

## 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

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

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 ( $N_{\text{annual}} = 16$ ;  $N_{\text{non-annual}} = 13$ ;  $N = 400$   
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  $\mu$ ; however, as growth was linear (e.g. most species had  
133 not reached the plateau of the growth phase) during the measurement period,  $\mu$  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  $\mu$   
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



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 ( $N_{\text{annual}} = 12$ ;  $N_{\text{non-annual}} = 10$ ), 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 ( $N_{\text{annual}} = 11$ ;  
164  $N_{\text{non-annual}} = 8$ ; 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-

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

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

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 <sub>MCMC</sub>
<i>Fixed Effects</i>				
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
<i>Random Effects</i>				
Growth Rate (Phylogeny)	7.93*10 <sup>-07</sup>	3.17*10 <sup>-11</sup>	0.000254	-
Maturation Rate (Phylogeny)	2.28	6.59*10 <sup>-07</sup>	317	-
Reproductive Rate (Phylogeny)	0.0157	2.05*10 <sup>-07</sup>	1.56	-
Growth Rate (Species)	8.41*10 <sup>-07</sup>	4.60*10 <sup>-11</sup>	0.000154	-
Maturation Rate (Species)	0.863	9.08*10 <sup>-06</sup>	174	-
Reproductive Rate (Species)	0.0048	1.30*10 <sup>-07</sup>	0.892	-
Growth (Residual Variance)	0.000122	9.87*10 <sup>-05</sup>	0.00014	-
Maturation Rate (Residual Variance)	139	108	199	-
Reproductive Rate (Residual Variance)	0.274	0.163	0.502	-

302

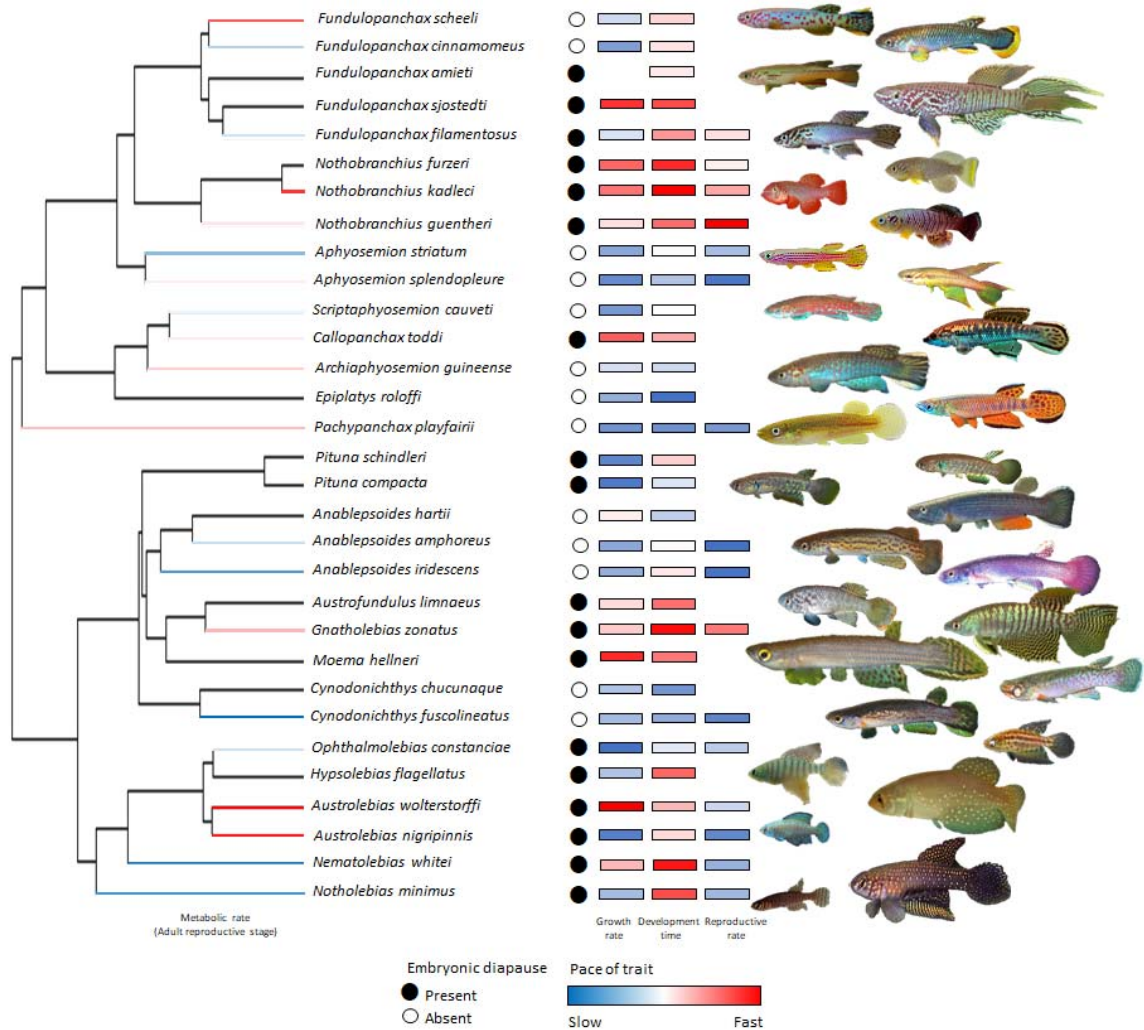
303 **Table 1: Results of the Bayesian phylogenetic mixed model testing how the pace of life-**  
 304 **history traits (growth, maturity and reproductive rates) depends on life-history strategy**  
 305 **(annual or non-annual), with life-history strategy and the interactions between life-history**  
 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**



