmospheric air. Again, the fish were left to recover from handling stress

174   for another 15 minutes, during which time oxygen concentration did not appreciably

175   decrease. Then, we bubbled the water with compressed nitrogen gas to decrease the

176   oxygen partial pressure of the water from 21 kPa to 0.5 kPa over 10 minutes, at which

177   point it was held constant at 0.5 kPa for the remainder of the experiment (Regan and

178   Richards, 2017; Regan et al., 2017). We recorded the time until the fish lost equilibrium,

179   that is when the fish did not respond to the physical stimulus of three gentle prods.

180

181   *Series 2 - Emergence behaviour and hypoxia sensitivity*

182   To test the prediction that hypoxia acclimation should increase the frequency of

183   emergence and subsequent decrease hypoxia tolerance, we acclimated fish (7d) to one

184   of three oxygen levels: normoxia (21 kPa oxygen; n = 20), hypoxia (2.1 kPa oxygen, n

185   =20) or extreme hypoxia (0.5 kPa; n = 15). Within each group, fish were acclimated to

186   either water or air for 7d immediately before the experiment began to determine if

187   differences in gill morphology caused by air- versus water-acclimation influence

188   emergence behaviour in hypoxia. Fish were held individually in a two-part container with

189   a platform for emergence as described previously (Livingston et al., 2018). These

190   containers were similar to the ones used to assess the hypoxic emergence response

191   (described above), except the lower, water-filled portion of the containers were mesh-

192   sided and submerged in a larger water bath, allowing for free circulation of water

193   maintained at one of three oxygen levels: We maintained oxygen levels within the basin

194   with an oxygen control system (OxyCTRL, Loligo Systems, Copenhagen, Denmark) that

195   automatically bubbled compressed nitrogen gas into a header tank when oxygen

196   concentrations exceeded the experimental setpoint. We video recorded fish behaviour

197   from above using a webcam during the 12 hours of light per day, as we previously found

198   that there is no difference in emergence behaviour between day and night (Turko et al.,

199   2011), and subsequently measured the proportion of time each fish spent out of water

200   per day. At the conclusion of the 7d experiment, we tested the acute emergence

201   response or time to LOE on random subsets of fish as described above.

202   We assessed gill surface area in a subsample of fish to determine if there was a

203   relationship between behaviour and gill morphology. Fish were euthanized using MS-

204   222 and processed for histology as described previously (Turko et al., 2011). We

205   measured the height of the ILCMs in 5 randomly selected gill filaments for each fish and

206   calculated the average proportion of ILCM cover per fish.

207

208   *Series 3 - Aquatic surface respiration*

209   We noticed that some fish in the extreme hypoxia treatment (0.5 kPa O_2) never

210   lost equilibrium despite remaining in water for lengths of time that far exceeded the

211   degree of tolerance we measured in our LOE experiments, and hypothesised the fish

212   were obtaining supplemental oxygen using aquatic surface respiration (ASR; Kramer

213   and McClure, 1982). To test this hypothesis, we placed fish (air-acclimated n = 4, water-

214   acclimated n=5) in a 30 cm upright mesh tube, inside of a 5-gallon glass aquarium filled

215   halfway with water at 25°C and allowed them to recover for 15 minutes. The sides of the

216   aquarium were covered, except for one viewing window, to limit external influences on

217   fish behaviour. After the recovery from handling stress, we slowly introduced nitrogen

218   gas into the water to reduce oxygen levels at a constant rate (-0.4 kPa oxygen/min),

219   mixed thoroughly with stir bars, over the span of 50 minutes. Each trial was video

220   recorded for its duration, and we subsequently quantified the proportion of time fish

221   used ASR as a function of oxygen saturation in the water.

222

223   *Statistical Analysis*

224   All statistics were calculated using R version 4.0.2. To compare oxygen

225   saturation at emergence and time to LOE in water- versus air-acclimated fish, we used

226   Student's t-tests (Series 1). To compare oxygen saturation at emergence, time to LOE,

227   and gill ILCM coverage in fish after acclimation to normoxic, hypoxic, or extremely

228   hypoxic water (Series 2), we used two-way ANOVA, followed by Tukey's post-hoc tests.

229   To assess changes in emergence behaviour over time within each acclimation

230   condition, we used permutational two-way ANOVA (1,000,000 permutations, package

231   *permuco*; Frossard and Renaud 2019) because our data did not meet assumptions of

232   normality or homoscedasticity. Permutational ANOVA is similar to a parametric ANOVA,

233   but rather than assuming a Gaussian distribution, the dataset is repeatedly randomized,

234   and the proportion of randomly ordered datasets with greater treatment effects than the

235   actual dataset is calculated. We used a series of two-way ANOVAs (one per oxygen

236   tension) rather than a single three-way ANOVA because we found a marginal 3-way

237   interaction in our exploratory analysis (F = 1.73, p = 0.056) and because the key

238   predictions made by the positive feedback hypothesis relate to patterns over time within

239   each treatment rather than statistical comparison among treatments. Post-hoc

240   comparisons were calculated using permutational t-tests (1,000,000 permutations)

241   implemented using the pairwise.perm.t.test function (package *RVAideMemoire*, Hervé

242   2020), and p-values were adjusted for multiple comparisons using the Benjamini and

243   Yekutieli correction which controls the false discovery rate (Benjamini & Yekutieli,

244   2001). We used similar permutational ANOVA and post-hoc permutation t-tests (with

245   Benjamini and Yekutieli corrected p-values) to assess changes in the proportion of time

246   fish used aquatic surface respiration during the induction of hypoxia (Series 3), as these

247   data did not meet parametric assumptions of normality or homoscedasticity.

