

1 **Abbreviated title: Ontogeny of jumping performance**

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3 **ONTOGENY OF THE MORPHOLOGY-PERFORMANCE AXIS IN AN**
4 **AMPHIBIOUS FISH (*KRYPTOLEBIAS MARMORATUS*)**

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23 **Abstract**

24 Establishing links between morphology and performance is important for understanding the
25 functional, ecological, and evolutionary implications of morphological diversity. Relationships
26 between morphology and performance are expected to be age-dependent if, at different points
27 during ontogeny, animals must perform in different capacities to achieve high fitness returns.
28 Few studies have examined how the relationship between form and function changes across
29 ontogeny. Here, we assess this relationship in the amphibious mangrove rivulus (*Kryptolebias*
30 *marmoratus*) fish, a species that is both capable of and reliant on “tail-flip jumping” for
31 terrestrial locomotion. Tail-flip jumping entails an individual transferring its weight to the caudal
32 region of the body, launching itself from the substrate to navigate to new aquatic or semi-aquatic
33 habitats. By combining repeated trials of jumping performance in 237 individuals from distinct
34 age classes with a clearing and staining procedure to visualize bones in the caudal region, we test
35 the hypotheses that as age increases: i) average jumping performance (body lengths jumped) will
36 increase, ii) the amount of variation for each trait will change, and iii) the patterns of
37 covariation/correlation among traits, which tell us about the integration of form with function,
38 will also change. We find a significant increase in size-adjusted jumping performance with age,
39 and modification to the correlation structure among traits across ontogeny. However, we also
40 find that significant links between form and function evident in young animals disappear at later
41 ontogenetic stages. Our study suggests that different functional mechanisms may be associated
42 with high performance at different stages of development.

43
44 **Keywords:** development, *Kryptolebias*, morphology, performance, terrestrial locomotion

45 **Introduction**

46 Animals must rely on numerous fitness-related performance characteristics (e.g., running,
47 jumping, biting, swimming) to survive, reach adulthood, and ultimately reproduce
48 (Irschick, Meyers, Husak, & Le Galliard, 2008; Wilson, Husak, Halsey, & Clemente, 2015;
49 Meyers and Irschick, 2015). Among-individual variation in these performance characteristics can
50 result from variation in the underlying morphology (Huckins, 1997). However, the link between
51 morphology and performance is often quite complex, as many different anatomical arrangements
52 among traits may provide the same level of performance (Wainwright, Alfaro, Bolnick, &
53 Hulseley, 2005). As a result, the evolution of performance characteristics may depend on the
54 extent to which suites of morphological traits are integrated with one another and with
55 performance (Stadler, Stadler, Wagner, & Fontana, 2001).

56 The link between form and function is further complicated by ontogeny because both
57 performance and morphology can vary as organisms grow and develop (Gignac and Santana,
58 2016; Herrel et al., 2016). Size is one factor that can lead to performance variation across
59 ontogeny (Webb, Kostecki, & Stevens, 1984). Juveniles always have a much smaller absolute
60 body size, which can lead to lower levels of performance relative to adults (Carrier, 1996).
61 Independent of body size, however, physiological limitations may also impose performance
62 constraints on young animals; for instance, young vertebrates may have yet to develop fully
63 ossified bones, strong muscles, and complex neural networks needed for high performance
64 (Carrier, 1996). Indeed, studies have found that underdeveloped physiology may be associated
65 with lower levels of performance in young animals (Erickson, Lappin, & Vliet, 2003; Noren,
66 Bidenbach, & Edwards, 2006; Galloway, Anderson, Wilga, & Summers, 2015). Whether due to
67 a smaller body size or some physiological limitation, lower levels of performance in juveniles

68 often makes them more prone to predation and less likely to acquire scarce resources (Fernandez
69 and Motta, 1997; Herrel and Gibb, 2006).

70 Estimating links between morphology and adult performance in a quantitative fashion has
71 enabled a greater understanding of the functional, ecological, and evolutionary implications of
72 morphological diversity (Carroll, Wainwright, Huskey, Collar, & Turingan, 2004).

73 Characterizing age-dependence in patterns of morphological and performance (co)variation
74 should add to such insights by highlighting how key aspects of life history (e.g., reproductive
75 status (Bauwens and Thoen, 1981) or ontogenetic stage-dependent diet (Hale, 1999; Gignac and
76 Erickson, 2015)) can alter the relationship between form and function. For instance, Herrel, Van
77 Damme, & Vanhooydonck (2001) found that as bite performance increases and head shape
78 changes across ontogeny in the western green lizard (*Lacerta bilineata*), so too does their
79 preferred diet (i.e. individuals shift from eating grasshoppers and crickets as juveniles to beetles
80 and isopods as adults). Without a thorough understanding of the relationships between
81 morphology and performance across ontogeny, we may miss key ecological and functional
82 insights into morphological diversity, and few studies have investigated this question (McHenry
83 and Jed, 2003).

84 An ideal model organism for examining ontogenetic variance in form and function is the
85 mangrove rivulus fish, *Kryptolebias marmoratus*. This species shows considerable
86 morphological variation across development (Grageda, Sakakura, & Hagiwara, 2004), and also
87 exhibits an extraordinary, and understudied, mode of terrestrial locomotion known as the ‘tail-
88 flip jump’ (Fig. 1). This behavior may have evolved in response to high tidal fluctuations
89 (Taylor, 2012; Magellan, 2016), as a means to escape potential aquatic predators (Taylor, 2012)
90 and/or intraspecific competition (Taylor, 2000), or to exploit high abundance of terrestrial prey

91 (Pronko, Perlman, & Ashley-Ross, 2013). Jumping performance can also be easily quantified in
92 a laboratory environment by simply probing the fish while on a moist substrate (Gibb, Ashley-
93 Ross, Pace, & Long, 2011; Gibb, Ashley-Ross, Hsieh, 2013; Ashley-Ross, Perlman, Gibb, &
94 Long, 2014).

95 Although the specific mechanism associated with the evolution of the tail-flip jump is not
96 clear since many divergent, ‘non-amphibious’ species (*Danio*, *Fundulus*, *Gambusia*, and *Betta*)
97 possess the ability to tail-flip jump (Gibb, Ashley-Ross, Hsieh, 2013, Ashley-Ross, Perlman,
98 Gibb, & Long, 2014, Bressman, Farina, & Gibb, 2016), the incredible jumping ability of the
99 mangrove rivulus is thought to be an adaptation to life on land within strongly tidal mangrove
100 ecosystems (Pronko, Perlman, & Ashley-Ross, 2013). In times when water quality is poor (e.g.
101 high hydrogen sulfide levels, low level of dissolved oxygen), mangrove rivulus may jettison
102 from aquatic habitats onto land, where the tail-flip jump allows fish an effective means of
103 locomotion until another temporary pool (or wet leaf litter) is found (Abel, Koenig, & Davis,
104 1987). The tail-flip jump is characterized by the fish bending its axial skeleton into a “C” shaped
105 position, transferring its body weight to the caudal peduncle (i.e. the area of the body from the
106 end of the dorsal and anal fins to the caudal fin) and launching off the substrate through the air,
107 often many times its body length (Ashley-Ross, Perlman, Gibb, & Long, 2014) (Fig. 1).

108 Here, we aim to determine how the relationship between morphological form and
109 function changes throughout ontogeny in mangrove rivulus. We focus on morphological
110 variation associated with the parahypural, epural, and hypural bones (Fig. S1) because force must
111 be transmitted through these structures to the substrate just prior to the animal leaving the ground
112 (Gibb, Ashley-Ross, Hsieh, 2013). Thus, changes in the dimensions of these bones (in both their
113 lengths and the angles between them) may increase the surface area of the caudal peduncle in

114 contact with the ground, thereby allowing for the displacement of greater ground reaction forces
115 (Dial and Jackson 2011). We hypothesize that, as age increases, i) jumping performance (in body
116 lengths) will increase with the growth and development of performance-related morphological
117 characteristics, ii) the amount of variation for each trait will change, and iii) the patterns of
118 covariation/correlation among traits – which tell us about the integration of form with function –
119 will also change. We take a multivariate approach to estimating (co)variance among a set of
120 seven morphological traits, and determining how this covariance structure is associated with
121 performance across three age groups.

122

123 **Materials and methods**

124 *Animal Care and Specimen Collection*

125 Mangrove rivulus fish (*Kryptolebias marmoratus* Poey 1880) are one of only two known self-
126 fertilizing hermaphroditic vertebrates, the other being its recently described sister species *K.*
127 *hermaphroditus* (Costa, 2016); however, this species may in fact be a species complex
128 containing two other species (Tatarenkov et al. 2017). With each generation of exclusive selfing,
129 heterozygosity is reduced by, on average, 50% and ultimately produces completely homozygous
130 individuals whose progeny are genetically identical to the parent and all siblings (i.e., an isogenic
131 line). A total of 237 *K. marmoratus* aged 24 to 1716 days post hatching (DPH) were obtained
132 from progenitor (F1-F12 generation) lines as a result of self-fertilization and were incorporated
133 into the experiment. We divided these individuals into 3 groups based on age: <120 DPH (n=54),
134 250-500 DPH (n=99), and >500 DPH (n=84). These age groups were chosen because they reflect
135 important transitions in the life history of *K. marmoratus*: before reproductive maturity (<120
136 DPH; referred to as ‘young’ below), ‘peak’ reproduction (250-500 DPH; referred to as ‘mature’

137 below), and declining investment towards reproduction (>500 DPH; referred to as 'old' below),
138 based on 10 years of lab data (R.L. Earley, unpublished data). Progenitors were kept under
139 common garden conditions (12 hours light: 12 hours dark photoperiod at $26\pm 1^\circ\text{C}$ and in 25 ppt
140 saltwater).