315

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

321

322

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 <sub>MCMC</sub>
<i>Fixed Effects</i>				
(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
<i>Random Effects</i>				
Species	4.69*10 <sup>-05</sup>	7.01*10 <sup>-10</sup>	0.00799	-
Phylogeny	4.51*10 <sup>-05</sup>	8.41*10 <sup>-09</sup>	0.0134	-
Ontogenetic Stage : Species	0.00206	0.000665	0.00437	-
Residual Variance	0.0111	0.00962	0.0123	-

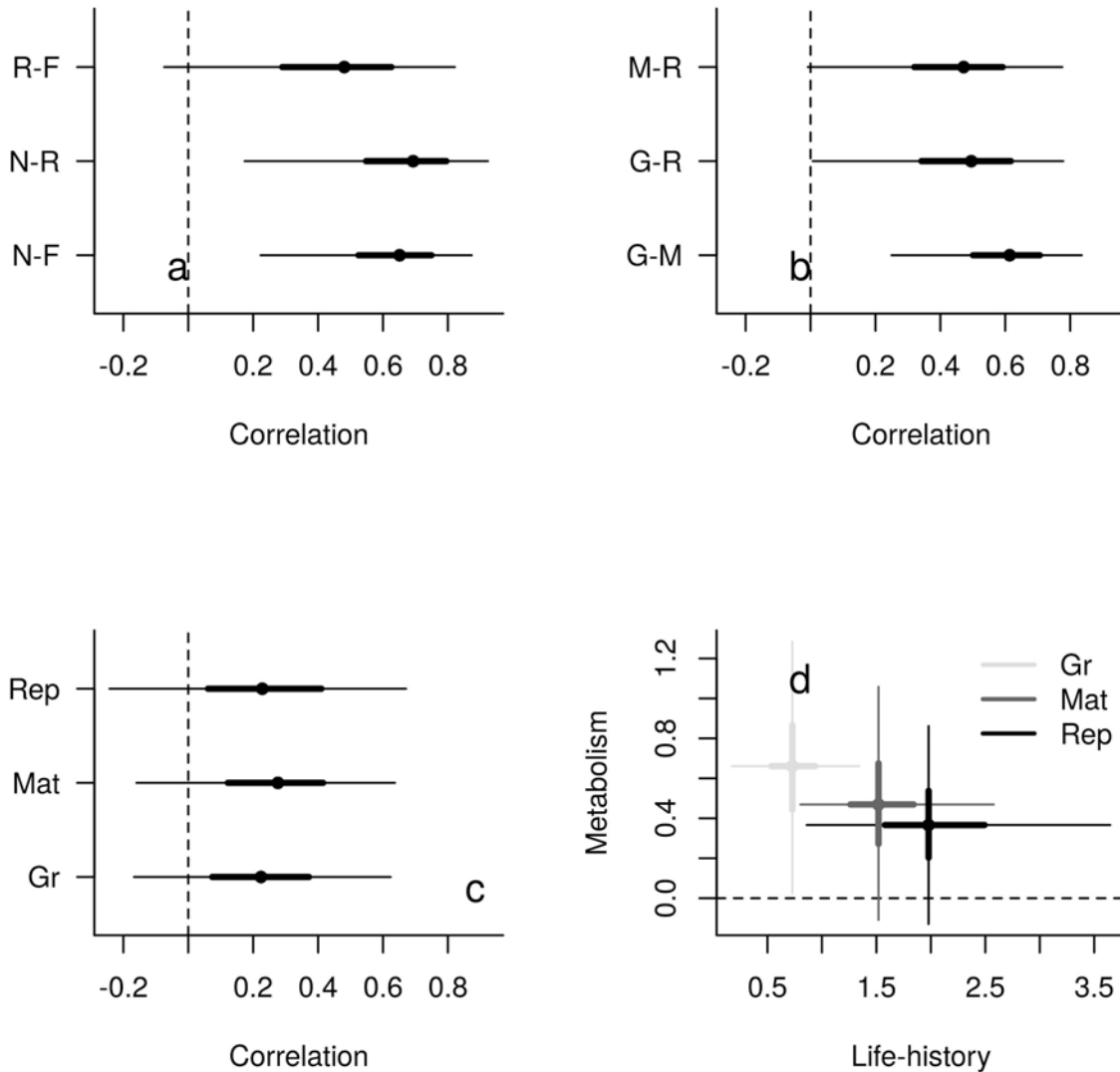
335  
336 **Table 2: Results of the Bayesian phylogenetic mixed model on standard metabolic rate, with**  
337 **ontogenetic stage (juvenile, non-reproducing adult, reproducing adult), life-history strategy**  
338 **(annual or non-annual), fish mass (log10 transformed) and the interaction between**  
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

