Coevolution between life-history and metabolic rate depends on ontogenetic stage

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 (1–5)

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1   **Abstract**

2

3   Metabolic rate is considered to determine the energetic investment placed into life-history

4   traits, regulating the speed of an organism's life-cycle and forming the so called "pace-of-life".

5   However, how metabolic rate and life-history traits co-evolve remains unclear. For instance, the

6   energetic demands of life-history traits, including the number of energy allocation trade-offs, is

7   unlikely to remain constant over ontogeny. Therefore, the predicted coevolution between

8   metabolic rate and life-history could be specific to particular ontogenetic stages, rather than a

9   stable property of an organism. Here, we test the ontogenetic dependency of the coevolution

10   between metabolic rate and the pace of life-history, under strictly standardized conditions

11   using 30 species of killifish, which are either annual species adapted to ephemeral pools or non-

12   annual species inhabiting more permanent waterbodies. Standard metabolic rates were

13   estimated at three ontogenetic stages, together with relevant life-history traits, i.e. growth

14   (juveniles), maturity (young adults), and reproductive rate (reproductive adults). Life-history

15   traits largely followed predicted pace-of-life patterns, with overall faster/higher rates in annual

16   species. The divergences in life-history traits across species tended to increase over ontogeny,

17   being smallest during juvenile growth and largest in reproductive adults. However, associations

18   between life-history strategy and metabolic rate followed a reversed pattern, being strongest in

19   juveniles, but lowest in reproductive adults. Our results are concordant with the number of

20   energetic trade-offs increasing over ontogeny, which results in a stronger covariation between

21   physiology and life-history traits earlier in ontogeny.

22

23   **Introduction**

24

25   An organism's position along the fast-slow life-history continuum is typically linked to

26   differential investment into energetically costly traits (e.g. growth, development, reproductive

27   rate; 1–5). Metabolic rate is considered to be an important functional trait in determining the

28   rate at which resources are converted into the energy available for life-history traits (6,7), and

29   the maintenance of somatic tissues (8). While presumably not under direct selection (9),

30   evolutionary shifts in metabolic rate are thought to follow concomitant changes in the pace of

31   life-histories (10). However, despite clear theoretical expectations, empirical evidence for the

32   co-evolution between metabolic rate and the pace of life-history remains ambiguous, with

33   mixed results from both experimental and comparative studies (e.g. 10–12).

34

35   Life-history traits and metabolic rate have commonly been measured on individuals sampled

36   from the wild, rather than individuals reared under standardised experimental settings (10).

37   This lack of standardisation has potentially obscured the coevolutionary associations between

38   the pace of life-history and metabolism, due to plasticity in either life-history traits or metabolic

39   rate (life-history traits: 13; metabolic rates: 14,15). Furthermore, the relationship between

40   metabolic rate and life-history is likely to vary over the course of an organism's life-cycle, rather

41   than remaining a stable property of an organism (13). For instance, the more energetically

42   costly elements of a life-history, which vary substantially across species (15,16), should be most

43   prominent at particular ontogenetic stages (e.g. juvenile - growth; adult - reproduction). In

44   contrast, for a stable relationship between life-history and metabolism to occur throughout

2

45   ontogeny, the amount of energy invested into somatic growth should be equal to that later

46   invested into reproduction, resulting in a constant energy allocation toward these relatively

47   costly life-history traits. However, a fast rate of growth does not necessarily lead to a high

48   reproductive rate, and vice versa (i.e. the pace of a life-history will not predict all variation in a

49   system; 1). Partly, this is due to an organism not being a tightly integrated entity, but rather

50   consisting of quasi-independent parts, which are tightly integrated (16). This modularity

51   reduces the probability for trade-offs to influence evolutionary change (17), as a finite amount

52   of energy being allocated among processes (e.g. homeostasis), results in the number of trade-

53   offs (18) typically increasing over ontogeny (19). Therefore, this increasing number of trade-offs

54   may mask associations between life-history traits and metabolic rate, and result in the latter

55   being influenced by a complex co-regulation between life-history characters and physiology

56   that can change over an organism's lifespan. As a consequence, we argue that there is a need

57   to integrate ontogeny into the pace-of-life framework, as the coevolution between metabolic

58   rate and the pace of life-history could remain undetected, if changes in energy allocation

59   towards different life-history traits over ontogeny are not properly considered.

60

61   Here, we test the association between metabolic rate and life-history traits (somatic growth

62   rate, maturity and reproductive rate) across different ontogenetic stages (juveniles, young non-

63   reproductive adults, and reproducing adults). We do this by conducting a large-scale

64   comparative, common garden study of 30 killifish species from the Aplocheiloidei suborder,

65   focusing on rates of growth, reproduction, maturity, and standard metabolic rate (SMR). Within

66   this clade there has been several independent evolutionary transitions between annual and

67   non-annual life-history strategies, where annual species have evolved egg-stages capable of

68   entering embryonic diapause to adapt to the regular desiccation of ephemeral aquatic habitats

69   (20). In contrast, non-annual killifishes inhabit more permanent, stable habitats, allowing these

70   species to live and breed over comparatively longer time-scales. Adaptations to time-

71   constrained environments, such as ephemeral habitats, typically include correlated selection on

72   increased rates of growth (21), quicker development periods (21,22); including in killifishes (23),

73   as well as higher reproductive rates (24). Moreover, annual killifishes have very short life-spans

74   in comparison to other similar vertebrates, which is likely a consequence of reduced selection

75   on later-life fitness (25,26,23,27–30). As such, we expect the independent evolutionary

76   transitions between annual and non-annual life-history strategies in killifishes (20) to be

77   accompanied by the evolution of life-history traits along the fast-slow life-history continuum.

78   Hence, killifishes present an unparalleled system for conducting comparative analyses on

79   organisms which are ecologically similar, yet potentially diverge substantially in regard to the

80   pace of life-history traits.