248

249   **Results**

250   The hypoxic emergence response of air-acclimated fish was almost twice as

251   sensitive as that of water-acclimated fish (t-test p = 0.004; Fig. 1A). Hypoxia tolerance

252   (time to LOE) of air-acclimated fish was approximately half that of water-acclimated fish

253   (t-test p = 0.0007; Fig. 1B).

254   In our habitat choice experiment, exposure to aquatic hypoxia dramatically

255   increased the amount of time fish spent out of water (Fig. 2). Fish in normoxia rarely

256   emerged from water over the course of the trial, and emergence frequency in previously

257   air-acclimated fish slightly but significantly decreased over the 7d trial (time *

258   acclimation p = 0.0069; Fig. 2A). In moderate hypoxia, emergence frequency similarly

259   decreased over time, and previously air-acclimated fish initially spent significantly more

260   time out of water than water-acclimated fish (time * acclimation p = 0.0072; Fig. 2B). In

261   extreme hypoxia, fish regularly emerged from water (~30% of the time) but this did not

262   depend on previous acclimation condition (p = 0.12) nor time (p = 0.057, interaction p =

263   0.11; Fig. 2C).

264   At the end of the habitat choice experiment, hypoxia-acclimated fish had a

265   significantly more sensitive hypoxic emergence response compared to fish acclimated

266   to normoxic conditions (two-way ANOVA, p < 0.0001; Fig. 3A). Acclimation to aquatic

267   hypoxia also decreased hypoxia tolerance (time to LOE) by about 50% (two-way

268   ANOVA, p < 0.00001; Fig. 3B). Finally, acclimation to extreme aquatic hypoxia almost

269   doubled the proportion of gill lamellae that were covered by an inter-lamellar cell mass

270   compared to normoxic conditions (two-way ANOVA, p = 0.002; Fig. 2C). In all cases,

271   there was no significant effect of previous acclimation to air versus water (all p > 0.05).

272   Mangrove rivulus significantly increased their use of aquatic surface respiration

273   when oxygen tension fell below 10 kPa (P<0.0005; Fig. 4). At the lowest P_{O2} we tested

274   (1.5 kPa), fish used aquatic surface respiration for 95% of the recording period. This

275   pattern of aquatic surface respiration was not affected by prior acclimation to air or

276   water (P>0.05; Fig. 4).

277

Discussion

279   Our results are generally consistent with the hypothesis that hypoxia-induced

280   emergence behaviour becomes self-reinforcing via positive feedback as gill remodelling

281   on land decreases respiratory function in water and promotes further emergence

282   behaviour. We found three lines of evidence that support this hypothesis. First, air-

283   acclimation increased the sensitivity to hypoxia as fish emerged at higher oxygen levels

284   relative to water-acclimated fish. Second, when reintroduced to an aquatic environment,

285   air-acclimated fish emerged more frequently and for longer durations than water-

286   acclimated fish across all oxygen treatment groups. These are both necessary

287   conditions for the establishment of a positive feedback loop. Finally, after a week of

288   exposure to hypoxic water with access to land, hypoxia sensitivity increased while

289   hypoxia tolerance decreased. Overall, our data indicate that emergence behaviour is an

290   important strategy for coping with adverse aquatic conditions in this species, consistent

291   with previous studies (Rossi et al., 2019; Turko et al., 2018). Importantly, our data also

292   indicates that this emergence behaviour makes it increasingly difficult for these fish to

293   maintain homeostasis in hypoxic water. We suggest that this sort of positive feedback

294   between plasticity and habitat choice has widespread potential to generate extreme

295   phenotypes in animals. Furthermore, we speculate that if habitat choice-plasticity

296   feedback results in genetic assimilation (Crispo, 2007; Pigliucci et al., 2006; Schneider

297   and Meyer, 2017), this process may accelerate evolutionary habitat transitions such as

298   the invasion of land by fishes.

299   *Requirements for positive feedback*

300   There are two key elements necessary to generate positive feedback between

301   habitat choice and phenotypic plasticity. First, expressions of plasticity that improve

302   performance in one habitat must also decrease performance in alternative habitats (i.e.

303   an antagonistic trade-off). Second, an animal must be capable of assessing its own

304   phenotype and using this information to choose suitable habitats (i.e. matching habitat

305   choice; Camacho et al., 2020). Our data indicate that both elements exist for mangrove

306   rivulus moving between water and land, suggesting that the positive feedback

307   hypothesis is plausible.

308   Mangrove rivulus face a suite of physiological trade-offs when moving between

309   aquatic and terrestrial environments, the first requirement for positive feedback to occur.

310   Many of these trade-offs involve the oxygen transport cascade. Gill remodelling on land

311   may reduce evaporative water loss in air but also decreases the capacity for oxygen

312   uptake in water (Turko et al., 2012). Angiogenesis of epithelial capillaries on land

313   enhances aerial oxygen uptake (Blanchard et al., 2019) perhaps at the cost of oxygen

314   loss to hypoxic water (Scott et al., 2017). The oxygen binding affinity of hemoglobin also

315   increases in air, but in water this may impair oxygen offloading to metabolically active

316   tissues (Tunnah et al., 2021; Turko et al., 2014). Terrestrially induced hypertrophy of

317   oxidative trunk muscle (Brunt et al., 2016; Rossi et al., 2018) may also detrimentally

318   increase metabolic oxygen demand in hypoxic aquatic environments. In this study, we

319   found that terrestrial acclimation decreased overall aquatic hypoxia tolerance (i.e. time

320   to loss of equilibrium) by over 50%, indicating that the combined effects of these

321   physiological trade-offs have a dramatic organism-level consequence.