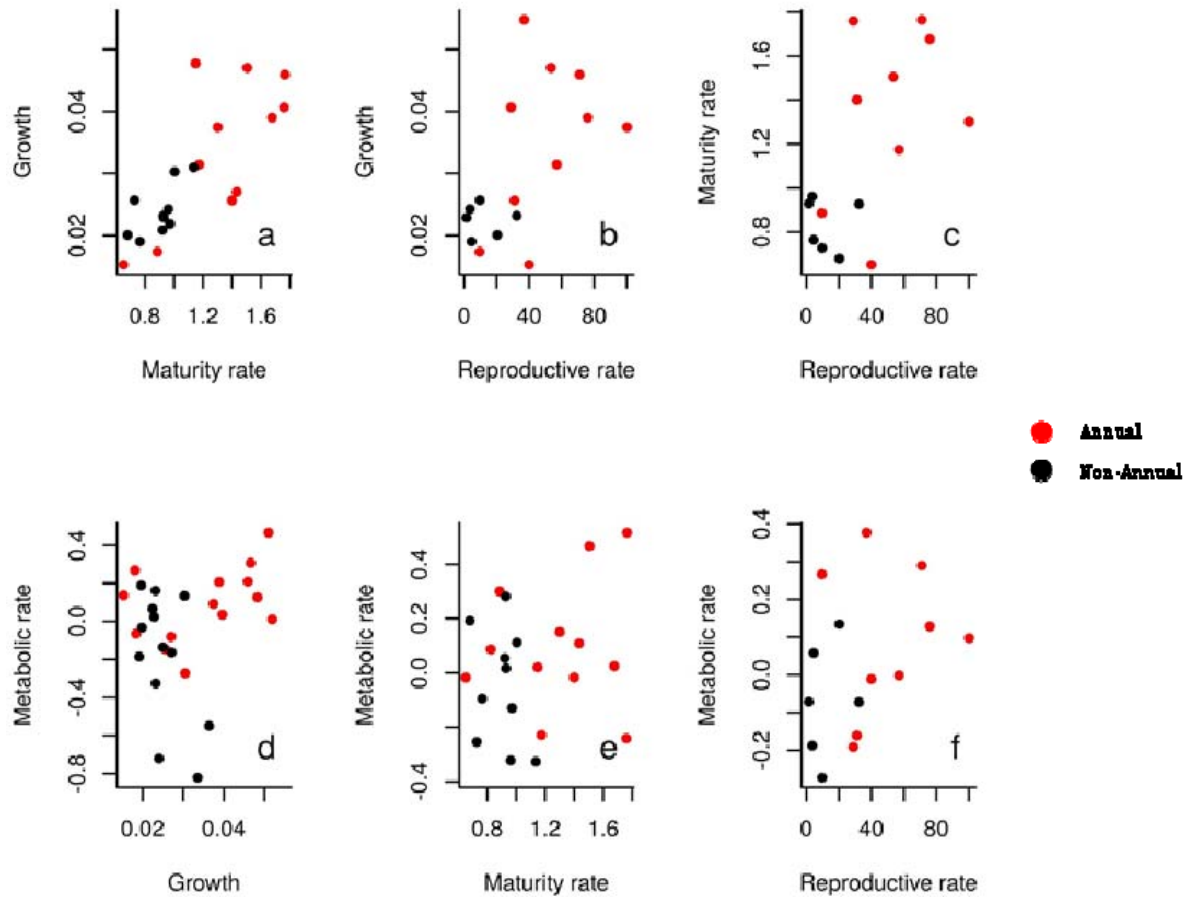
379 largest difference (Figure 3d). Correlations between life-history traits and metabolic rate were

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,

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

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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