81

82   In killifishes, we purposely investigate life-history traits that are directly linked to biosynthesis,

83   and are therefore governed by an allocated energetic budget, predicted to be positively

84   correlated with metabolic rate (31–36). We use a strictly standardized common garden

85   experimental set-up, as plasticity in both metabolic rate and life-history traits has been

86   suggested as a potential source of ambiguity in the empirical evidence previously collected on

87   the pace of life-histories (10). We predict that life-history traits and metabolic rate in killifishes

88   should evolve in a correlated manner, and that annual species should on average exhibit faster

4

89   life-history traits and have a higher metabolic rate, compared to non-annual species.

90   Furthermore, if metabolic rate evolves to fuel life-histories, we predict that the maximum

91   divergence between comparable species with fast and slow life-histories will coincide with the

92   ontogenetic time point when corresponding life-history trait divergences are also at their

93   maximum.

94

95   **Methods**

96

97   *Study system*

98   Life-history strategy (annual or non-annual) is characterised in killifishes according to the

99   presence or absence of eggs capable of entering embryonic diapausing (20). Here, we reared 30

100   species of killifish, 13 non-annual and 17 annual species (see Supplementary Table 1 for the full

101   species list), which we selected based on their phylogenetic position (20), and to represent

102   multiple independent evolutionary transitions to an annual life-history. Diapausing eggs have

103   both lower expressions of growth hormones (37,38) and lower metabolic rates (39,40)

104   compared to directly developing eggs, implying that the diapause stage is an adaptation to

105   ephemeral habitats that regularly desiccate for extended periods, and is not mechanistically

106   linked to traits related to the pace-of-life.

107

108   Fish were housed under laboratory conditions (average 24.3°C; 12-hour day:night cycle), and

109   were fed newly hatched *Artemia*, supplemented with frozen bloodworms when they reached

110   adulthood, to satiation three times daily (once daily during weekends). All individuals were

111   hatched from eggs under our laboratory conditions, with eggs either produced from our own

112   stock populations, or sourced from dedicated aquarists. Fry were initially housed in small plastic

113   containers (9 × 9 × 9 cm) and were moved to bigger aquaria as they grew larger (13 L; furnished

114   with gravel, clay pots and bundles of wool yarn). All fish were initially raised in solitary

115   conditions, but after sexual maturity a subset were housed as pairs, or trios (1 male and 2

116   females, to reduce male aggression) and allowed to breed, with the remainder kept in isolation

117   and unable to breed, until the end of the experimental period.

118

119   *Growth rate*

120   We measured the growth rate of 29 species (N_{annual} = 16; $N_{non-annual}$ = 13; N = 400

121   individuals; Supplementary Table 1), during the linear juvenile growth phase, by photographing

122   each individual every 7 to 10 days, from hatching until an age of ca. 3 months or sexual

123   maturity. Standard body length was measured from these photographs using the software

124   ImageJ (41). As the level of replication in our analysis was at the individual, any imprecisions in

125   measurements due to minor distortions in images could have substantial inferential impact.

126   Therefore, to avoid incorporating high amounts of noise in the data, we fitted Gompertz growth

127   models to each individual, and removed outliers that had absolute values of residuals >0.11.

128   Outlier removal was script-based and hence blind to species identity. Using data on length, with

129   outliers removed, we fitted a linear model for each individual, in the interval between 10 and

130   60 days of age, and used the slope of this regression as a measure of the growth rate of the

131   individual, which was predominantly linear (see supplementary material). We also assessed

132   growth as the Gompertz-parameter μ; however, as growth was linear (e.g. most species had

133   not reached the plateau of the growth phase) during the measurement period, μ was estimated

134   outside the range of the data in more than half of the individuals. Hence, here we use the slope

135   of a linear regression of time on length as a proxy for individual growth, while analysis of μ

136   yielded congruent results (results not presented).

137

138   *Maturity*

139   Killifishes are sexually dimorphic in both shape and colour (27; Sowersby et al. under review),

140   which we exploited to assess developmental rates. Specifically, as juveniles are typically more

141   similar in appearance to females than males, we noted the time point (in days since hatching)

142   at which we were able to determine if an individual was a male (based on the appearance of

143   species-specific male colour patterns) using the photos taken weekly for growth estimation.

144   Our measure of developmental rate was hence sex-specific and assumed that both females and

145   males mature at similar time points. This should be a valid assumption, given that the life-

146   history evolution of these fishes is likely to be predominantly determined by time-constrained

147   environmental conditions, and therefore similar for both sexes. Furthermore, in other studies

148   we have dissected killifishes at various time points in their life-cycle and have found that sex

149   identification based on visual inspection always aligns with the correct male or female

150   reproductive tissue being observed during dissection (Sowersby et al. under review). Our

151   predictions were that annual species would have higher values (i.e. higher rates) in all

152   measured traits, compared to non-annual species. Therefore, for ease of interpretation, we

153   subtracted each individual's time to maturity observation from the overall mean time to

7

154   maturity, across all species, creating an index where small values correspond to fast

155   development and large values with slow development time (i.e. an additive inversion of the

156   data, henceforth "rate of maturity"). The analysis was performed on 113 individuals from 22

157   species (N_{annual} = 12; $N_{non-annual}$ = 10), where the sample size ranged from 1 to 10

158   individuals per species (median: 4.5; Supplementary Table 1). As killifishes sometimes show

159   significant bias in sex-ratios (Sowersby et al. under review), sample sizes differed among

160   species, depending on the number of males available.

161

162   *Reproduction*

163   We used previously estimated reproductive rates (24). Briefly, for 19 species (N_{annual} = 11;

164    $N_{non-annual}$ = 8; Supplementary Table 1), of which 16 overlap with species used in the growth

165   measurements, we estimated reproductive rates by counting the number of eggs deposited by

166   each female per month. Females that did not reproduce during this month, where considered

167   to be reproductively inactive, and were excluded from the data.

168

169   *Standard Metabolic Rate*

170   Oxygen consumption was measured over time using an intermittent-flow respirometry setup

171   (Loligo Systems, Viborg, Denmark), set at 24°C, under a 12:12 hour day:night regime (light:

172   07:00 – 19:00). Specifically, we measured oxygen uptake rates (as a proxy for estimated SMR)

173   at three different biologically relevant ontological time points (juveniles: N = 187, from 13 slow-

8

174 living and 16 fast-living species; young adults N = 141, from 13 slow-living and 17 fast-living

175 species; reproducing adults: N = 223, from 10 slow-living and 10 fast-living species; see

176 Supplementary Table 2 for details). Prior to the measurements, fish were fasted for 15 hours (8

177 hours in home tanks, 7 hours during acclimation in the respirometry chambers) and weighed

178 (prior to acclimation). Trials were run for ca. 17 h, overnight, starting approximately at 17:00.