322   We also found evidence that habitat choice depends on physiological state (i.e. self-

323   assessment), fulfilling the second requirement for positive feedback. When acutely

324   challenged with progressive aquatic hypoxia, air-acclimated fish emerged at oxygen

325   concentrations that were more than double that of water-acclimated fish (1.8 vs. 4.2

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326   kPa). This result implies that mangrove rivulus can self-assess their physiological

327   tolerance of aquatic hypoxia and make adaptive anticipatory decisions, as the

328   concentrations of oxygen that elicited emergence were ~3-8 fold higher than the

329   concentrations that these fish could tolerate for 20-40 min in our LOE experiments. The

330   mechanism(s) used for self-assessment of hypoxia sensitivity are not known, but

331   previous work has found that the hypoxic emergence response of this species is

332   regulated by oxygen-sensitive neuroepithelial cells (Regan et al., 2011). One possibility

333   is that self-assessment occurs via internally oriented neuroepithelial cells that sense

334   blood P_{O2} (Perry et al., 2009), as impaired respiratory function in air-acclimated fish

335   would presumably cause a faster decrease in blood P_{O2} compared to water-acclimated

336   fish. Understanding the mechanism of self-assessment is an exciting area for future

337   research.

338   *Establishment of an experimental positive feedback loop*

339   Our habitat choice experiment provides further evidence for positive feedback

340   between gill plasticity and habitat choice. We found that fish acclimated to extreme

341   aquatic hypoxia spent substantial amounts of time out of water (~30% of the

342   experiment, 70-fold more than controls in normoxic water) and were more sensitive to

343   aquatic hypoxia than fish acclimated to normoxia or moderate hypoxia. Fish in the

344   extreme aquatic hypoxia group were also less hypoxia tolerant (i.e. time to LOE was

345   reduced by ~40%) and had reduced functional gill surface area. Notably, these plastic

346   changes are opposite to those typically observed with hypoxia acclimation in fishes

347   (Perry et al., 2009; Richards, 2009). For example, when mangrove rivulus were

348   prevented from leaving water during hypoxia acclimation, respiratory function improved

349   and gill surface area increased (Turko et al., 2012). Overall, our results indicate that

350   when mangrove rivulus are given the choice between living in extremely hypoxic water

351   or on land, the respiratory phenotype is shaped by the terrestrial habitat. Thus,

352   behavioural habitat choice may be a key factor that determines physiological tolerance

353   of extreme environments.

354   While fish in the extreme aquatic hypoxia treatment spent much more time out of

355   water than fish in the other treatments, as expected, we did not find any change in

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356 emergence rates over the course of the 7d experiment. This result is inconsistent with

357 the positive feedback hypothesis, which predicts an increase in emergence over time.

358 Emergence was also surprisingly low in the moderate hypoxia treatment considering

359 that that this oxygen concentration (2.1 kPa) acutely elicited an emergence response in

360 our experiments and is lower than the critical oxygen tension of this species (Turko et

361 al., 2012). Wild mangrove rivulus under similarly hypoxic conditions spent ~90% of their

362 time on land (Turko et al., 2018). One explanation for the low rates of emergence in our

363 experiment was the extensive use of aquatic surface respiration (Chapman and

364 Mckenzie, 2009; Kramer and McClure, 1982). At the levels of aquatic hypoxia we used,

365 mangrove rivulus spent more than 80% of the time displaying this behaviour. We

366 suggest that this extensive use of aquatic surface respiration may be a laboratory

367 artefact, as in the wild ASR exposes fish to predation (Domenici et al., 2007; Kramer,

368 1983; Riesch et al., 2010) and mangrove rivulus do not appear to frequently use this

369 behaviour in the wild (A. Turko, personal observation). Another possibility is that the low

370 rates of emergence at moderate hypoxia occurred because our experimental conditions

371 were more benign than typical conditions in the field. In addition to hypoxia, wild

372 mangrove rivulus are exposed to intraspecific aggression, high temperatures,

373 hypercarbia, and high levels of hydrogen sulfide; variables all known to cause fish to

374 leave water (Cochrane et al., 2019; Gibson et al., 2015; Robertson et al., 2015; Taylor,

375 1990). These additional factors may additively increase the overall amount of time

376 rivulus spend out of water. Nonetheless, our results illustrate the interactive effects of

377 habitat choice and physiological plasticity. Even subtle habitat choice, i.e. a shift up in

378 the water column to use ASR, minimized the severity of hypoxia experienced by

379 mangrove rivulus and blunted the plastic responses to gill morphology and respiratory

380 function that occur when fish cannot access the air-water interface.

381 We noticed a high degree of variation in the tendency of individual fish to leave

382 water in our long-term behaviour experiment, especially in the extreme hypoxia

383 treatment. A similar degree of individual variation in emergence behaviour has been

384 previously reported for this species (Turko et al., 2011). We hypothesise that positive

385 feedback between habitat choice and phenotypic plasticity acts to accentuate individual

386 differences in behaviour and may in part explain the high degree of variation we

13

387   observed. For example, some individuals may be slightly more likely to leave water as

388   adults (e.g. due to developmental plasticity or epigenetic effects). This behaviour in turn

389   causes an increase in the size of the ILCM and may therefore strengthen the tendency

390   to emerge from water, which could ultimately lead to highly divergent behavioural

391   phenotypes among individuals. Previous work has shown that relatively bold birds

392   choose habitats with higher levels of disturbance, and these habitats may reinforce bold

393   phenotypes (Holtmann et al., 2017). Currently, little is known about how matching

394   habitat choice and variances in individual behaviour affects ecological outcomes, but we

395   think this is an important area for future work.