141 Progenitors were housed in 750mL Rubbermaid® TakeAlong® Deep Squares with a small
142 piece of spawning substrate (i.e. Poly-Fil® pillow fuzz), and were fed 4 mL of brine shrimp
143 nauplii (*Artemia* spp.) daily. The spawning substrate was checked weekly for eggs. Each clutch
144 of eggs was stored in a 59 mL clear, polystyrene cup filled with 25 ppt water until hatching. Eggs
145 were grouped according to the date on which they were laid, their generation, and the parental
146 line. We conducted complete water changes on each egg cup once per week to refresh the water.
147 Egg cups were checked for hatchlings daily. Any hatchlings were removed from the cup and
148 placed in a 750 mL Rubbermaid® TakeAlong® Deep Squares filled with 25 ppt water. All
149 pertinent information (population, genotype, generation, date egg laid, and date hatched) was
150 maintained with each hatchling upon relocation to the 750 mL containers. Although the genotype
151 is known, we did not have enough replicates per genotype (46 genotypes with 1-6 individuals per
152 genotype) to characterize genetic variance within and covariance among traits. As a result, we
153 focus the current study on questions of phenotypic integration (i.e. patterns of correlations
154 among functionally related traits) only.

155 Because the time spent developing within the egg affects locomotor performance in other
156 systems (Shine and Olsson, 2003), we recorded this parameter for all individuals and
157 subsequently used it as a covariate in our statistical models (as described in full below). All eggs
158 and hatchlings were kept under the same common garden conditions as the progenitors. Animals

159 were incorporated into the experiment at various ages, spanning a wide developmental spectrum
160 from 24-1716 DPH.

161

162 *Jumping performance trials*

163 *K. marmoratus* individuals exist as either self-fertilizing hermaphrodites or as males in natural
164 populations (Harrington, 1961). Because performance can be sex-specific (Berwaerts,
165 Matthysen, & Dyck, 2008) and because self-fertilizing hermaphrodites predominate in natural
166 populations (Turner, Fisher, Taylor, Davis, & Jarrett, 2006), we excluded any males from the
167 jumping trials. Males were excluded based on sexually dimorphic characteristics (i.e. the
168 presence of orange freckling anywhere on the body) (Soto and Noakes, 1994; Scarsella,
169 Gresham, & Earley, 2018).

170 Jumping trials were conducted in a Sizzlin' Cool Blue Wading Kiddie Pool[®] (914.4 mm
171 diameter) lined with damp paper towels. The damp paper towels were used to simulate moist
172 substrate (i.e. leaf litter) commonly found in the mangrove habitat and also to prevent desiccation
173 during timed trials (Gibb, Ashley-Ross, Pace, & Long, 2011). To capture jumping performance
174 for all individuals, we used a Kodak[®] PlaySport camera, which was supported by PVC pipes, and
175 positioned directly above the pool at a distance of 145 cm. The PVC pipes were lowered to 102
176 cm for especially small fish that could not be seen on camera at a distance of 145 cm. We used
177 the pool, which had a diameter of 914.4 mm, as a scale in each video. A tarp, which minimized
178 any external stimuli, except from the prober (see below), bordered the jumping arena. We began
179 filming when one fish was transferred by netting from its individual housing unit onto the moist
180 paper towels in the center of the pool. Each trial lasted a total of two minutes. For the first
181 minute, fish were allowed to acclimate to the kiddie pool. During this time, no jumps were

182 recorded. During the second minute, fish were continually probed using the handle of an
183 aquarium fish net (25.4 cm JW[®] Pet SmartNet Fine Nylon Mesh Aquarium Fish Net) to
184 stimulate jumping. The vast majority of fish jumped when approached by the handle. However,
185 if the fish failed to jump when approached, they were gently touched on the snout to elicit a
186 jump. Following the performance trial, fish were immersed in a lethal dose of sodium
187 bicarbonate buffered pharmaceutical-grade MS-222 (Finquel[®]), and stored individually in 15 mL
188 centrifuge tubes filled with 100% ethanol until morphological assessment. All animal care was
189 done in accordance with The University of Alabama's Institutional Animal Care and Use
190 Committee (IACUC) (Protocol #:14-05-0070).

191

192 *Jumping performance assessment*

193 Jumping performance videos were converted from .MP4 into .AVI format using iSkysoft[®] Video
194 Converter and decompressed using VirtualDub, an open source software available via
195 SourceForge (www.virtualdub.org). The decompressed videos were imported into ImageJ
196 (Schneider, Rasband, & Eliceiri, 2012) as a collection of individual frames (i.e. virtual stack).
197 Each video was scaled to mm using the known diameter of the pool (914.4 mm) and advanced
198 frame by frame until just prior to the first jump ('Preparation' in Fig. S2). The location of the
199 head and tail were landmarked using the 'point' function in ImageJ. The video was then
200 advanced until the first jump was completed ('Post-Jump' in Fig. S2), and the location of the
201 head and tail were landmarked again. The process continued until the locations of the head and
202 tail before and after each jump were recorded during the probing segments of the trial. These
203 coordinate data were then exported into Microsoft Excel[®], where jumping distance (mm) was
204 calculated as the sum of the squared differences between the pre-jump and post-jump locations

205 of the head (DHM) or tail (DTM). For each jump, we obtained 2 measures: distance head moved
206 (DHM) and distance tail moved (DTM). However, given a strong correlation between these two
207 measures ($r=0.833$, $DF=5953$, $P<0.0001$), we elected to present analyses of DHM only in full
208 here. Results obtained from analyses (as described below) of DTM were very similar (see
209 Appendix 1).

210

211 *Morphological assessment*

212 A clearing and staining technique, modified from Webb and Byrd (1994), which yields a
213 transparent specimen with bones stained deep red, was used to assess morphological traits (Fig.
214 S1). Specimens first were placed into a 1:1:18 staining solution of 0.1% Alcian blue: 0.1%
215 Alizarin red S: 70% EtOH buffered with 40g of potassium hydroxide phthalate for 48 hours.
216 Specimens were then transferred to a 1.5% KOH solution for 48 hours and a 2:2:1 solution of
217 glycerol: 70% EtOH: Benzyl alcohol for 24 hours before being transferred to a 1:1 solution of
218 glycerol: 70% EtOH for storage.

219 Specimens were photographed using a Canon® Powershot G-9® camera under a Zeiss
220 Stemi-C 2000 stereoscope in standard ichthyological position next to a metric ruler. Photographs
221 were imported into ImageJ and a scale was set to mm using the metric ruler within the image
222 (Schneider, Rasband, & Eliceiri, 2012). Standard length (SL), length and angle of the epural
223 (EPL and EPA), length and angle of the parahypural (PHPL and PHPA), and length and width of
224 the hypurals (HYPL and HYPW) were measured (Fig. S1). To maintain the integrity of bones,
225 we did not assess individual reproductive status because this would require dissection. We
226 acknowledge that this may have influenced variation in jumping performance among individuals
227 (Iraeta, Salvador, Monasterio, & Diaz, 2010).

228

229 *Overview of statistical analysis*

230 We analyzed all data using univariate and multivariate linear mixed effects models, fit with
231 ASreml-R 3.0 (Gilmour, Cullis, Gogel, Welham, & Thompson, 2002; Butler, 2009) in R version
232 3.4.1 (R Core Team, 2017). Univariate models were used to test our hypothesis that jumping
233 performance (in body lengths) changes with age. We then used multivariate models to estimate
234 the among-trait phenotypic (co)variance matrices (subsequently denoted \mathbf{P}). We tested
235 hypotheses about trait integration (among morphological traits, and between all morphological
236 traits and performance) and its stability across age by summarizing and comparing age-specific
237 estimates of \mathbf{P} . These analyses are described in detail below.

238 A feature of our mixed model analyses is that they allowed all jumping performance data
239 to be utilized while accounting for non-independence of repeated jumps (within-trials) made by
240 individual fish. Although some studies utilize a single maximum measure of performance when
241 linking form to function (e.g. Herrel, Podos, Huber, & Hendry, 2005), we prefer to include
242 multiple jumps for each individual for two reasons. First, maximum performance values can bias
243 correlation estimates, especially when individuals differ in the number of repeated measures
244 (Careau and Wilson, 2017a). Second, ‘trade-offs’ (negative correlations between traits that
245 contribute positively to fitness) that occur within individuals may be hidden by among-individual
246 variation (Careau and Wilson, 2017b). Having obtained repeated measures of DHM for each
247 individual, we were able to partition among- from within-individual variation in jumping
248 performance and estimate the relationships between morphology and the repeatable part of
249 jumping performance using procedures outlined in Houslay and Wilson (2017).

250 Prior to analysis, all traits were scaled to standard deviation units, with standard
251 deviations determined from observations of all fish (i.e. across all ages). Scaling traits in this way
252 assists multivariate model fitting as all response variables are on a common scale (where a single
253 unit of a scaled trait is equivalent to a standard deviation in the raw data). Pragmatically, by
254 simply dividing all trait values by the relevant standard deviation, we retain any differences in
255 trait means and/or (co)variation structure among ages. It also prevents trait-specific scaling from
256 complicating the interpretation of estimated among-trait phenotypic covariance matrices (**P**) as
257 described below.

258

259 *Univariate models to test age effects on performance*

260 First, we tested whether jumping performance (in body lengths) changed as a function of age
261 independent of time spent in embryonic development. For this analysis we used a size-adjusted
262 measure of jumping performance, DHM in body lengths (i.e. DHM/SL) as the response variable,
263 and age and developmental time within egg as fixed (continuous) predictors. Individual ID was
264 included as a random effect. We scaled our measure of jumping performance by body size
265 instead of including SL as a covariate in our model because age and SL were significantly
266 correlated ($r=0.43$, $DF=234$, $p<0.0001$). We note that scaling performance by body size may
267 complicate any biological interpretation of random effect variance in this case (see Wilson
268 2018). However, here we simply seek to control for non-independence because, while each
269 individual was observed at a single age only, the data set contains multiple jumps per individual
270 trial. We also report on a model that included non-scaled measures of jumping performance
271 (DHM) as the response variable, and age, standard length (SL), and developmental time within
272 egg as fixed (continuous) predictors (see Results) to determine whether age remains a significant

273 predictor of jumping performance despite variation in body size. We tested for a significant
274 effect of age using a Wald F-test.