179 Oxygen consumption was measured in total darkness, in 30-minute cycles (between 00:00 -

180 05:00, when fish were likely to be least active), by estimating the slope of the decreasing

181 oxygen concentration over time. Out of ten slopes obtained per individual, the three lowest-

182 valued slopes (the majority being R^2 > 0.9) were retained for further analysis. Of these, all

183 analyses were performed on the lowest of these values. The lowest estimate of oxygen

184 consumption was strongly correlated to the mean of the 3 lowest values (r = 0.998), and

185 analysis on the mean of the three lowest values yielded highly congruent results. The decrease

186 in oxygen consumption over time was highly linear (typically R^2 > 0.98), suggesting that fish

187 were consistently inactive during this period. The rate of background respiration (due to the

188 presence of microorganisms in the respirometry set-up) was accounted for by taking blank tests

189 (i.e. with no fish) before and after SMR measurements, and subtracting the extrapolated value

190 from the total oxygen consumption. The respirometry analysis, including correction for

191 background respiration, was conducted using the R package FishResp (42). We performed a

192 Cook's D outlier analysis on a model with log_{10} oxygen consumption as response, and log_{10} mass

193 as a covariate, including species as a random effect (fit using restricted maximum likelihood in

194 the R-library lme4), and the most deviating observations (i.e. the 5% quantile; 17 observations)

195 were removed. In a subset of analyses we used residual metabolic rate, which was calculated as

9

196   the residuals from a regression on $\log_{10}$ oxygen consumption as the response, and $\log_{10}$ mass as

197   a covariate, $\log_{10}$ oxygen consumption as a response, $\log_{10}$ mass, ontogenetic stage, and their

198   interaction as explanatory variables.

199

200   *Phylogeny*

201   In order to control for phylogenetic non-independence of data points, we included information

202   on shared ancestry based on a dated phylogeny (20). We added missing taxa to the dated

203   phylogeny by utilizing other previously published phylogenies (see supporting information),

204   using the add.species.to.genus and bind.tip functions in the R package phytools (43). The

205   resulting final phylogeny was included as a random factor in all Bayesian linear mixed models

206   (see below).

207

208   *Statistical analysis*

209   We aimed to test how life-history traits and metabolic rate co-evolved across species, as well as

210   whether the association between life-history and metabolic rate is dependent on ontogenetic

211   stages. Given the difficulty in disentangling correlations between species means into a

212   component caused by phylogenetic signal and a component caused by evolutionary processes

213   independent from ancestry, we analysed our data utilizing our *a priori* sampling regime, i.e.

214   species that were sampled from repeated, independent evolutionary transitions between two

215   states: absence-presence of diapausing eggs (a specific adaptation to ephemeral habitats). By

216   employing phylogenetically informed sampling we were able to obtain a number of

217   phylogenetically independent contrasts, between species that differed in the pace of life-

218   history traits associated with adaptations to ephemeral or permanent habitats. However, as

219   outlined in the introduction, theory predicts correlations among species, inferences from our

220   models therefore assume that any divergences across annual and non-annual life-history

221   groups arise due to correlations among species. In order to validate this assumption, we also

222   analysed species-level correlations. Further, as theory predicts that metabolic rate evolves as a

223   correlated response to the energy requirements of particular life-history traits, we should

224   expect that standardised differences in life-histories will be comparable to standardised

225   differences in metabolic rates. To assess this possibility, we compared standardised contrasts in

226   life-history traits, across annual and non-annual species, with standardized contrasts in the

227   corresponding metabolic rates, at the matching ontogenetic stage.

228

229   *Contrasts between annual and non-annual life-history strategy*

230

231   We first tested the effect of life-history on rates of growth, maturity and reproduction, as

232   response variables in a multivariate model, with the trait specific means, life-history strategy

233   (annual or non-annual) and their interaction as fixed effects. Species and phylogeny were added

234   as random effects, as well as separate residual variances for each response variable.

235

236   Then, to test if metabolic rate differed between annual and non-annual species, we fit the

237   lowest measured value of oxygen consumption (i.e. standard metabolic rate) per individual as a

238   log_{10} transformed response variable, with log_{10} transformed body size, the presence or absence

239   of diapausing eggs (i.e. annual or non-annual), ontogenetic stage, and the interaction between

11

240   ontogenetic stage and presence or absence of diapausing eggs added as fixed effects, species,

241   phylogeny, and the interaction variance of species and ontogenetic stage were added as

242   random effects. We did not explicitly focus on any sex differences, as the life-history trait

243   variables we measured were either independent of sex (e.g. juvenile growth rate), or were

244   purposely defined by only one sex (e.g. we used the secondary sexual traits of males to assess

245   sexual maturity and the number of eggs laid by females were used as a proxy for reproductive

246   rates, see (24). However, it has been previously suggested that any coevolution between

247   metabolic rate and life-history could be sex-specific (44,45). Therefore, we tested this

248   hypothesis by modelling $\log_{10}$ transformed metabolic rate as a response variable, and $\log_{10}$

249   transformed body size, the presence/absence of diapausing eggs, ontogenetic stage, and sex as

250   fixed effects. No interactions among the fixed effects were significant, and were hence not

251   included. Species, phylogeny, and the interaction variance of species and ontogenetic stage

252   were added as random effects.

253

254   *Correlations among traits and ontogenetic stages*

255   To assess species level correlations between metabolic rate, life-history traits, and ontogenetic

256   stages, we analysed *i)* a multivariate model with rates of growth, development and

257   reproduction as responses), *ii)* specific life-history traits (in total three bivariate models, growth

258   - juvenile; maturity rate - young adults; reproductive rate - reproductive adults), *iii)* a univariate

259   model of residual metabolic rate as a response variable and ontogeny included as a fixed effect.