396   *Perspectives*

397   A fundamental goal of physiological ecology is understanding the processes that

398   promote phenotype-environment matching (Bolnick and Otto, 2013; Edelaar et al.,

399   2008). At the level of individual animals, the two most important mechanisms are

400   behavioural habitat choice and phenotypic plasticity. These mechanisms are often

401   studied in isolation, and modelling studies have even suggested that selection should

402   favour one or the other depending on the type of environmental variation an animal

403   experiences (Botero et al., 2015; Edelaar et al., 2017; Scheiner, 2016). However, our

404   results combined with other empirical studies (Boyle and Start, 2020; Lowe and Addis,

405   2019) indicate that plasticity and matching habitat choice interact and even positively

406   feed back on one another. Understanding these interactions can be important for

407   interpreting patterns of phenotypic variation and predicting how animals will respond to

408   changing environments. For example, our data showing that hypoxia-acclimated

409   mangrove rivulus have relatively low gill surface area and hypoxia tolerance cannot be

410   explained without considering both plasticity and habitat choice. Understanding the

411   conditions that give rise to habitat choice-plasticity feedback interactions versus habitat

412   choice or plasticity alone is an important area for future research.

413   Positive feedback moves systems away from equilibrium and as a result this

414   feedback been hypothesized to facilitate major evolutionary transitions (Crespi, 2004;

415   Hendry, 2016) such as the invasion of land. For example, on evolutionary timescales,

416   phenotypic change coupled with matching habitat choice is thought to promote local

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417   adaptation and potentially speciation (Camacho et al., 2020; Jacob et al., 2017; Muñoz

418   and Losos, 2018; Nicolaus and Edelaar, 2018). Our results indicate that analogous

419   feedback between plasticity and habitat choice can cause phenotypic divergence within

420   the lifetime of individuals. Given that expressions of plasticity can cause evolutionary

421   change through genetic assimilation (Crispo, 2007; Pigliucci et al., 2006; Schneider and

422   Meyer, 2017), we speculate that positive feedback between habitat choice and plasticity

423   may be an important first step in generating similar evolutionary feedback between

424   these processes. For example, populations of mangrove rivulus that inhabit moderately

425   versus severely hypoxic habitats should differ in emergence rates and thus respiratory

426   phenotype. If these environmental differences persist, evolutionary processes (e.g.

427   genetic assimilation and/or the altered selective environment) may reinforce the

428   phenotypic divergence between populations. Over time, this mechanism may therefore

429   promote a major ecological transition by producing terrestrially adapted fishes that have

430   poor aquatic performance due to respiratory trade-offs.

431

Acknowledgements

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Figure 1. Sensitivity and tolerance of *Kryptolebias marmoratus* to aquatic hypoxia after acclimation to water or air. (A) Partial pressure of oxygen at the point of emergence (n=10/treatment). (B) Time until loss of equilibrium at 0.5 kPa (n=6/treatment). Water-acclimated fish are shown in blue, air-acclimated fish are shown in orange. Small points denote raw values, large points denote group means, and error bars show standard error. An asterisk denotes significant difference between acclimation treatments (p<0.05).

Figure 2. Habitat choice in *Kryptolebias marmoratus* acclimated to varying levels of aquatic hypoxia. Proportion of time air- or water-acclimated fish spent on land during exposure to water of one of three oxygen pressures: (A) normoxia (21 kPa; n=20), (B) hypoxia (2.1 kPa, n=20, and (C) extreme hypoxia (0.5 kPa, n=15). Water-acclimated fish are shown in blue, air-acclimated fish are shown in orange. Small points denote raw values, large points denote group means, and error bars show standard error. Different letters within an acclimation group denote statistical differences over time (p < 0.05). An asterisk denotes significant difference between acclimation treatments (p<0.05).

Figure 3. Phenotypic plasticity of *Kryptolebias marmoratus* after acclimation to varying levels of aquatic hypoxia with access to terrestrial habitats. Water oxygen pressure at the point of emergence (A) and time until loss of equilibrium (B) and the proportion of the gill covered by an ILCM (C) between *K. marmoratus* acclimated to air or water for seven days, followed by a return to water with one of three oxygen pressures, over another seven days. Water-acclimated fish are shown in blue, air-acclimated fish are shown in orange. Small points denote raw values, large points denote group means, and error bars show standard error. Different letters denote a significant difference between oxygen pressure treatment.

Figure 4. Use of aquatic surface respiration by *Kryptolebias marmoratus*. Proportion of time water- or air-acclimated fish spent at the surface in response to decreasing oxygen concentrations (n=6/treatment; note the reversed x-axis). Water-acclimated fish are shown in blue, air-acclimated fish are shown in orange. Small points

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467   denote raw values, large points denote group means, and error bars show standard

468   error. Different letters denote overall statistical differences between levels of oxygen (p
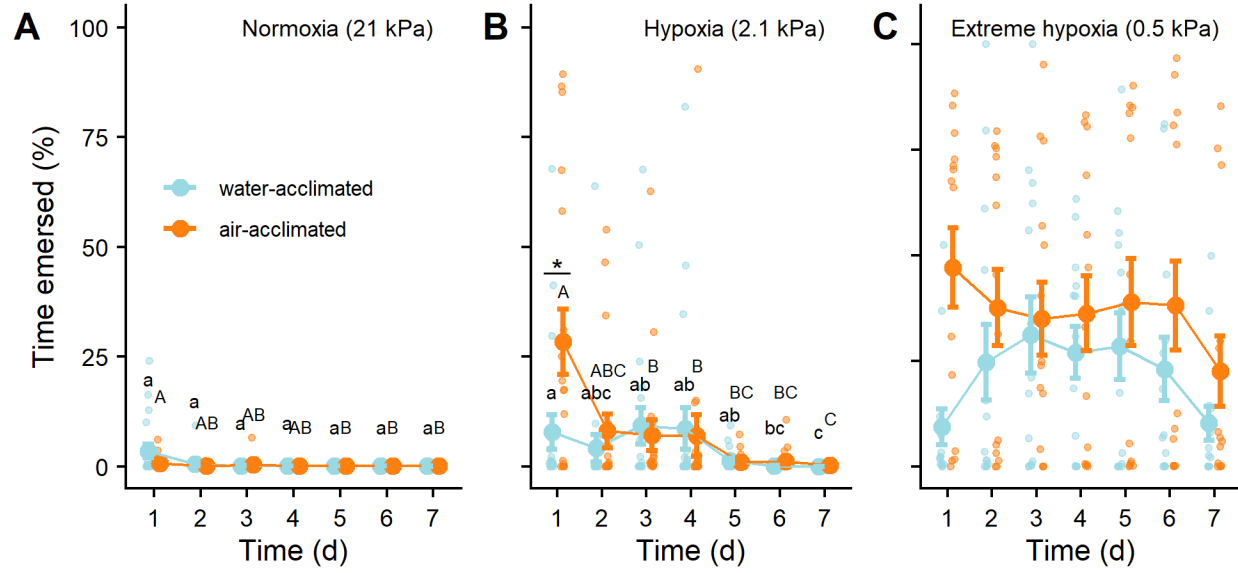
469   < 0.05).