275

276 *Estimation of age-class specific \mathbf{P} matrices*

277 To test our second and third hypotheses that variance/covariance within and among
278 morphological and jumping performance traits changes over development, we fitted a
279 multivariate mixed model with all seven morphological traits and DHM as response variables for
280 each of our 3 age classes. This enabled us to estimate the among-trait phenotypic variance-
281 covariance matrix (8x8 matrix) which we denote for age class X as \mathbf{P}_x . \mathbf{P} -matrices contain
282 estimates of the phenotypic variance for each trait on the diagonal and phenotypic covariances
283 among traits on the off-diagonals, and can be used to determine how the combined effect of the
284 environment and genes has influenced variance within and covariance among traits (Arnold,
285 1981). Fixed effects included a mean on each trait, time spent developing within the egg, and
286 jumping sequence (for DHM only), because fish appeared to tire (i.e. produce shorter jumps) as
287 they continued to jump (Fig. S3). We did not include the total number of jumps made by each
288 individual in the model because these data may be the result of ‘prober bias’ (i.e. the ability of
289 the prober to, in a timely manner, immediately probe the fish as it changed locations within the
290 jumping arena). We included standard length (SL) as one of our response variables in our
291 multivariate mixed models. By doing so, we are also able to estimate how body size variation
292 within each age group related to performance and morphological variation.

293 A random effect of individual was included on all traits, with the among-trait covariance
294 (conditional on fixed effects) then estimated as \mathbf{P}_x for each age class x . Note that as repeat
295 measures are available for DHM only, within-individual variances for (and covariances with) all

296 morphological traits cannot be estimated and therefore were constrained to zero. The within-
297 individual variance for DHM is estimable and attributable to behavioral plasticity and/or
298 measurement error. In practice, including the random effect of individual allows us to partition
299 out within-individual variance as a residual term for DHM. This allows us to interpret \mathbf{P}_x as an
300 among-individual covariance matrix, although we note that, strictly speaking, variance in
301 morphological traits will also include any measurement error as they are assayed only once per
302 individual. Each estimate of \mathbf{P}_x (i.e., $\mathbf{P}_{\text{young}}$, $\mathbf{P}_{\text{mature}}$ and \mathbf{P}_{old}) was also scaled to its corresponding
303 correlation matrix to further aid interpretation. To obtain approximate 95% confidence intervals
304 around each element of \mathbf{P}_x (and the corresponding correlation matrices) we used a parametric
305 bootstrap approach (described in Boulton et al., 2015) to generate 5,000 random draws for each
306 matrix. The resulting confidence intervals are necessarily approximate and rely on an assumption
307 of multivariate normality (see Boulton et al., 2015; Houle and Meyer, 2015 for discussion).

308

309 *Structure of \mathbf{P}_x within and among age classes*

310 Having estimated \mathbf{P}_x at each age several approaches were taken to summarize the
311 covariance/correlation structure and to compare it across age-groups. Bootstrapped samples (as
312 described above) were used to obtain 95% confidence intervals on all metrics of matrix
313 differentiation (see below) and statistical significance at a nominal value of $\alpha=0.05$ concluded
314 when the range of these intervals did not include zero. First, we used the mean squared
315 correlation as a measure of morphological trait integration. This was estimated at each age using
316 the correlations among morphological traits only (i.e., excluding correlations involving DHM).
317 We then estimated the difference (with 95% CI) in mean squared correlations between each pair
318 of age classes. Second, we repeated these steps but using the morphology-DHM correlations, to

319 test for significant integration of morphology and performance within each age group and to test
320 for differences among ages. Third, using the bootstrap samples of each \mathbf{P}_x we tested for
321 significant differences in pairwise trait-correlations (r) between age groups (x). Note that for any
322 pair of traits (i, j) stability of correlation structure across ages leads to the simple null expectation
323 that, for instance, $r_{\text{young}(i,j)} - r_{\text{young}(i,j)} = 0$ (see Houslay, Vierbuchen, Grimmer, Young, &
324 Wilson, 2017). Because pairwise trait-correlations can differ under similar overall levels of
325 integration (i.e. mean squared correlations), this test allowed us to describe finer scale
326 differences between the ages. Fourthly, we estimated and compared the total amounts of
327 phenotypic variation across ages, noting that total (multivariate) phenotypic variance is equal to
328 the trace (i.e. sum of diagonal elements) of each matrix \mathbf{P}_x . Finally, for jumping performance
329 only (DHM), partitioning among- and within-individual variance components in the multivariate
330 models allowed us to estimate repeatability in each age class and compare repeatabilities across
331 age classes. Age-specific repeatabilities of jumping performance were estimated conditional on
332 fixed (effects of jumping sequence and time spent developing in the egg) as the ratio of among-
333 individual variance to the sum of among- and within-individual variance.

334

335 **Results**

336 *Age effects on jumping performance in body lengths*

337 Our univariate mixed model indicated that, as hypothesized, there was a significant positive
338 relationship between jumping performance (body lengths) and age in *K. marmoratus* (Slope
339 $=6.1 \times 10^{-4}$; Wald's $F_{1,5952} = 31.8$, $p < 0.0001$) (Fig. 2). Within this mixed model, time spent in
340 embryonic development did not have a significant effect on jumping performance (Slope=
341 7.7×10^{-4} ; Wald's $F_{1,5952} = 0.552$, $p = 0.45$). We note that when jumping performance (DHM) is

342 not size-adjusted and SL is included in the model as a covariate, age remains significant (Wald's
343 $F_{1,5951}=59.6$, $p<0.0001$), despite a very strong effect of SL (Wald's $F_{1,5951}=40.0$, $p<0.0001$).

344

345 *Integration of morphology traits*

346 Within each age group, estimates of \mathbf{P} provided evidence of extensive covariance/correlation
347 structure among morphological traits (Table 1) consistent with significant integration.

348 Summarized as the mean-squared correlation among morphological traits, integration differed
349 among classes (Fig. 3A), being highest in young fish (Estimate:0.353, bootstrapped 95% C.I.:
350 0.287-0.439) and lowest (Estimate:0.211, bootstrapped 95% C.I.: 0.178-0.254) in mature fish.

351 The difference between mean squared correlations was significant (i.e. the bootstrapped
352 confidence interval did not cross zero) in this comparison (95% C.I.: -0.272 to -0.072), but not
353 between either of these age groups and the old fish, which exhibited an intermediate mean
354 squared correlation (95% C.I. for the differences were -0.211 to 0.006 for young vs. old and -
355 0.005 to 0.123 for mature vs. old).

356

357 *Association of form and function*

358 We found significant correlation structure between morphological traits and the repeatable (i.e.
359 among-individual) component of jumping performance (DHM) only in the young age class
360 (Table 1). In this age group, jumping performance (measured in absolute distance, not body
361 lengths) was significantly higher in individuals with longer EPL and HYPL, but smaller EPA
362 (Fig. 4). Although the magnitude of correlations between jumping performance and SL and
363 PHPL (estimated as 0.24 and 0.27 respectively) in the young age group were similar to the
364 magnitude of correlations between jumping performance and EPL, HYPL and EPA, the 95%

365 confidence interval included zero in both former cases so we considered these relationships non-
366 significant. Although morphology and performance were only correlated in the young group,
367 there was not a significant difference among age classes in levels of integration (mean squared
368 correlation) between morphology (all traits) and performance: young-mature (-0.19 to 0.050),
369 young-old (-0.192 to 0.034), and mature-old (-0.069 to 0.049) (Fig. 3B).

370

371 *Further comparisons of **P** among-age groups*

372 Comparison of individual elements of the age-specific correlation matrices provided further
373 statistical support for differences in **P** among ages (Tables 1 and 2). Of the 28 pairwise trait
374 correlations, 12 differed significantly between young and mature, 4 between young and old and
375 10 between mature and old. One notable source of variation across age classes stems from the
376 correlations of EPA with other traits. These were strongly negative and significant in the young
377 age class, but tended to be weak and not significant in mature and old fish (Table 1). All of the
378 pairwise correlation estimates involving EPA differed significantly between young and mature
379 fish, while three of seven also differed between young and old age groups (Table 2). However,
380 many relationships, especially among morphological traits, were at least qualitatively conserved.
381 For instance, in each age group: i) HYPL was significantly positively correlated with EPL,
382 PHPL, HYPW, and SL; ii) HYPW was positively correlated with EPL, PHPL, and SL; iii) PHPL
383 was positively correlated with EPL and SL; and iv) EPL was positively correlated with SL
384 (Table 1). A significant negative correlation between parahypural and epural angles (PHPA and
385 EPA) was also maintained across ontogeny (Table 1).

386 There were no significant differences among ages in the traces (i.e. sum of the variance
387 components across all traits) of **P** (bootstrapped 95% C.I. of trace differences were; -2.38 to 1.62

388 young vs. mature; -1.06 to 3.43 young vs. old; -0.200 to 3.26 mature vs. old). Thus, each age
389 group displays similar levels of total variance in the full multivariate phenotype (Fig. S4).
390 However, for jumping performance, we found that the repeatability (i.e. ratio of among
391 individual variance to the sum of among- and within-individual variance) was significantly
392 higher in the young group, as determined by no overlap in the 95% CI between young and
393 old/mature: ($R_{\text{young}} = 0.582$ (95% CI: 0.529-0.636); $R_{\text{mature}} = 0.317$ (95% CI: 0.279-0.355); $R_{\text{old}} =$
394 0.287 (95% CI: 0.247-0.327) (Fig. 5).