260   In these models, we fitted the full covariance matrices associated with species-specific and

261   phylogenetic effects. We examined the amount of variation explained by species using intra

262   class correlations. The putative species-level correlations among traits, were calculated from

263   the species-level covariance matrix, calculated as the sum of the phylogenetic covariance

264   matrix, and the species-specific covariance matrix (46).

265

266   *Standardized contrasts across annual and non-annual species*

267   To compare contrasts across life-history strategies (i.e. annual and non-annual) based on traits

268   with different means and variances, we calculated effect sizes (Hedge's D) from the posterior

269   distributions. For life-history traits, the means were extracted from the multivariate model

270   testing the effect of annual and non-annual strategy on measured life-history traits. For

271   metabolic rate, means and variances were extracted from a model where residual metabolic

272   rate was fit as the response variable, and ontogenetic stage, life-history and their interaction

273   were fit as fixed effects, with species and phylogeny fit as random effects.

274

275   All models were fit within a (multivariate) Bayesian phylogenetic mixed model framework, using

276   the R package MCMCglmm (47), with flat priors for the fixed effects, and correlations. Three

277   chains were fit to each model, and the posterior modes were fused across these models. Within

278   chains, autocorrelations were in the interval >-0.1 and <0.1, and the Gelman diagnostic across

279   the three replicate chains was always <1.2, both suggesting that the Bayesian models

280   converged. Flat priors were used for the fixed effects, and inverse Wishart priors, flat for the

281   correlation, were used for the random effects.

282

283

284 **Results**

285

286 *Life-history traits*

287 In congruence with our predictions, annual species exhibited an overall faster pace-of-life, with

288 faster rates of growth, maturity rates, and higher reproductive rates (see 24), in comparison to

289 non-annual species (Table 1; Figure 1). All life-history traits exhibited variation explained

290 significantly by species, where species explained 77.9% of the variation in growth rates (95% CI:

291 67.4-86.6), 64.6% in maturity rate (i.e. inversed time to maturity; 95% CI: 47.4-78.8), and 82.2%

292 in reproductive rate (95% CI: 61.8-92.7) (Supplementary Table 2). Life-history traits further

293 exhibited significant correlations across species (Figure 2b). Importantly, sample sizes for

294 growth rates were higher than both maturity and reproductive rates, meaning that growth data

295 may exhibit higher precision for species-specific estimates. However, growth rates had the

296 lowest intra-class correlation for species, which indicates that the other two life-history rates

297 had larger underlying biological effect sizes. Hence, it is unlikely that any results were driven by

298 varying statistical power across the three traits.

299

300   **Table 1**

301

| Parameter	Estimate	Lower CI	Upper CI	P_{MCMC}
Fixed Effects				
Growth Rate	0.0355	0.0268	0.0453	0.000333
Maturation Rate	6.78	-4.51	18.9	0.177
Reproductive Rate	3.93	2.94	4.78	0.000333
Life-History	-0.0133	-0.0213	-0.00284	0.0153
Maturation Rate : Life-History (Non-Annual)	-23.6	-34.2	-12.9	0.000333
Reproductive Rate : Life-History (Non-Annual)	-1.93	-2.92	-0.769	0.000667
Random Effects				
Growth Rate (Phyolgeny)	$7.93*10^{-07}$	$3.17*10^{-11}$	0.000254	-
Maturation Rate (Phylogeny)	2.28	$6.59*10^{-07}$	317	-
Reproductive Rate (Phylogeny)	0.0157	$2.05*10^{-07}$	1.56	-
Growth Rate (Species)	$8.41*10^{-07}$	$4.60*10^{-11}$	0.000154	-
Maturation Rate (Species)	0.863	$9.08*10^{-06}$	174	-
Reproductive Rate (Species)	0.0048	$1.30*10^{-07}$	0.892	-
Growth (Residual Variance)	0.000122	$9.87*10^{-05}$	0.00014	-
Maturation Rate (Residual Variance)	139	108	199	-
Reproductive Rate (Residual Variance)	0.274	0.163	0.502	-

302
303   **Table 1: Results of the Bayesian phylogenetic mixed model testing how the pace of life-**
304   **history traits (growth, maturity and reproductive rates) depends on life-history strategy**
305   **(annual or non-annual), with life-history strategy and the interactions between life-history**
306   **traits and life-history strategy as fixed effects. The model was run with the specific species**
307   **and phylogeny used in each life-history trait as random effects. Where, "species" signifies the**
308   **variance explained by species, "phylogeny" signifies the variance explained by the phylogeny,**
309   **and "residual variance" is the variance that is not explained by the mode. Lower and upper**
310   **CIs represent 95 % credible intervals. The table is structured according to the standard output**
311   **from R.**
312