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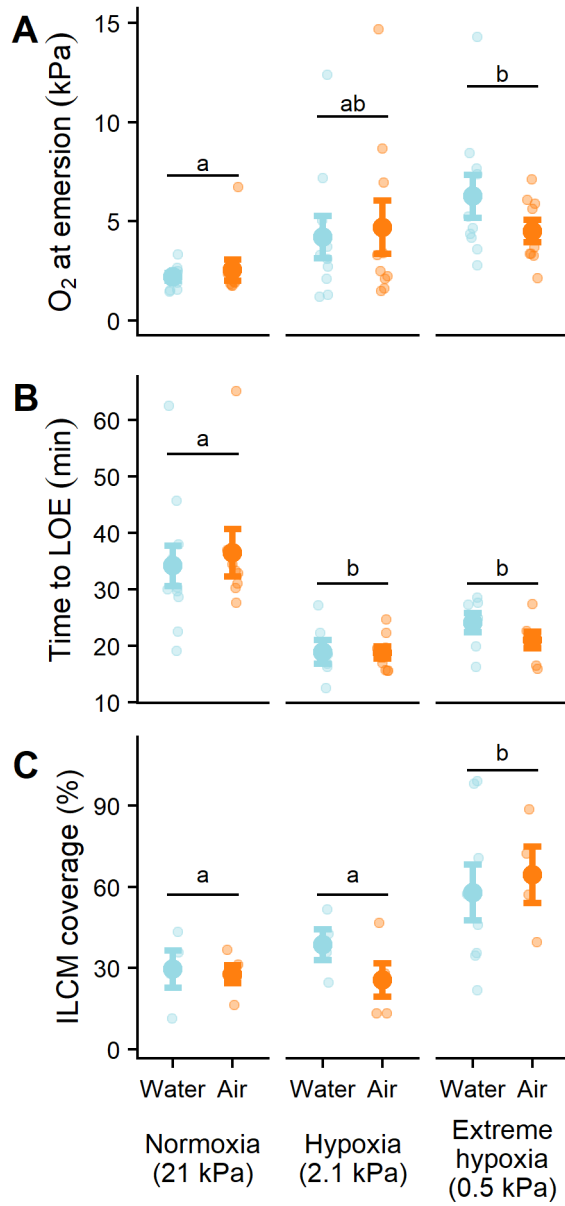
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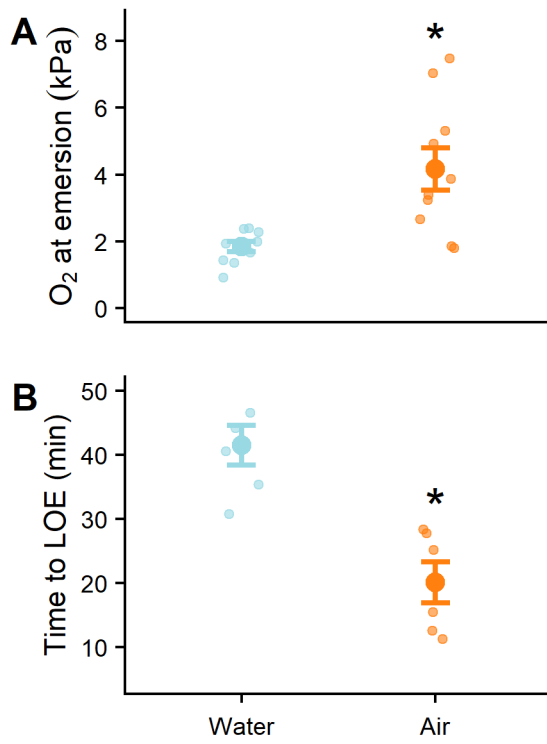
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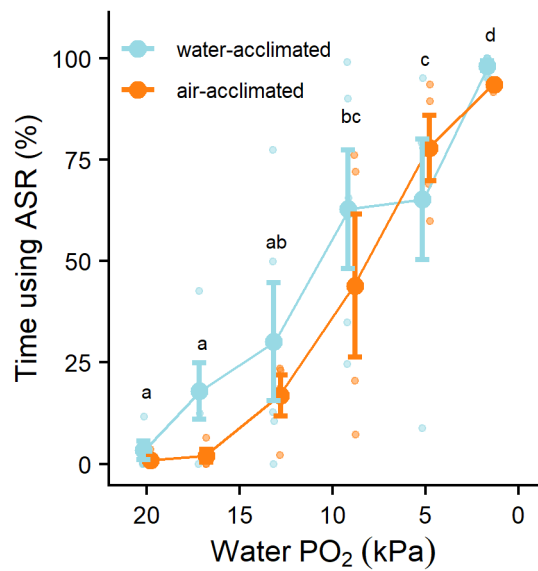
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487 **Agrawal, A. A.** (2020). A scale-dependent framework for trade-offs, syndromes, and
488   specialization in organismal biology. *Ecology* **101**, 1–24.