395

396 **Discussion**

397 Overall, we found that age had a strong influence on variation in, and covariation among,
398 morphological and performance traits. Integration (i.e. strength of correlation structure among
399 functionally related traits) across morphological traits was found in all age groups, but was
400 highest in young fish, and the association of morphology with performance was also strongest in
401 this age group. In contrast, morphology-performance associations were less evident, and not
402 statistically significant, in mature or old fish classes (although we note our overall estimates of
403 integration between form and function do not themselves differ significantly among age-classes).

404 Age was also a significant predictor of jumping performance means. In particular we
405 found evidence that jumping performance (as measured in body lengths) increases as a function
406 of age. Although young fish had lower jumping performance relative to mature or old fish, they
407 still were able to jump several to many times their own body length. The maintenance of high
408 jumping performance in *K. marmoratus* across ontogeny can reflect strong selection on jumping
409 performance in young as well as adult (i.e. mature and old) fish. This seems likely considering
410 high jumping performance would provide a means to escape predators (Carrier, 1996; Herrel and

411 Gibb, 2006), and also to find new aquatic habitats during low tide or when water conditions
412 become suboptimal (Taylor, 2012). Repeatability (i.e. proportion of total phenotypic variance
413 due to among-individual differences) of jumping performance was high for young fish (Fig. 5),
414 indicating that variance in this trait may be due to genetic differences among individuals at this
415 age. At the same time, correlations among functionally related traits (i.e. EPA, EPL, and HYPL)
416 were strong and in a direction that would facilitate future phenotypic responses to selection on
417 jumping performance. EPL/HYPL were negatively related to EPA (a trait that was negatively
418 correlated with jumping performance) and EPL was positively related to HYPL (traits that are
419 both positively related to jumping performance). Taken together, these results provide strong
420 evidence that future positive selection on jumping performance may be facilitated by abundant
421 genetic variance and favorable integration among functionally important traits in young fish.

422 Several studies have found that strong integration among traits may constrain future
423 responses to selection (Goswami, 2006; Wroe and Milne 2007). However, when integration
424 among traits is in the direction of selection (e.g. all traits are positively correlated to one another
425 and are under positive selection), it may facilitate evolution. For example, Vincent, Dang, Herrel,
426 & Kley (2006) demonstrated that high integration among head width, jaw length, and out-lever
427 length may have facilitated the evolution of high feeding performance in terms of maximum
428 consumed prey mass in macrostomatan snakes (Vincent, Dang, Herrel, & Kley, 2006). Future
429 work should look to determine if variation in jumping performance is due to genetic factors and
430 if genetic correlations among these functionally important traits might also facilitate future
431 responses to natural selection.

432 The loss of strong correlations between the axial skeleton and terrestrial jumping in the
433 adults (i.e. mature and old groups) suggests the potential for performance-related, physiological

434 differences between juveniles and adults. One possibility is that age-dependent variance in
435 musculoskeletal morphology might drive age-dependent jumping performance (Stiller and
436 McBrayer, 2013). Given that young fish are still developing complex sensory-motor systems and
437 anaerobic power in fast muscle fibers (Blaxter 1986; Wieser 1995), the skeletal system in young
438 individuals may be playing a greater role in influencing jumping performance than in older age
439 groups. At maturity, however, once complex functionally-related systems have formed (i.e.
440 efficient fast muscle fibers and connective tissue), the skeletal system may be less involved,
441 which could lead to a decrease in phenotypic integration (manifested as the loss or weakening of
442 correlations) between the axial skeleton and jumping performance as the animals mature. It is
443 important to note that, in our study, fish were raised solely in water with no access to a terrestrial
444 habitat. Thus, bone and muscle development could have influenced how we have come to
445 understand the ontogenetic relationship between skeletal morphology and jumping performance
446 in this species. A further avenue of study might be to assess the link between form and function
447 when animals are permitted to emerge (i.e. leave the water) and locomote on land at will
448 throughout ontogeny.

449 While there was among-individual variation in performance in juveniles, this was also
450 true in mature and old fish. Variation in performance at adulthood might also be subject to other
451 influences, both internal and external. For instance, an individual's investment in reproduction
452 once it reaches sexual maturity may limit its locomotor abilities, reflecting a life history 'trade-
453 off' between reproductive investment and performance. It is possible that we might also observe
454 a life history 'trade-off' between reproduction and terrestrial jumping performance in the
455 mangrove rivulus. In this species, reproduction can occur year-round (Lomax, Carlson, Wells,
456 Crawford, & Earley, 2017), and the gonad can make up 12% of their body mass (Johnson,

457 Weinersmith, & Earley, 2016). Likewise, because hermaphrodites tend to lay single to several
458 embryos on a regular basis (Lomax, Carlson, Wells, Crawford, & Earley, 2017), high
459 reproductive investment may place a continual burden on jumping performance in sexually
460 mature individuals. There may also be seasonal variation in rates of egg laying in *K.*
461 *marmoratus*, so this ‘trade-off’ may be more apparent at certain times of year (Harrington,
462 1968). ‘Trade-offs’ appear to explain at least some age-dependent phenotypic variance in other
463 model systems. For instance, under laboratory conditions, Ghalambor, Reznick, & Walker
464 (2004) found that escape performance was negatively correlated with reproductive investment in
465 Trinidadian guppies from high predation sites, reflecting a ‘trade-off’ between swimming
466 performance and reproduction. Future studies could include variation in body condition or
467 reproductive status as a covariate in their statistical models when assessing performance variance
468 among age classes.

469 In young animals, we found that better jumpers had longer hypurals and epurals and
470 shorter angles between the epural and vertebral column (Fig. 4). These relationships were most
471 often either reversed or not significant in the mature and old age groups, respectively. One study
472 (Ashley-Ross, Perlman, Gibb, & Long, 2014) alluded to the importance of large, ‘localized
473 structures’, specifically the hypurals (in our study, HYPL and HYPW), in tail-flip jumping.
474 Ashley-Ross, Perlman, Gibb, & Long (2014) emphasized that larger (i.e. more ‘robust’) hypurals
475 may provide a larger, more stable platform needed for adequate propulsion off of a terrestrial
476 substrate. Our study, in large part, seems to be in agreement with the hypothesis proposed by
477 Ashley-Ross, Perlman, Gibb, & Long (2014), at least for young animals. Additionally, however,
478 our study found evidence that long epurals are also associated with longer jumps (Fig. 4). As a
479 result, in young fish, long hypurals may facilitate strong jumping performance only when they

480 are close to long epurals. In this way, the epural may act as a critical extension to the jumping
481 platform created by the hypurals. An extended jumping platform may increase the area of the
482 caudal peduncle in contact with the ground just prior to jumping, and thus, increase ground
483 reaction forces.

484 Determining how terrestrial jumping performance is related to other performance
485 characteristics, such as swimming and emersion (i.e. transitioning from water to land) should be
486 another goal of future research. Terrestrial jumping may be positively related to some aspects of
487 swimming performance like the C-start escape, which appears qualitatively similar to the tail-flip
488 jump (Perlman and Ashley-Ross, 2016), but negatively related to others like burst swimming,
489 which involve morphologies that might not be conducive to jumping (i.e. more streamlined
490 caudal peduncles and narrower heads) (Langerhans, Layman, Shokrollahi, & DeWitt, 2004).
491 Other performance characteristics related to emersion, such as launches and ‘squiggles’, a form
492 of terrestrial locomotion in which the fish oscillates its head and tail over moist substrate
493 (Pronko, Perlman, & Ashley-Ross, 2013), may require a wholly different functional architecture,
494 and may limit other locomotor performances (Pronko Pronko, Perlman, & Ashley-Ross, 2013).

495 Jumping performance is a complex trait involving multiple physiological systems (e.g.
496 respiratory, cardiovascular, and musculoskeletal). Here, we concentrated on relating variance in
497 one system (i.e. the skeletal system) to terrestrial jumping performance across ontogeny, and we
498 found significant correlations between bones and jumping performance in young fish. Future
499 research should detail how components of other physiological systems affect the kinematics of
500 terrestrial jumping performance in *K. marmoratus* (Perlman and Ashley-Ross, 2016), especially
501 in the mature and old age groups where the skeletal system seems to be playing a minimal role.
502 For instance, it might be profitable to examine mitochondrial density in white skeletal muscle

503 tissue and rates of fatty acid metabolism (Moyes, Mathieu-Costello, Brill, & Hochachka, 1992)
504 across ontogeny to see if they may be related to differences in jumping performance. All of these
505 avenues should give us a better idea of how ontogenetic variance in jumping performance has
506 evolved, and a clearer understanding of the morphological variance associated with locomotion
507 in fish.

508 We uncovered three important findings. First, performance in terms of body lengths
509 jumped changes with age. Second, morphological integration changes with age. Third,
510 integration among form and function is only convincingly present in younger fish. These results
511 highlight the need to take an ontogenetic approach in evolutionary studies if we are to understand
512 the functional, ecological, and evolutionary implications of morphological diversity. As
513 ontogenetic variance in all types of phenotypic traits (i.e. behavior, morphology, physiology, and
514 life-history) continues to be described, ultimate explanations (e.g. life history ‘trade-offs’,
515 differential selection, etc.) are required to explain the origin of such changes across development.