313

314 **Figure 1**
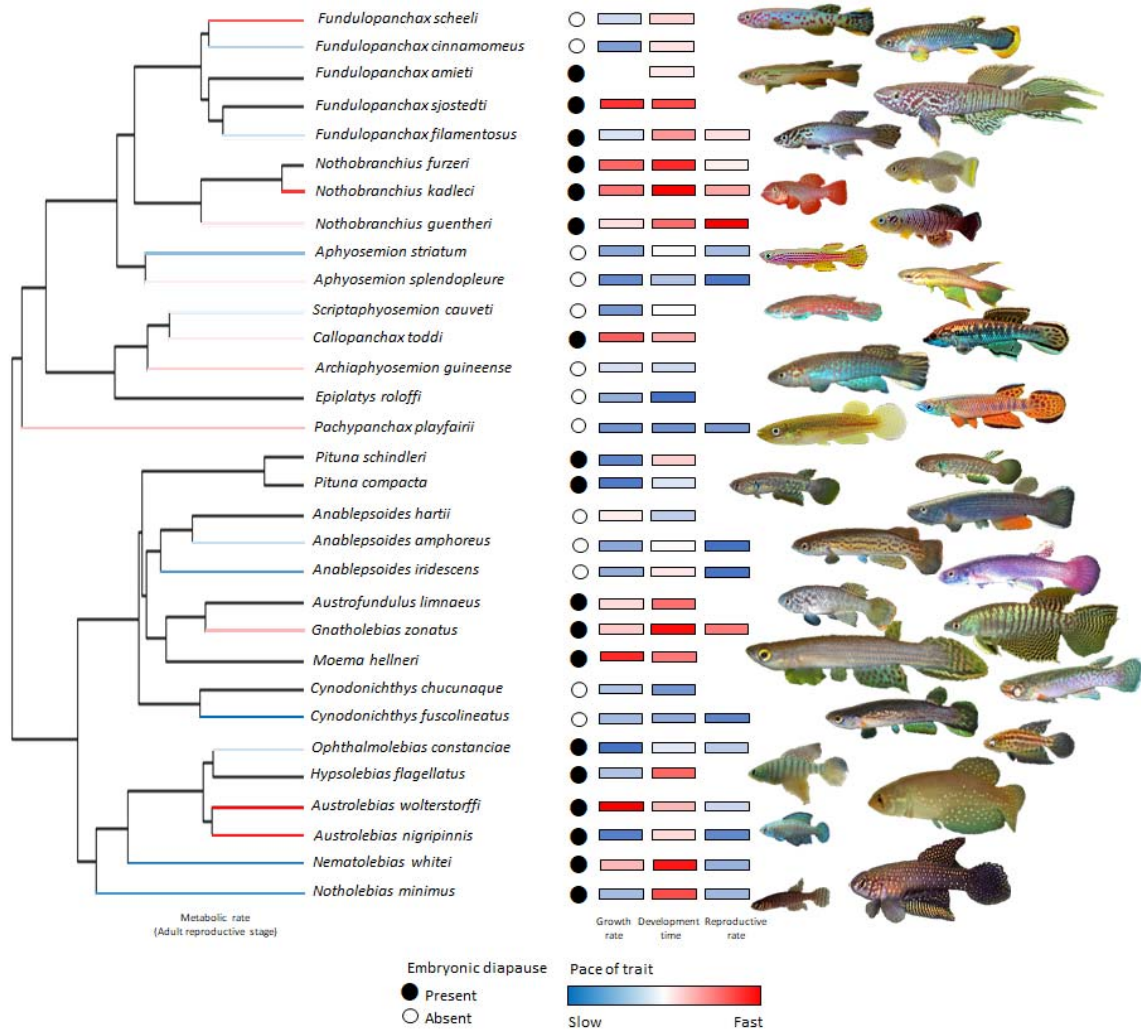
Figure 1. Updated phylogenetic tree (utilizing Furness et al. 2015) of the species used in the study. Adult breeding stage residual standard metabolic rate (SMR was highly correlated across ontogenetic stages) is displayed on the tips of the tree. The intensity of red (fast) and blue (slow) colour represents the mean pace of measured life-history traits (growth rate, development time, and reproductive rate), per species.

16

323 *Metabolic rates*

324 We found that for a given body size, metabolic rate differed between annual and non-annual

325 species, with annual species having overall higher metabolic rates than non-annual species

326 [beta = -0.081 (95% CI: -0.16 - -0.018), P = 0.02, Table 2]. Further, we found that metabolic

327 rates, in general, decreased over ontogeny, being highest in fry and lowest in reproductive

328 adults (Table 2). The allometric slope for metabolic rate was 0.88 (95% CI 0.83 - 0.92) and we

329 found no significant differences in the allometric slope of metabolic rate between the two life-

330 history strategies or between the ontogenetic stages. We found no significant differences

331 among the sexes in metabolic rate (Supplementary Table 3).

332

333 **Table 2**
334

| Parameter	Estimate	Lower CI	Upper CI	P_{MCMC}
Fixed Effects				
(Intercept)	-0.812	-0.895	-0.726	0.000333
Ontogenetic Stage (Non-Reproducing Adult)	-0.109	-0.171	-0.0598	0.000667
Ontogenetic Stage (Reproducing Adult)	-0.15	-0.218	-0.103	0.000333
Life-History (Non-Annual)	-0.105	-0.176	-0.0198	0.0147
Fish Mass	0.884	0.828	0.922	0.000333
Ontogenetic Stage (Non-Reproducing Adult) : Life-History (Non-Annual)	0.0242	-0.0362	0.0994	0.415
Ontogenetic Stage (Reproducing Adult) : Life-History (Non-Annual)	0.0202	-0.0419	0.11	0.336
Random Effects				
Species	$4.69*10^{-05}$	$7.01*10^{-10}$	0.00799	-
Phylogeny	$4.51*10^{-05}$	$8.41*10^{-09}$	0.0134	-
Ontogenetic Stage : Species	0.00206	0.000665	0.00437	-
Residual Variance	0.0111	0.00962	0.0123	-

335
336 **Table 2: Results of the Bayesian phylogenetic mixed model on standard metabolic rate, with**
337 **ontogenetic stage (juvenile, non-reproducing adult, reproducing adult), life-history strategy**
338 **(annual or non-annual), fish mass (log10 transformed) and the interaction between**
339 **ontogenetic stage and life-history as fixed effects. The model was run with species, phylogeny**
340 **and the interaction variance of ontogenetic stage and species as random effects. Where,**
341 **"species" signifies the variance explained by species, "phylogeny" signifies the variance**
342 **explained by the phylogeny, "ontogenetic stage : species (interaction variance)" represents**
343 **the variance explained by ontogenetic stage and species and "Residual Variance" is the**
344 **variance that is not explained by the model. Lower and upper CIs represent 95 % credible**
345 **intervals.**
346

347

348   When assessing species effects in the different ontogenetic stages, we found significant species

349   effects across all stages of ontogeny, but the variation explained by species decreased over

350   ontogeny. Species explained 63.2 % (95% CI: 44.9 – 78.6) of the variation in juveniles, 53.8%

351   (95% CI: 37.5 – 69.8) in non-reproducing young adults, and 31.3% (95% CI: 14.8 – 53.9) in

352   reproductive adults, due to lower among-species variation at later stages of ontogeny

353   (Supplementary Table 4). Further, species-specific metabolic rates were strongly correlated

354   across ontogenetic stages, suggesting that the clustering into high vs low metabolic rates is a

355   species-specific property that is stable over ontogeny (Figure 2a). Species level correlation in

356   metabolic rate was 64.5 between juveniles and young adults (95% CI: 20.5 – 86.8), 47.7

357   between juveniles and reproductive adults (95% CI: -9.25 – 81.9), and 68.4 between young

358   adults and reproductive adults (95% CI: 16.6 – 92) (Supplementary Table 4).