489 **Asbury, D. A. and Adolph, S. C.** (2007). Behavioural plasticity in an ecological
490   generalist: Microhabitat use by western fence lizards. *Evol. Ecol. Res.* **9**, 801–815.

491 **Blanchard, T. S., Whitehead, A., Dong, Y. W. and Wright, P. A.** (2019). Phenotypic
492   flexibility in respiratory traits is associated with improved aerial respiration in an
493   amphibious fish out of water. *J. Exp. Biol.* **222**,.

494 **Bolnick, D. I. and Otto, S. P.** (2013). The magnitude of local adaptation under
495   genotype-dependent dispersal. *Ecol. Evol.* **3**, 4722–4735.

496 **Botero, C. A., Weissing, F. J., Wright, J. and Rubenstein, D. R.** (2015). Evolutionary
497   tipping points in the capacity to adapt to environmental change. *Proc. Natl. Acad.*
498   *Sci. U. S. A.* **112**, 184–189.

499 **Boyle, J. and Start, D.** (2020). Plasticity and habitat choice match colour to function in
500   an ambush bug. *Funct. Ecol.* **34**, 822–829.

501 **Brunt, E. M., Turko, A. J., Scott, G. R. and Wright, P. A.** (2016). Amphibious fish
502   jump better on land after acclimation to a terrestrial environment. *J. Exp. Biol.* **219**,
503   3204–3207.

504 **Camacho, C., Sanabria-Fernández, A., Baños-Villalba, A. and Edelaar, P.** (2020).
505   Experimental evidence that matching habitat choice drives local adaptation in a wild
506   population. *Proc. R. Soc. B* **287**,.

507 **Chapman, L. J. and Mckenzie, D. J.** (2009). Chapter 2 Behavioral Responses and
508   Ecological Consequences. *Fish Physiol.* **27**, 25–77.

509 **Cochrane, P. V., Rossi, G. S., Tunnah, L., Jonz, M. G. and Wright, P. A.** (2019).
510   Hydrogen sulphide toxicity and the importance of amphibious behaviour in a
511   mangrove fish inhabiting sulphide-rich habitats. *J. Comp. Physiol. B Biochem. Syst.*
512   *Environ. Physiol.* **189**, 223–235.

513   **Crespi, B. J.** (2004). Vicious circles: Positive feedback in major evolutionary and
514   ecological transitions. *Trends Ecol. Evol.* **19**, 627–633.

515   **Crispo, E.** (2007). The Baldwin effect and genetic assimilation: Revisiting two
516   mechanisms of evolutionary change mediated by phenotypic plasticity. *Evolution*
517   *(N. Y).* **61**, 2469–2479.

518   **Damsgaard, C., Baliga, V. B., Bates, E., Burggren, W., McKenzie, D. J., Taylor, E.**
519   **and Wright, P. A.** (2019). Evolutionary and Cardio-Respiratory Physiology of
520   Air-breathing and Amphibious Fishes. *Acta Physiol.*

521   **Dejours, P.** (1989). Respiration in Water and Air in Intertidal Fishes, pp. 54–78.

522   **Dingemanse, N. J. and Wolf, M.** (2010). Recent models for adaptive personality
523   differences: A review. *Philos. Trans. R. Soc. B Biol. Sci.* **365**, 3947–3958.

524   **Domenici, P., Lefrançois, C. and Shingles, A.** (2007). Hypoxia and the antipredator
525   behaviours of fishes. *Philos. Trans. R. Soc. B Biol. Sci.* **362**, 2105–2121.

526   **Drummond, G. B., & Vowler, S. L.** (2012). Different tests for a difference: How do we
527   do research? Journal of Physiology-London, 590, 235–238.

528   **Edelaar, P. and Bolnick, D. I.** (2019). Appreciating the Multiple Processes Increasing
529   Individual or Population Fitness. *Trends Ecol. Evol.* **34**, 435–446.

530   **Edelaar, P., Siepielski, A. M. and Clobert, J.** (2008). Matching habitat choice causes
531   directed gene flow: A neglected dimension in evolution and ecology. *Evolution (N.*
532   *Y).* **62**, 2462–2472.

533   **Edelaar, P., Jovani, R. and Gomez-Mestre, I.** (2017). Should I change or should I go?
534   Phenotypic plasticity and matching habitat choice in the adaptation to
535   environmental heterogeneity. *Am. Nat.* **190**, 506–520.

536   **Frick, N. T. and Wright, P. A.** (2002). Nitrogen metabolism and excretion in the
537   mangrove killifish Rivulus marmoratus I. The influence of environmental salinity and
538   external ammonia. *J. Exp. Biol.* **205**, 79–89.

539   **Frossard, J and Renaud, O.** (2019). permuco: Permutation Tests for Regression,

540   (Repeated Measures) ANOVA/ANCOVA and Comparison of Signals. R package

541   version 1.1.0. https://CRAN.R-project.org/package=permuco

542

543   **Gibson, D. J., Sylvester, E. V. A., Turko, A. J., Tattersall, G. J. and Wright, P. A.**

544   (2015). Out of the frying pan into the air-emersion behaviour and evaporative heat

545   loss in an amphibious mangrove fish (Kryptolebias marmoratus). *Biol. Lett.* **11**,.

546   **Graham, J. B.** (1997). *Air-breathing fishes: evolution, diversity, and adaptation.*

547   **Hendry, A. P.** (2016). Key questions on the role of phenotypic plasticity in eco-

548   evolutionary dynamics. *J. Hered.* **107**, 25–41.

549   **Hervé, M.** (2020). RVAideMemoire: Testing and Plotting Procedures for Biostatistics. R

550   package version 0.9-77. https://CRAN.R-project.org/package=RVAideMemoire

551   **Holtmann, B., Santos, E. S. A., Lara, C. E. and Nakagawa, S.** (2017). Personality-

552   matching habitat choice, rather than behavioural plasticity, is a likely driver of a

553   phenotype–environment covariance. *Proc. R. Soc. B Biol. Sci.* **284**,.