516

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525

526 **Competing Interests:**

527 No competing interests declared.

528

529 **Data Accessibility**

530 Data and R script are deposited within Dryad (Link provided upon publication).

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713

714 **Table 1:** Age-dependent partial phenotypic (**P**) correlation matrices for: young (top), mature (middle), and old (bottom) age groups

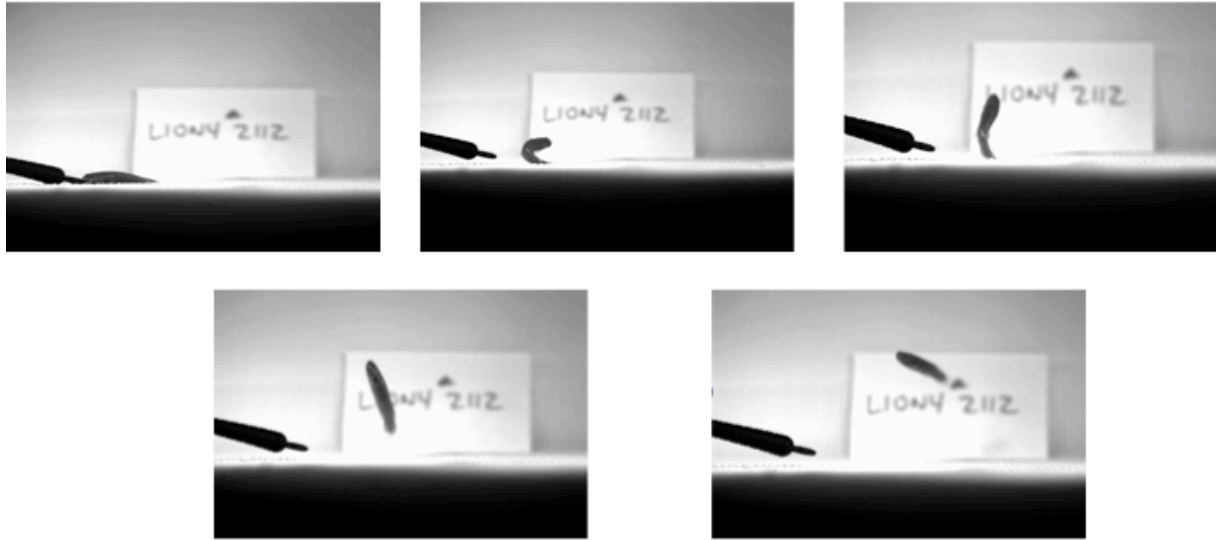
<u>Young</u>	EPL	EPA	PHPL	PHPA	HYPL	HYPW	SL	Jumping
EPL	0.89 (0.58,1.25)	-0.48 (-0.7,-0.27)*	0.92 (0.88,0.96)*	0.23 (-0.04,0.5)	0.92 (0.87,0.96)*	0.84 (0.75,0.92)*	0.9 (0.84,0.95)*	0.29 (0.02,0.54)*
EPA	-0.38 (-0.62,-0.14)	0.7 (0.43,0.98)	-0.47 (-0.7,-0.26)*	-0.59 (-0.78,-0.4)*	-0.4 (-0.62,-0.14)*	-0.39 (-0.62,-0.14)*	-0.44 (-0.66,-0.19)*	-0.29 (-0.55,-0.01)*
PHPL	0.79 (0.47,1.08)	-0.36 (-0.58,-0.13)	0.82 (0.52,1.13)	0.28 (0.01,0.54)*	0.92 (0.87,0.96)*	0.83 (0.73,0.91)*	0.88 (0.81,0.94)*	0.24 (-0.06,0.5)
PHPA	0.21 (-0.03,0.47)	-0.47 (-0.74,-0.22)	0.24 (-0.01,0.48)	0.91 (0.56,1.25)	0.25 (-0.01,0.52)	0.3 (0.02,0.55)*	0.34 (0.07,0.58)*	0.09 (-0.2,0.39)
HYPL	0.85 (0.53,1.19)	-0.33 (-0.56,-0.09)	0.81 (0.48,1.12)	0.24 (-0.02,0.51)	0.96 (0.58,1.31)	0.85 (0.76,0.92)*	0.91 (0.86,0.95)*	0.38 (0.11,0.62)*
HYPW	0.69 (0.4,0.97)	-0.29 (-0.5,-0.07)	0.66 (0.39,0.94)	0.25 (0.01,0.48)	0.73 (0.41,1.01)	0.76 (0.48,1.07)	0.81 (0.71,0.91)*	0.16 (-0.13,0.44)
SL	0.86 (0.54,1.21)	-0.37 (-0.63,-0.12)	0.81 (0.48,1.12)	0.32 (0.04,0.6)	0.91 (0.53,1.25)	0.72 (0.41,1.01)	1.03 (0.64,1.4)	0.27 (-0.01,0.54)
Jumping	0.22 (0,0.45)	-0.2 (-0.4,0)	0.18 (-0.04,0.38)	0.07 (-0.15,0.3)	0.31 (0.07,0.55)	0.11 (-0.09,0.32)	0.23 (0,0.47)	0.67 (0.39,0.95)
<u>Mature</u>	EPL	EPA	PHPL	PHPA	HYPL	HYPW	SL	Jumping
EPL	0.6 (0.43,0.76)	-0.03 (-0.24,0.17)	0.78 (0.69,0.85)*	-0.02 (-0.22,0.17)	0.78 (0.7,0.86)*	0.58 (0.43,0.7)*	0.76 (0.68,0.85)*	0.13 (-0.07,0.36)
EPA	-0.03 (-0.2,0.14)	1.25 (0.9,1.6)	0.05 (-0.17,0.25)	-0.74 (-0.83,-0.64)*	0.12 (-0.1,0.3)	0.15 (-0.05,0.35)	0.2 (0.01,0.4)*	0.11 (-0.12,0.32)
PHPL	0.48 (0.32,0.63)	0.04 (-0.13,0.22)	0.64 (0.46,0.81)	-0.04 (-0.25,0.15)	0.78 (0.69,0.85)*	0.6 (0.46,0.72)*	0.74 (0.65,0.83)*	0.1 (-0.12,0.31)
PHPA	-0.02 (-0.18,0.15)	-0.9 (-1.18,-0.59)	-0.04 (-0.21,0.13)	1.18 (0.86,1.52)	-0.14 (-0.35,0.04)	0.02 (-0.17,0.24)	-0.13 (-0.33,0.06)	-0.19 (-0.4,0.02)
HYPL	0.47 (0.32,0.62)	0.1 (-0.08,0.27)	0.48 (0.32,0.63)	-0.12 (-0.29,0.04)	0.6 (0.43,0.75)	0.62 (0.49,0.74)*	0.83 (0.76,0.89)*	0.1 (-0.13,0.31)
HYPW	0.41 (0.26,0.58)	0.15 (-0.05,0.36)	0.44 (0.27,0.6)	0.02 (-0.18,0.22)	0.44 (0.28,0.6)	0.84 (0.61,1.07)	0.65 (0.53,0.76)*	0.14 (-0.07,0.35)
SL	0.46 (0.31,0.61)	0.18 (-0.01,0.34)	0.46 (0.32,0.62)	-0.11 (-0.28,0.05)	0.5 (0.34,0.65)	0.47 (0.3,0.63)*	0.61 (0.44,0.78)	0.14 (-0.08,0.35)
Jumping	0.05 (-0.03,0.15)	0.07 (-0.06,0.2)	0.04 (-0.05,0.13)	-0.11 (-0.24,0.01)	0.04 (-0.05,0.13)	0.07 (-0.03,0.18)	0.06 (-0.04,0.14)	0.29 (0.2,0.39)
<u>Old</u>	EPL	EPA	PHPL	PHPA	HYPL	HYPW	SL	Jumping
EPL	1.11 (0.77,1.42)	-0.15 (-0.35,0.06)	0.85 (0.78,0.9)*	0.15 (-0.07,0.35)	0.86 (0.81,0.92)*	0.72 (0.62,0.83)*	0.81 (0.74,0.88)*	0.01 (-0.23,0.26)
EPA	-0.15 (-0.36,0.06)	0.93 (0.65,1.2)	0 (-0.21,0.21)	-0.72 (-0.83,-0.62)*	-0.03 (-0.25,0.19)	0.1 (-0.11,0.31)	0.07 (-0.15,0.27)	-0.08 (-0.31,0.15)
PHPL	0.95 (0.63,1.26)	0 (-0.21,0.21)	1.14 (0.79,1.47)	-0.01 (-0.23,0.19)	0.87 (0.82,0.92)*	0.75 (0.66,0.85)*	0.81 (0.74,0.88)*	-0.1 (-0.33,0.16)
PHPA	0.15 (-0.07,0.35)	-0.65 (-0.89,-0.42)	-0.01 (-0.21,0.2)	0.87 (0.61,1.13)	0.03 (-0.19,0.23)	0.04 (-0.18,0.26)	-0.04 (-0.25,0.18)	0.19 (-0.04,0.42)
HYPL	0.94 (0.65,1.26)	-0.03 (-0.24,0.18)	0.97 (0.66,1.28)	0.02 (-0.17,0.23)	1.09 (0.78,1.43)	0.75 (0.65,0.84)*	0.88 (0.82,0.92)*	-0.02 (-0.26,0.24)
HYPW	0.88 (0.56,1.2)	0.11 (-0.13,0.34)	0.92 (0.61,1.26)	0.04 (-0.18,0.27)	0.9 (0.59,1.22)	1.33 (0.91,1.73)	0.73 (0.63,0.83)*	0.07 (-0.19,0.32)
SL	0.78 (0.52,1.04)	0.06 (-0.12,0.25)	0.79 (0.54,1.06)	-0.04 (-0.22,0.14)	0.84 (0.56,1.1)	0.77 (0.5,1.05)	0.83 (0.59,1.09)	0.08 (-0.17,0.33)
Jumping	0 (-0.13,0.15)	-0.04 (-0.16,0.07)	-0.06 (-0.2,0.08)	0.1 (-0.02,0.22)	-0.01 (-0.15,0.12)	0.05 (-0.1,0.21)	0.04 (-0.08,0.17)	0.3 (0.19,0.41)

*Phenotypic variances, covariances, and correlations are on the diagonals (bold), below the diagonals (gray) and above the diagonals, respectively. Significant results are asterisked. Bootstrapped 95% C.I. are given in parentheses.