359

360 **Figure 2**
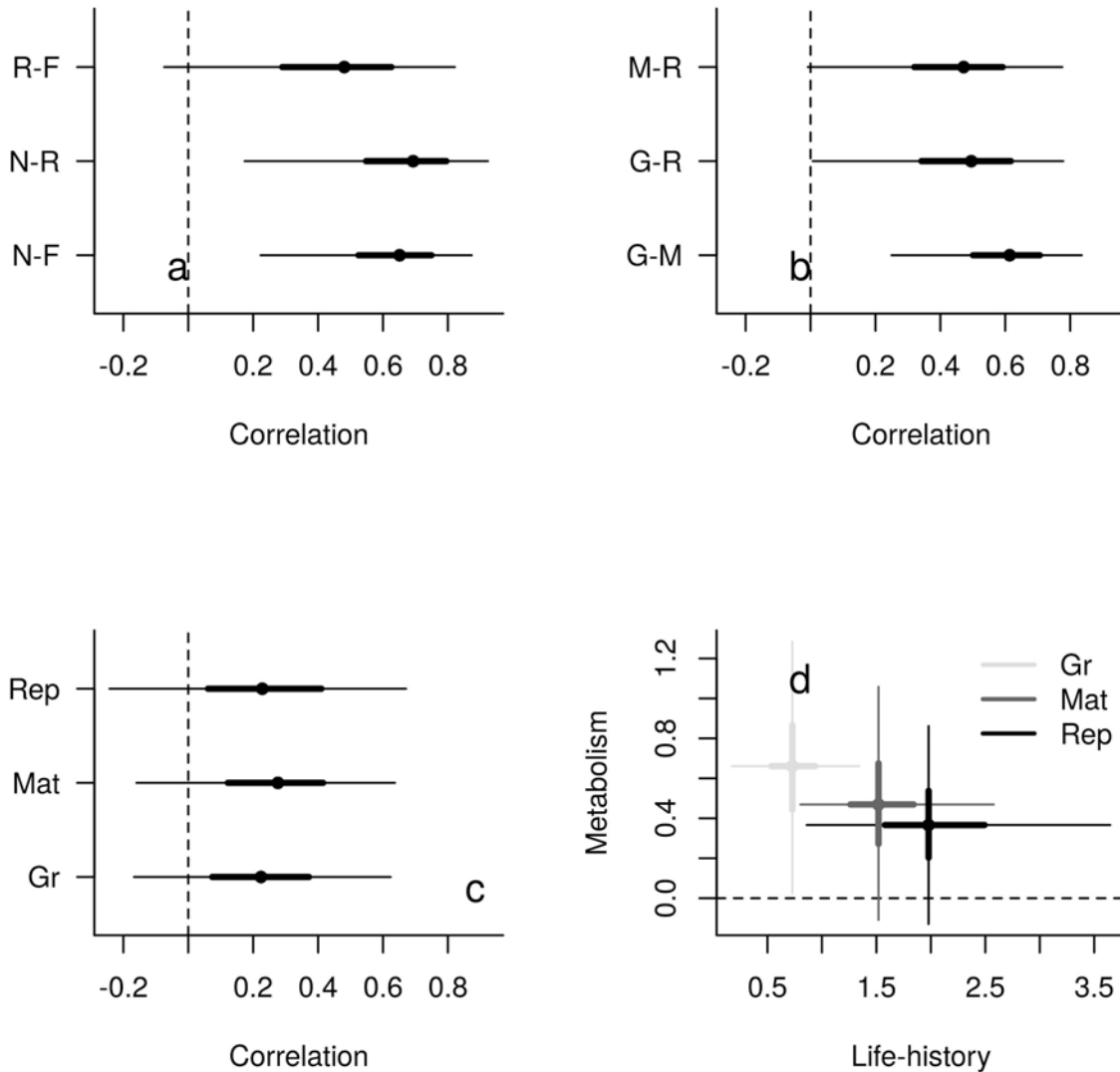
361
362 **Figure 2.** Correlations between a) species level metabolic rates across ontogenetic stages,
363 where R = reproductive adults, N = non-reproductive adults, and F = juveniles. b) The estimated
364 species level correlations between different life-history traits where G = growth, M = time to
365 maturity, and R = reproduction. c) The estimated species level correlations between metabolic
366 rate and reproduction (Rep), maturity rate (Mat), and growth (Gr). d) Effect sizes (Hedges d) of
367 divergences following adaptations to ephemeral environments for life-history traits and
368 ontogeny specific metabolic rates, for juvenile-growth, non-reproductive adults-maturity rate
369 and reproductive adults-reproductive rate. In all plots, the dot is the median of the posterior
370 distribution, narrow lines denote the 95% credibility intervals, and thick lines indicate the 50%
371 credibility intervals.

372

373　*Connections between life-history and metabolic rate*

374　When examining the contrasts between annual and non-annual species in metabolic rate

375　specific to the three life-history stages (Supplementary Table 4), to contrasts in life-history traits

376　expressed at these ontogenetic stages, we found trends towards diminishing contrasts in

377　metabolic rate over ontogeny, but increased contrasts in life-histories, where growth rate had

378　the smallest difference between annual and non-annual species and reproductive rate had the

379　largest difference (Figure 3d). Correlations between life-history traits and metabolic rate were

380　in general weak, and while none were significantly different from 0, growth rate had a stronger

381　correlation than development time and reproduction (Figure 3a; Supplementary Tables 5-7).