554   **Jacob, S., Legrand, D., Chaine, A. S., Bonte, D., Schtickzelle, N., Huet, M. and**

555   **Clobert, J.** (2017). Gene flow favours local adaptation under habitat choice in

556   ciliate microcosms. *Nat. Ecol. Evol.* **1**, 1407–1409.

557   **Kassen, R.** (2002). The experimental evolution of specialists, generalists, and the

558   maintenance of diversity. *J. Evol. Biol.* **15**, 173–190.

559   **Kramer, D. L.** (1983). The evolutionary ecology of respiratory mode in fishes: an

560   analysis based on the costs of breathing. *Environ. Biol. Fishes* **9**, 145–158.

561   **Kramer, D. L. and McClure, M.** (1982). Aquatic surface respiration, a widespread

562   adaptation to hypoxia in tropical freshwater fishes. *Environ. Biol. Fishes 1982 71* **7**,

563   47–55.

564   **Livingston, M. D., Bhargav, V. V., Turko, A. J., Wilson, J. M. and Wright, P. A.**

565   (2018). Widespread use of emersion and cutaneous ammonia excretion in

566   Aplocheiloid killifishes. *Proc. R. Soc. B Biol. Sci.* **285**,.

567 **Lowe, W. H. and Addis, B. R.** (2019). Matching habitat choice and plasticity contribute
568   to phenotype–environment covariation in a stream salamander. *Ecology* **100**, 1–14.

569 **Mandic, M., Sloman, K. A. and Richards, J. G.** (2009). Escaping to the surface: A
570   phylogenetically independent analysis of hypoxia-induced respiratory behaviors in
571   sculpins. *Physiol. Biochem. Zool.* **82**, 730–738.

572 **Manenti, R., Denoël, M. and Ficetola, G. F.** (2013). Foraging plasticity favours
573   adaptation to new habitats in fire salamanders. *Anim. Behav.* **86**, 375–382.

574 **Miner, B. G., Sultan, S. E., Morgan, S. G., Padilla, D. K. and Relyea, R. A.** (2005).
575   Ecological consequences of phenotypic plasticity. *Trends Ecol. Evol.* **20**, 685–692.

576 **Muñoz, M. M. and Losos, J. B.** (2018). Thermoregulatory behavior simultaneously
577   promotes and forestalls evolution in a tropical lizard. *Am. Nat.* **191**, E15–E26.

578 **Nicolaus, M. and Edelaar, P.** (2018). Comparing the consequences of natural
579   selection, adaptive phenotypic plasticity, and matching habitat choice for
580   phenotype–environment matching, population genetic structure, and reproductive
581   isolation in meta-populations. *Ecol. Evol.* **8**, 3815–3827.

582 **Nilsson, G. E., Dymowska, A. and Stecyk, J. A. W.** (2012). New insights into the
583   plasticity of gill structure. *Respir. Physiol. Neurobiol.* **184**, 214–222.

584 **Ong, K. J., Stevens, E. D. and Wright, P. A.** (2007). Gill morphology of the mangrove
585   killifish (Kryptolebias marmoratus) is plastic and changes in response to terrestrial
586   air exposure. *J. Exp. Biol.* **210**, 1109–1115.

587 **Ord, T. J. and Cooke, G. M.** (2016). Repeated evolution of amphibious behavior in fish
588   and its implications for the colonization of novel environments. *Evolution* **70**, 1747–
589   1759.

590 **Perry, S. F., Jonz, M. G. and Gilmour, K. M.** (2009). Chapter 5 Oxygen Sensing And
591   The Hypoxic Ventilatory Response. *Fish Physiol.* **27**, 193–253.

592 **Pfennig, D. W.** (2021). *Phenotypic Plasticity and Evolution; Causes, Consequences,*
593   *Controversies.*

594 **Pigliucci, M., Murren, C. J. and Schlichting, C. D.** (2006). Phenotypic plasticity and
595   evolution by genetic assimilation. *J. Exp. Biol.* **209**, 2362–2367.

596 **Randall, David, Burggren Warren, Farrell, A. P. S. H.** (1981). *The Evolution of Air*
597   *Breathing in Vertebrates .* Stony Brook Foundation, Inc.

598 **Regan, M. D. and Richards, J. G.** (2017). Rates of hypoxia induction alter mechanisms
599   of O_2 uptake and the critical O_2 tension of goldfish. *J. Exp. Biol.* **220**, 2536–2544.

600 **Regan, K. S., Jonz, M. G. and Wright, P. A.** (2011). Neuroepithelial cells and the
601   hypoxia emersion response in the amphibious fish Kryptolebias marmoratus. *J.*
602   *Exp. Biol.* **214**, 2560–2568.

603 **Regan, M. D., Gill, I. S. and Richards, J. G.** (2017). Metabolic depression and the
604   evolution of hypoxia tolerance in threespine stickleback, Gasterosteus aculeatus.
605   *Biol. Lett.* **13**,.

606 **Richards, J. G.** (2009). Chapter 10 Metabolic and Molecular Responses of Fish to
607   Hypoxia. *Fish Physiol.* **27**, 443–485.

608 **Riesch, R., Oranth, A., Dzienko, J., Karau, N., Schießl, A., Stadler, S., Wigh, A.,**
609   **Zimmer, C., Arias-Rodriguez, L., Schlupp, I., et al.** (2010). Extreme habitats are
610   not refuges: poeciliids suffer from increased aerial predation risk in sulphidic
611   southern Mexican habitats. *Biol. J. Linn. Soc.* **101**, 417–426.