716 **Table 2:** 95% Confidence intervals based on differences between bootstrapped **P** estimates between young and mature, mature and
 717 old, and young and old individuals.

<u>Young-Mature</u>	EPL	EPA	PHPL	PHPA	HYPL	HYPW	SL	Jumping
EPL	-0.09,0.66	-0.67,-0.08*	-0.04,0.64	-0.05,0.54	0.01,0.74	-0.05,0.61	0.02,0.76*	-0.08,0.4
EPA		-0.99,-0.11	-0.7,-0.12*	0.04,0.81*	-0.72,-0.13 *	-0.75,-0.16*	-0.85,-0.23*	-0.51,-0.03*
PHPL			-0.17,0.54	-0.01,0.58	-0.03,0.69	-0.08,0.55	-0.01,0.7	-0.1,0.36
PHPA				-0.77,0.18	0.07,0.68*	-0.08,0.54	0.11,0.75*	-0.09,0.43
HYPL					-0.05,0.75	-0.06,0.62	0.01,0.78*	0.01,0.51*
HYPW						-0.46,0.29	-0.09,0.6	-0.18,0.28
SL							0.01,0.86	-0.08,0.43
Jumping								0.08,0.66
<u>Mature-Old</u>	EPL	EPA	PHPL	PHPA	HYPL	HYPW	SL	Jumping
EPL	-0.88,-0.14	-0.15,0.39	-0.82,-0.13*	-0.42,0.1	-0.81,-0.15*	-0.82,-0.11*	-0.61,-0.02*	-0.11,0.22
EPA		-0.13,0.78	-0.24,0.32	-0.61,0.15	-0.15,0.4	-0.27,0.35	-0.15,0.36	-0.06,0.29
PHPL			-0.88,-0.11	-0.3,0.24	-0.85,-0.15*	-0.86,-0.12*	-0.64,-0.04 *	-0.06,0.27
PHPA				-0.11,0.72	-0.41,0.11	-0.3,0.3	-0.3,0.18	-0.39,-0.04*
HYPL					-0.87,-0.15	-0.83,-0.12*	-0.63,-0.02*	-0.11,0.22
HYPW						-0.97,-0.04	-0.62,0.02	-0.17,0.21
SL							-0.53,0.07	-0.13,0.17
Jumping								-0.16,0.14
<u>Young-Old</u>	EPL	EPA	PHPL	PHPA	HYPL	HYPW	SL	Jumping
EPL	-0.7,0.25	-0.58,0.07	-0.6,0.28	-0.26,0.39	-0.57,0.35	-0.63,0.23	-0.35,0.51	-0.05,0.48
EPA		-0.62,0.16	-0.67,-0.04*	-0.17,0.53	-0.62,0.01	-0.7,-0.06*	-0.74,-0.11*	-0.4,0.08
PHPL			-0.76,0.17	-0.07,0.56	-0.6,0.3	-0.68,0.18	-0.39,0.45	-0.01,0.51
PHPA				-0.43,0.47	-0.11,0.55	-0.13,0.52	0.04,0.69*	-0.29,0.22
HYPL					-0.61,0.37	-0.6,0.28	-0.36,0.53	0.04,0.59
HYPW						-1.06,-0.08	-0.45,0.36	-0.18,0.33
SL							-0.28,0.63	-0.1,0.44
Jumping								0.06,0.66

*Significant confidence intervals are asterisked. Confidence intervals associated with differences in variance components are bold.



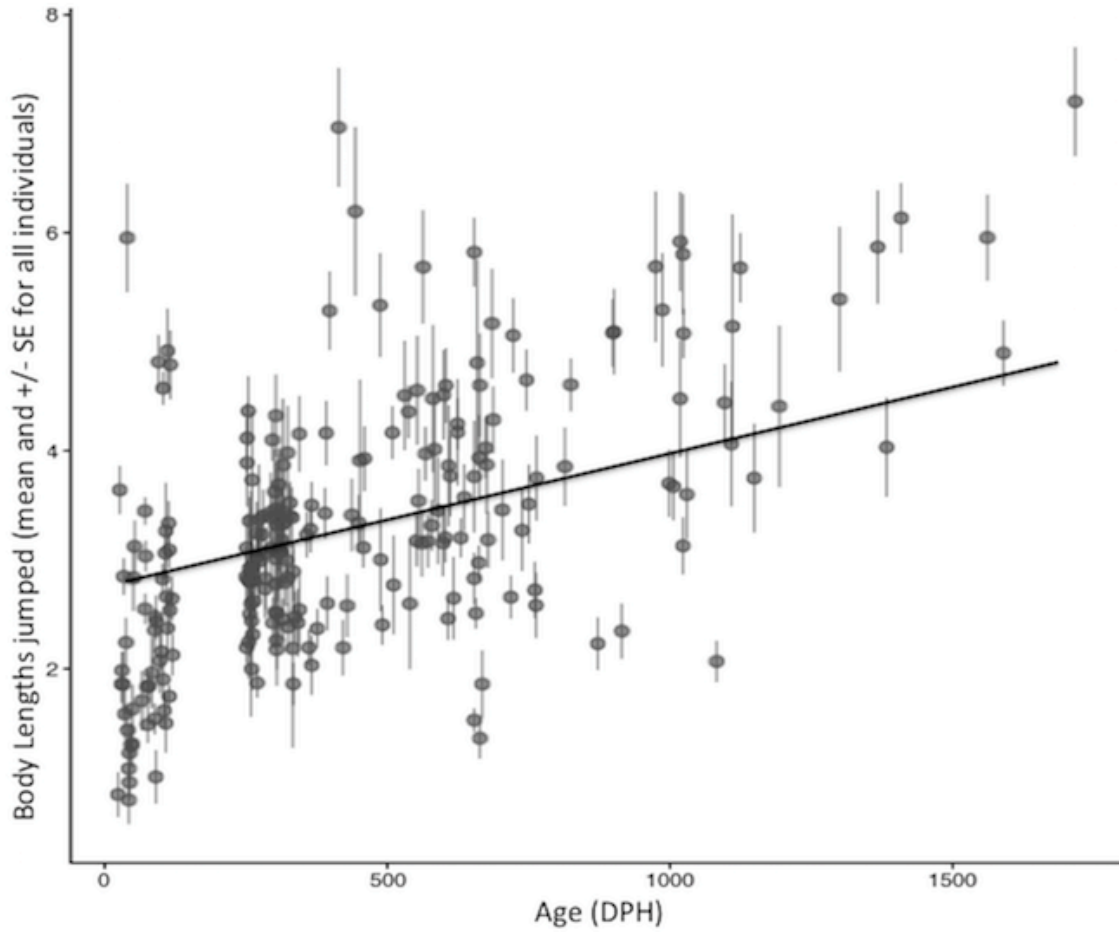
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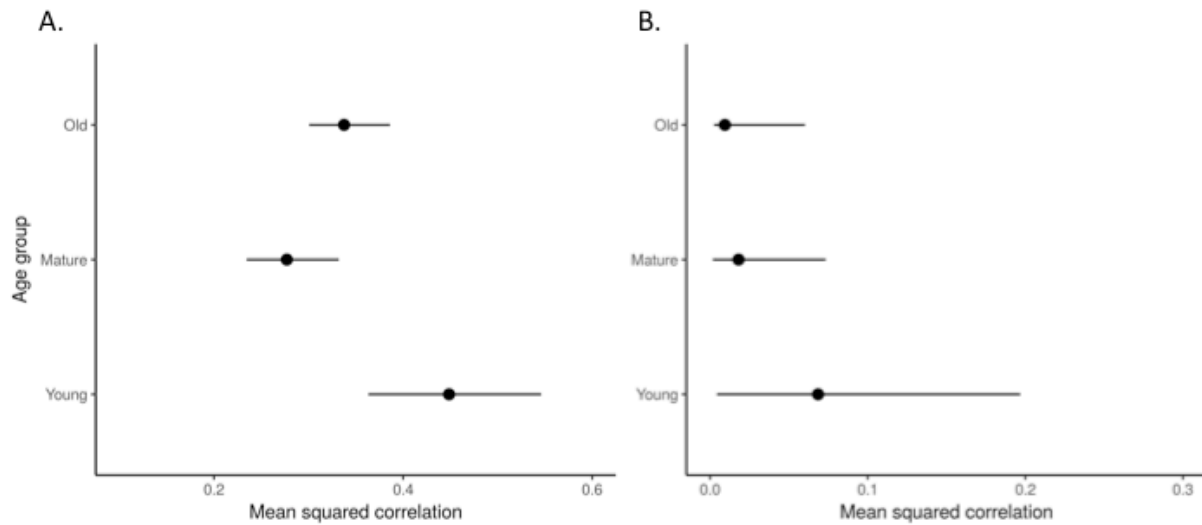
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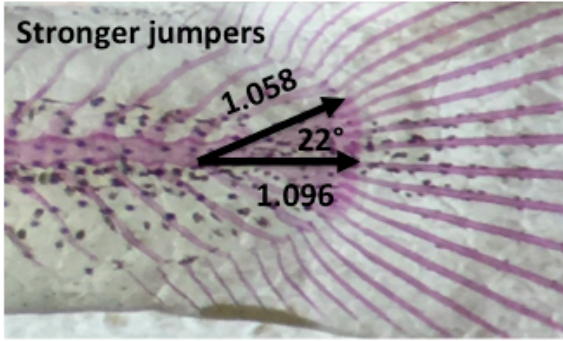
Figure 1: Typical 'tail-flip' jump exhibited by *K. marmoratus* in which the animal presses its caudal peduncle against the substrate, and launches itself through the air often many times its body length. Still shots were obtained from a video taken by Benjamin Perlman.