382

383   **Figure 3**
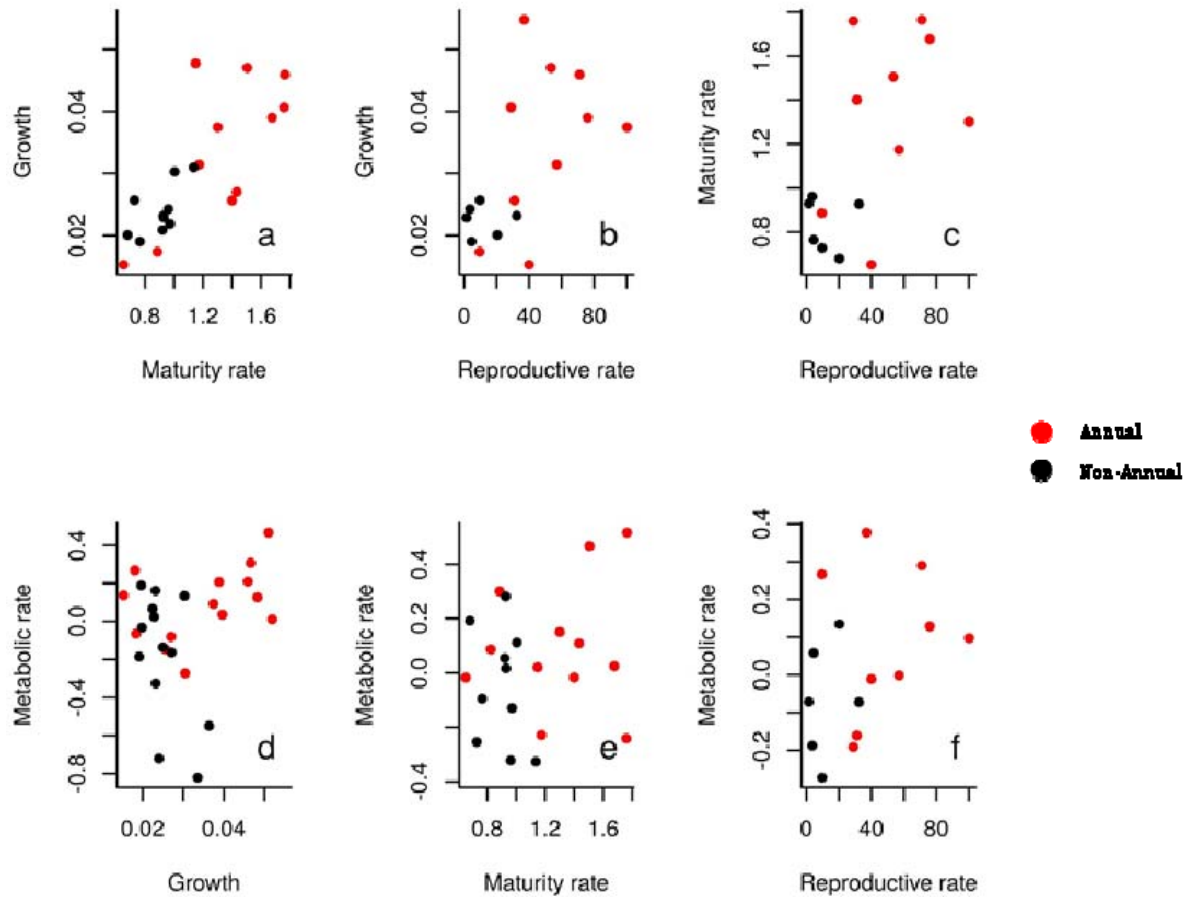
384

385   **Figure 3.** Scatterplots between a) rates of growth and maturity. b) rates of growth and
386   reproduction. c) rates of maturity and reproduction. d) growth rate and metabolic rate for
387   juveniles, e) maturity rate and metabolic rate for non-reproductive adults, and f) reproductive
388   rates and metabolic rates of reproductive adults. In all plots, red dots indicate annual species,
389   and black dots non-annual species.
390

391 **Discussion**

392

393 In accordance with the predictions made by pace-of-life theory, we found that rates of life-

394 history traits were correlated across species. Specifically, we found that annual species had

395 significantly faster rates of growth, maturity, and reproduction, in comparison to non-annual

396 species. The divergences between life-history strategies (annual and non-annual) tended to

397 increase over ontogeny, being smallest during juvenile growth, and largest in reproductive

398 adults. In general, metabolic rate was higher in annual fishes, and followed a similar pattern

399 with species means correlating positively over the three ontogenetic stages. Interestingly, we

400 found that the rank order of metabolic rate across species was relatively stable over ontogeny,

401 implying that placement along the axis of low to high metabolic rate can be considered a

402 species-specific trait.

403

404 However, while we found no significant interactions between life-history and ontogenetic

405 stage, we found that associations between metabolic rate and life-history strategy were

406 strongest at earlier ontogenetic stages (i.e. at the juvenile stage), and tended to decrease over

407 ontogeny. Rather than being a straightforward energetic trade-off between investment into

408 growth or reproduction, our results suggest a more complex relationship between metabolic

409 rate and life-history, where energetic allocations likely change over the course of an organism's

410 life-cycle. Furthermore, as our analyses were phylogenetically controlled, our results suggest

411 that pace-of-life is sustained across genetically diverged species, including independent

412 evolutionary transitions, e.g. suggesting parallel evolution.

413

414   *Life-histories co-evolve as predicted by pace-of-life theory*

415

416   To maximise reproductive success, life-history traits are predicted to evolve in response to

417   different biotic and abiotic environmental factors (15). According to life-history theory, species

418   with high rates of extrinsic mortality are typically selected to emphasise current over future

419   reproductive events, which often results in the co-evolution of life-history traits in the same

420   direction, along a fast-slow continuum (15). We found that key life-history traits did indeed

421   correlate in the direction predicted by pace-of-life theory. Specifically, annual killifish species,

422   which are adapted to time-constrained ephemeral habitats, exhibited significantly faster rates

423   of all measured life-history traits, compared to non-annual species. While evolution under

424   differential mortality rates has been identified as a key driver of the pace of life-histories

425   (15,48), annual and non-annual killifishes do often co-occur, for example in flood plain areas

426   (20; Sowersby et al. under review). Hence, we acknowledge that adult annual killifish could

427   possibly escape the desiccation of ephemeral habitats by seasonally migrating to more

428   permanent water-bodies, like some non-annual species (49). However, the strong divergences

429   we observed in the pace of life-histories among the two groups suggests that these migrations

430   may not occur, or do not occur at evolutionarily significant frequencies. In addition to having

431   eggs capable of entering diapause, annual killifishes are also known to age rapidly compared to

432   other similar vertebrate species, which is highly unlikely to be an adaptive trait if these fishes

433   are migrating away from ephemeral environments (26). Therefore, adaptations to ephemeral,

24

434   time-constrained environments, appear to have played a key role in shaping the evolution of

435   the pace of life-history, in this clade of fishes.