612 **Robertson, C. E., Turko, A. J., Jonz, M. G. and Wright, P. A.** (2015). Hypercapnia
613   and low pH induce neuroepithelial cell proliferation and emersion behaviour in the
614   amphibious fish Kryptolebias marmoratus. *J. Exp. Biol.* **218**, 2987–2990.

615 **Rossi, G. S., Turko, A. J. and Wright, P. A.** (2018). Oxygen drives skeletal muscle
616   remodeling in an amphibious fish out of water. *J. Exp. Biol.* **221**,.

617 **Rossi, G. S., Tunnah, L., Martin, K. E., Turko, A. J., Taylor, D. S., Currie, S. and**
618   **Wright, P. A.** (2019). Mangrove fishes rely on emersion behavior and physiological
619   tolerance to persist in sulfidic environments. *Physiol. Biochem. Zool.* **92**, 316–325.

620 **Sayer, M. D. J.** (2005). Adaptations of amphibious fish for surviving life out of water.

Fish Fish. **6**, 186–211.

Scheiner, S. M. (2016). Habitat Choice and Temporal Variation Alter the Balance between Adaptation by Genetic Differentiation, a Jack-of-All-Trades Strategy, and Phenotypic Plasticity. *Am. Nat.* **187**, 633–646.

Schneider, R. F. and Meyer, A. (2017). How plasticity, genetic assimilation and cryptic genetic variation may contribute to adaptive radiations. *Mol. Ecol.* **26**, 330–350.

Scott, G. R., Matey, V., Mendoza, J. A., Gilmour, K. M., Perry, S. F., Almeida-Val, V. M. F. and Val, A. L. (2017). Air breathing and aquatic gas exchange during hypoxia in armoured catfish. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **187**, 117–133.

Tatarenkov, A., Ring, B. C., Elder, J. F., Bechler, D. L. and Avise, J. C. (2010). Genetic composition of laboratory stocks of the self-fertilizing fish Kryptolebias marmoratus: A valuable resource for experimental research. *PLoS One* **5**, 1–9.

Taylor, D. S. (1990). Adaptive specializations of the Cyprinodont fish Rivulus marmoratus. *Florida Sci.* **53**, 239–248.

Taylor, D. S. (2012). Twenty-four years in the mud: What have we learned about the natural history and ecology of the mangrove rivulus, kryptolebias marmoratus? *Integr. Comp. Biol.* **52**, 724–736.

Tunnah, L., Robertson, C. E., Turko, A. J. and Wright, P. A. (2021). Acclimation to prolonged aquatic hypercarbia or air enhances hemoglobin-oxygen affinity in an amphibious fish. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **252**, 110848.

Turko, A. J. and Wright, P. A. (2015). Evolution, ecology and physiology of amphibious killifishes (Cyprinodontiformes). *J. Fish Biol.* **87**, 815–835.

Turko, A. J., Earley, R. L. and Wright, P. A. (2011). Behaviour drives morphology: Voluntary emersion patterns shape gill structure in genetically identical mangrove rivulus. *Anim. Behav.* **82**, 39–47.

Turko, A. J., Cooper, C. A. and Wright, P. A. (2012). Gill remodelling during terrestrial

648 acclimation reduces aquatic respiratory function of the amphibious fish Kryptolebias

649 marmoratus. *J. Exp. Biol.* **215**, 3973–3980.

650 **Turko, A. J., Robertson, C. E., Bianchini, K., Freeman, M. and Wright, P. A.** (2014).

651 The amphibious fish Kryptolebias marmoratus uses different strategies to maintain

652 oxygen delivery during aquatic hypoxia and air exposure. *J. Exp. Biol.* **217**, 3988–

653 3995.

654 **Turko, A. J., Tatarenkov, A., Currie, S., Earley, R. L., Platek, A., Taylor, D. S. and**

655 **Wright, P. A.** (2018). Emersion behaviour underlies variation in gill morphology and

656 aquatic respiratory function in the amphibious fish Kryptolebias marmoratus.

657 **Turko, A. J., Maini, P., Wright, P. A. and Standen, E. M.** (2019a). Gill remodelling

658 during terrestrial acclimation in the amphibious fish Polypterus senegalus. *J.*

659 *Morphol.* **280**, 329–338.

660 **Turko, A. J., Doherty, J. E., Yin-Liao, I., Levesque, K., Kruth, P., Holden, J. M.,**

661 **Earley, R. L. and Wright, P. A.** (2019b). Prolonged survival out of water is linked

662 to a slow pace of life in a self-fertilizing amphibious fish. *J. Exp. Biol.* **222**,.

663 **Urbina, M. A., Forster, M. E. and Glover, C. N.** (2011). Leap of faith: Voluntary

664 emersion behaviour and physiological adaptations to aerial exposure in a non-

665 aestivating freshwater fish in response to aquatic hypoxia. *Physiol. Behav.* **103**,

666 240–247.

667 **Wells, M. W., Turko, A. J. and Wright, P. A.** (2015). Fish embryos on land: Terrestrial

668 embryo deposition lowers oxygen uptake without altering growth or survival in the

669 amphibious fish Kryptolebias marmoratus. *J. Exp. Biol.* **218**, 3249–3256.

670 **West-Eberhard, M. J.** (1989). Phenotypic plasticity and the origins of diversity. *Annu.*

671 *Rev. Ecol. Syst. Vol. 20* 249–278.

672 **West-Eberhard, M. J.** (2003). Developmental Plasticity and Evolution. 794.

673 **Wright, P. A.** (2012). Environmental physiology of the mangrove rivulus, kryptolebias

674 marmoratus, a cutaneously breathing fish that survives for weeks out of water.

675 *Integr. Comp. Biol.* **52**, 792–800.

676   **Wright, P. A. and Turko, A. J.** (2016). Amphibious fishes: evolution and phenotypic

677   plasticity. *J. Exp. Biol.* **219**, 2245–2259.

678

679

680

681