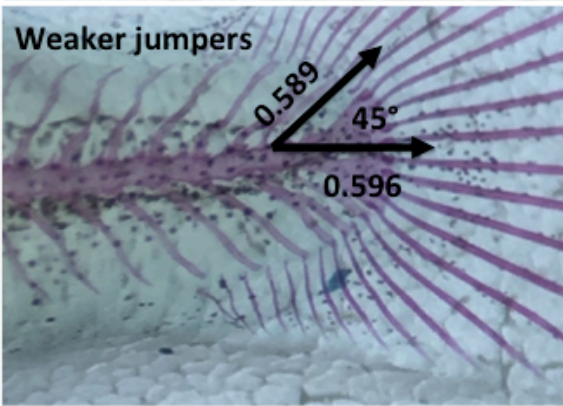
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725 Figure 2: The relationship between distance jumped (Body Lengths) and age (DPH). The mean
726 and standard error across all jumps for each individual is depicted. Line of best fit for the
727 relationship between age and body lengths jumped shown in black. Model predictions were
728 based on averaging over our fixed and random effects.



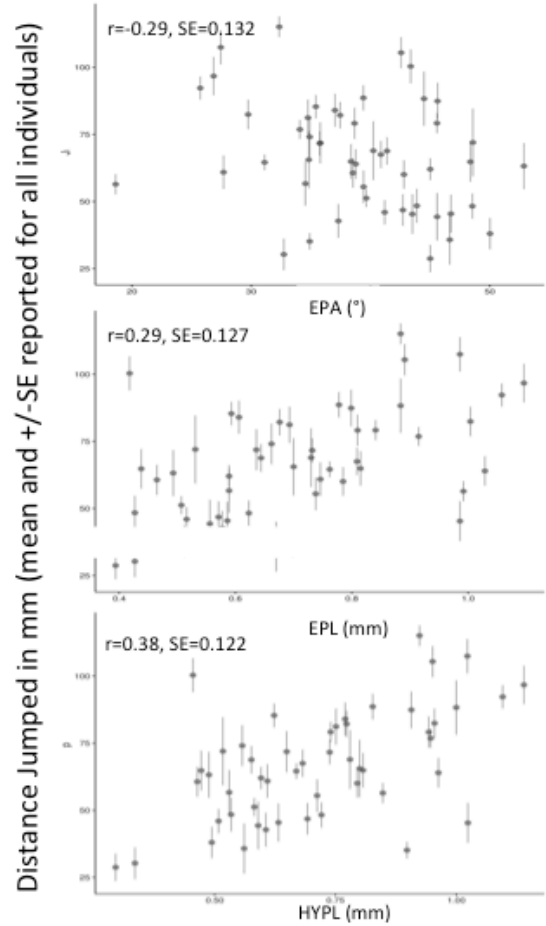
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 730 Figure 3: Phenotypic integration, as measured by the mean squared correlation: A.) among all
 731 morphological characters, and B.) between morphological characters (all) and jumping
 732 performance separated by age group for *K. marmoratus*. Age groups are significantly different
 733 when 95% confidence intervals are not overlapping. Note: square correlations will always be >0
 734 by necessity.



Long hypural (e.g. 1.096 mm)/epural length (e.g. 1.058), small epural angle (e.g. 22°)

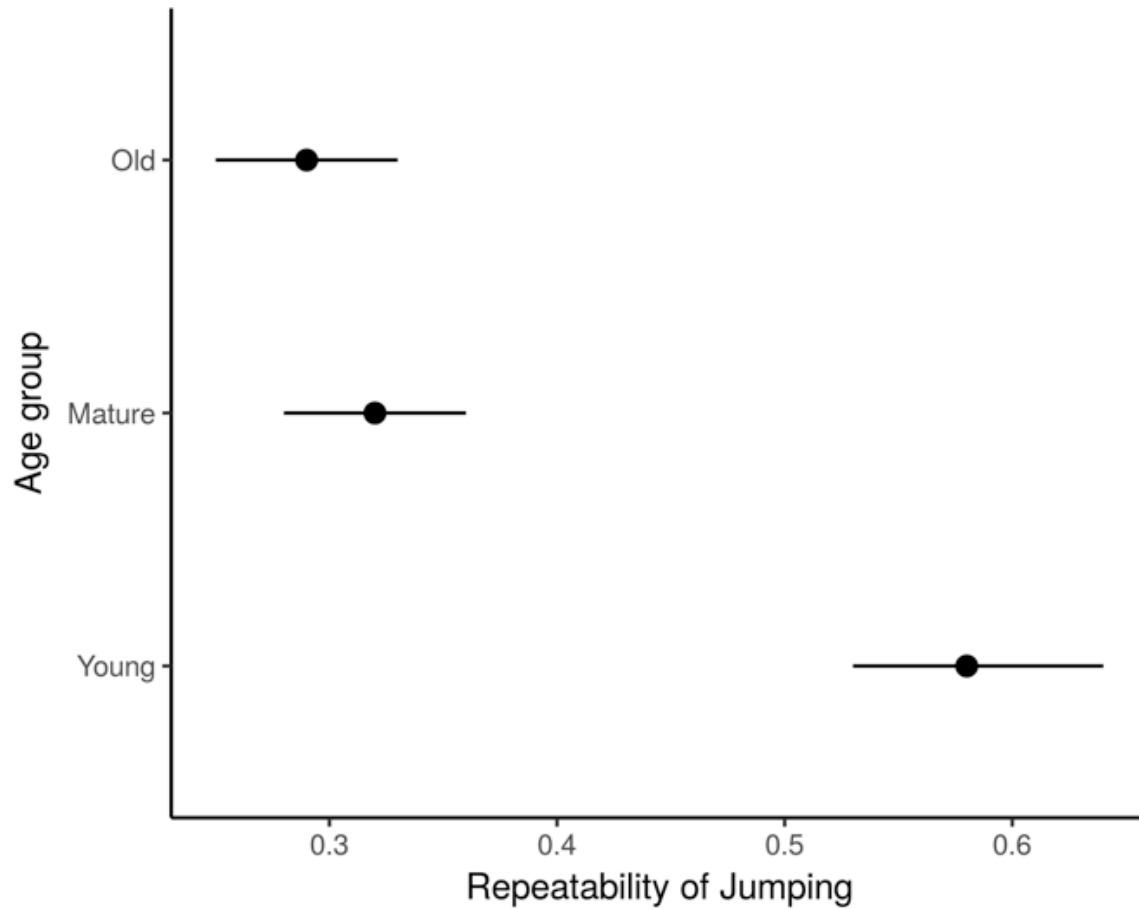


Short hypural (e.g. 0.596)/epural length (e.g. 0.589), large epural angle (e.g. 45°)