436

437   *A faster life-history is associated with a higher metabolic rate*

438

439   One hypothesis that has received substantial interest stipulates that life-history strategies

440   characterised by high rates of growth and reproduction, should have co-evolved corresponding

441   physiological mechanisms to fuel these energetically demanding processes (4). In this context,

442   the pace-of-life hypothesis has been proposed as a framework explaining the expected

443   coevolution of metabolic rate and life-history strategies (3,50,6,10). However, the empirical

444   evidence for this relationship between metabolic rate and the pace of life-histories has

445   remained weak (13; including in killifishes, Eckerström-Liedholm et al. under review). One

446   plausible explanation for this disparity between theoretical predictions and empirical results

447   may be because both metabolic rate and life-history traits have considerable plasticity

448   (51,14,52,53), meaning any coevolutionary associations are potentially distorted by

449   environmental effects (10,54; see 13). Here, we controlled for potential confounds generated

450   by environmental effects, by employing a common garden approach with strictly standardised

451   environmental conditions. Under these conditions we found that metabolic rates were indeed

452   higher in species with an annual life-history, compared to non-annual species. Further,

453   metabolic rate generally decreased over ontogeny, which has been attributed to a decrease in

454   the relative size of metabolically costly tissues (e.g. the liver) over ontogeny (55). For example, a

455   decreasing proportion of organs with high mass-specific metabolic rates throughout ontogeny

456   provides a proximate mechanistic explanation for ontogenetic declines in metabolic rate (56–

457   58). Although metabolic rate often displays plasticity and decreases over ontogeny, we found

458   that species level correlations between metabolic rate, measured at three different ontogenetic

459   stages, were positive and rather strong. This implies that species level rank orders of metabolic

460   rate remained rather consistent, which is in congruence with previous research on metabolic

461   allometries across fishes (59).

462

463   The total energy budget of an organism is distributed amongst key functions, such as activity,

464   biosynthesis (growth and reproduction), and somatic maintenance (59). As resources are

465   typically finite, energy used for one function diminishes that available for another, creating

466   energetic conflicts. For instance, both theoretical and empirical evidence indicates that

467   organisms with fast rates of growth and reproduction have shorter lifespans, suggesting that

468   fast and slow-living organisms invest different amounts of their energy budget into the

469   maintenance of somatic tissues (59). Indeed, annual killifishes, which we found to have

470   generally fast rates of growth and reproduction, do have short lifespans, with some

471   *Nothobranchius* species having among the shortest lifespans recorded for any vertebrate (60).

472   Therefore, it is possible that slow-living non-annual killifish species could have overall similar

473   metabolic requirements as annual fast-living killifishes, if non-annual species invest a greater

474   amount of energy into somatic maintenance. However, we found that annual species, which

475   had faster rates of both growth and reproduction, also had significantly higher metabolic rates.

476   This pattern indicates an association between the life-history traits directly involved in

477   biosynthesis (e.g. growth and reproduction) and energetic demands, which is presumably in

478   excess of the energy invested by non-annual species into somatic maintenance. Our results are

479   hence largely congruent with Pettersen et al. (61), who found that bryozoans with a higher

480   metabolic rate have shorter developmental times and life-spans, in contrast to bryozoans with

481   a lower metabolic rate.

482

483   *Across species, pace-of-life patterns are most apparent early in ontogeny, when difference in*

484   *metabolic rate are the smallest*

485

486   When assessing standardised differences in life-history traits, we found that divergences across

487   annual and non-annual species increased over ontogeny. This is not an unexpected pattern, as

488   evolutionary trajectories frequently occur through changes in the timing and the rate of

489   developmental events, leading to an accumulation of divergence throughout life (62,63). As a

490   consequence, evolutionary divergence has been found to increase over ontogenetic

491   development (64,65). However, surprisingly, patterns of pace-of-life have not typically (if at all)

492   been assessed over ontogeny, and ours is the first to do so at a macro-evolutionary scale.

493

494   If differences in metabolic rate evolve as a correlated response to selection on key life-history

495   traits (9), we would expect that differences in metabolic rate between life-history strategies

496   would become increasingly evident during the later stages of ontogeny. However, we found

497   that associations between life-history strategy and metabolic rate followed a reversed pattern,

498   being significantly different in juveniles, but tending to decrease over ontogeny (i.e. being

499   lowest in reproductive adults). More specifically, standardized divergences (between annual

27

500   and non-annual species) in metabolic rate and growth, were of roughly the same magnitude in

501   juveniles, while divergence in reproductive rate was approximately five times higher than the

502   divergence in metabolic rate in adults. This pattern suggests that the number of energetic

503   trade-offs within an organism increases over ontogeny (19), with the energetic trade-offs that

504   exist earlier in development potentially involving lower modular complexity. If the number of

505   potential energetic trade-offs does increase over time within an organism, pace-of-life patterns

506   may be most apparent earlier in ontogeny. Overall, our results suggest that the relationship

507   between metabolic rate and life-history is likely to be influenced by a complex interaction

508   between life-history characters and physiology, which is modulated over ontogeny.

509

510   *Conclusion*

511

512   In conclusion, we found that annual killifishes that are adapted to ephemeral environments had

513   overall faster life-histories, both in terms of life-history traits and in associated changes in

514   metabolic rate. This is in comparison to non-annual killifishes that are adapted to more stable,

515   constant environments. We had predicted that differences between fast and slow life-history

516   strategies, in regard to the association between life-history traits and physiology, would be

517   most apparent at distinct points during ontogeny. Indeed, we found that associations between

518   life-history and metabolic rate were higher during periods of juvenile growth. Our results show

519   that the covariance between metabolism and life-history traits can change over ontogeny, likely

520   due to an increase in the number of trade-off components as an organism develops into a

521   reproductive stage.

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