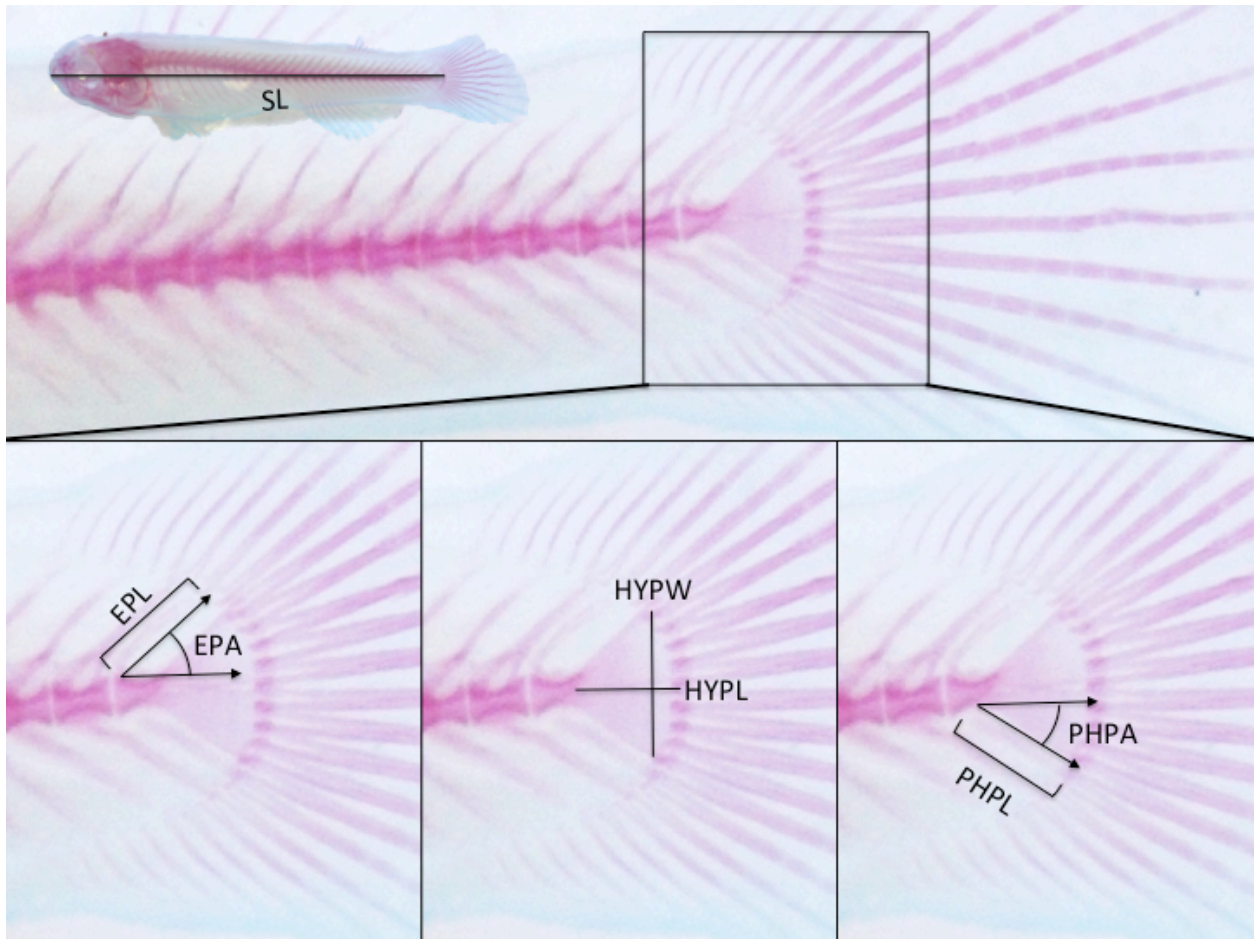


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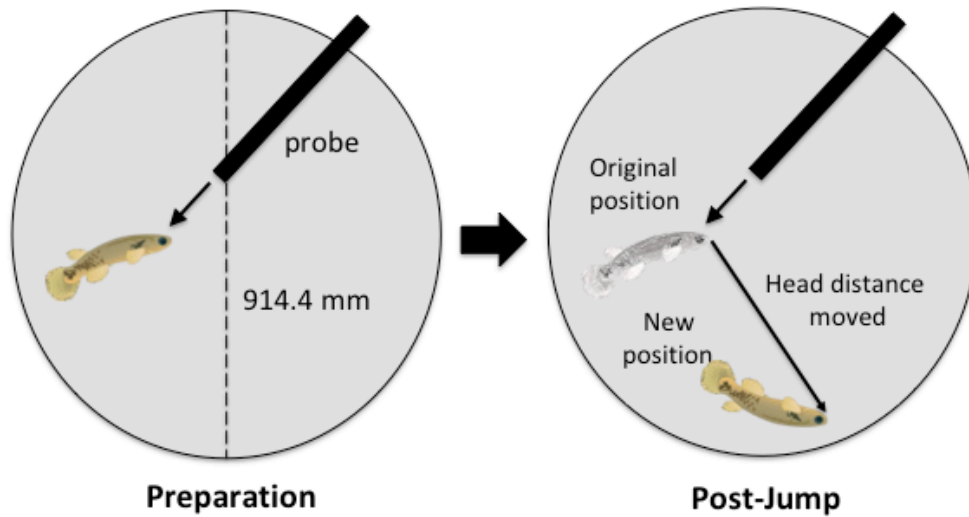
Figure 4: Visual depiction of morphological variation associated with variation in jumping performance in the young age group (left). Significant relationships between morphology (EPA, EPL, and HYPL) and jumping performance (distance jumped in mm) are depicted (right). Mean and +/- standard errors are included for all individuals. Correlations are also given in the upper left corner.



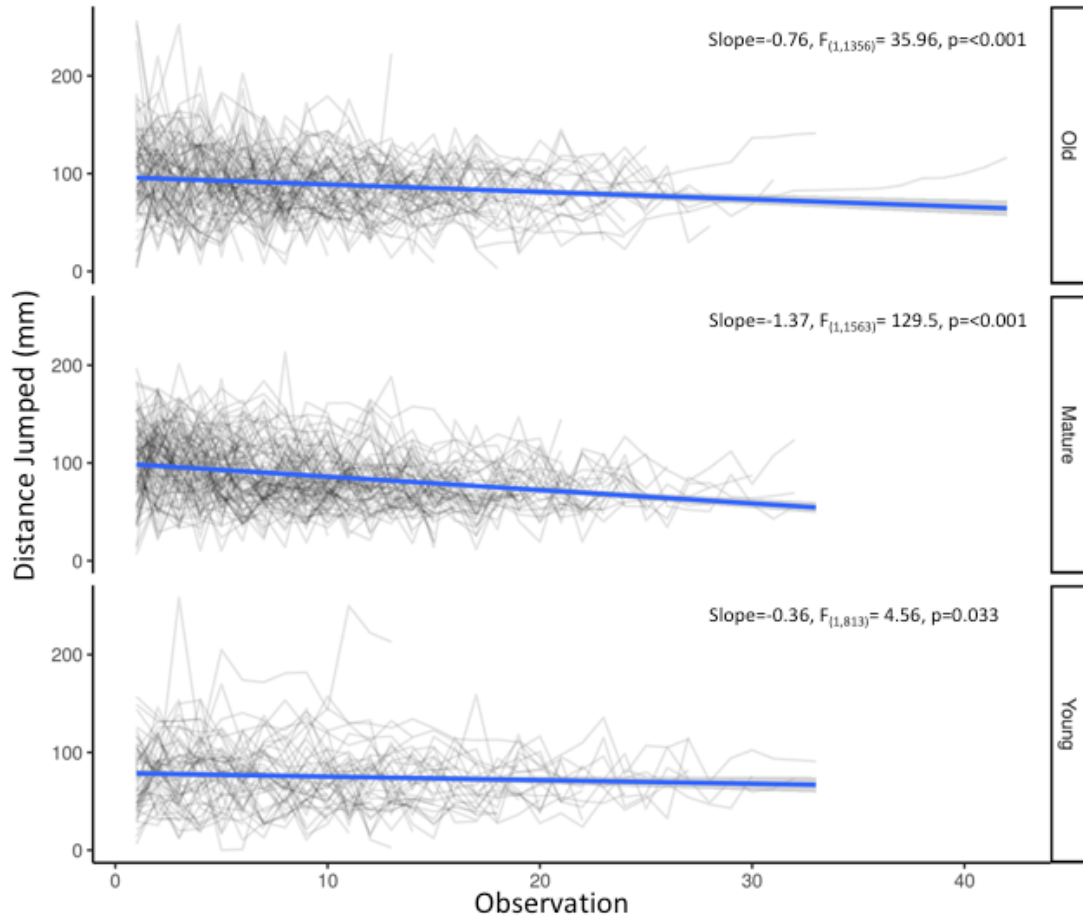
741
 742 Figure 5: Among individual variance divided by the sum of the among-and within-individual
 743 variance in jumping performance (i.e. repeatability) separated by age group for *K. marmoratus*.
 744 95% confidence intervals based on 5,000 bootstrap draws are also depicted around each estimate.
 745 Age groups are significantly different when 95% confidence intervals are not overlapping.



746
 747 Figure S1: Cleared and stained *K. marmoratus* specimen. EPA=epural angle, EPL=epural length,
 748 HYPL=length of the hypurals, HYPW=width of the hypurals, PHPA=parahypural angle,
 749 PHPL=parahypural length, and SL=standard length measurements are shown.

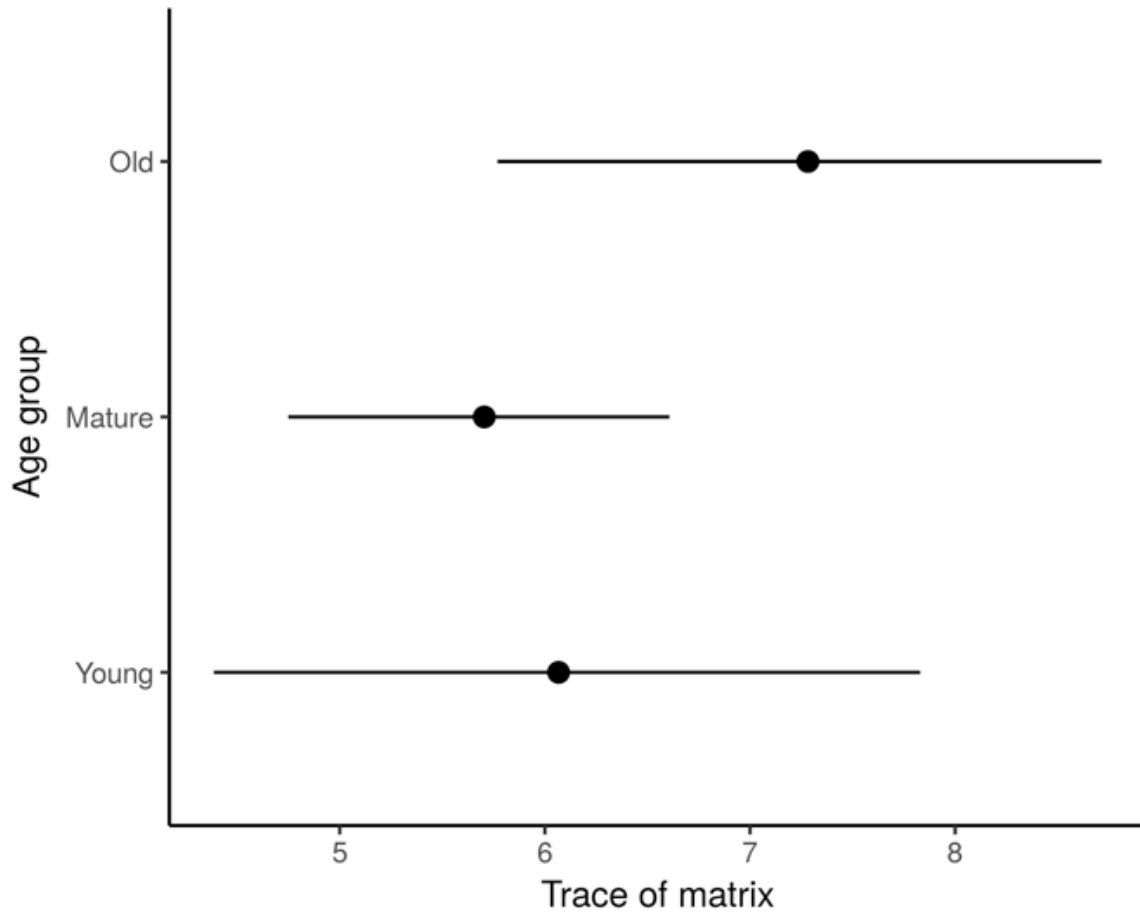


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751 Figure S2: Jumping performance trials on *K. marmoratus*. Fish were stimulated to jump via
752 probing during the 'preparatory' phase. Head and tail distance moved (not shown) were then
753 calculated from the original location of the head or tail in the 'preparation' phase and the new
754 position seen in the 'post-jump' phase. All jumps were scaled according to the size of the
755 jumping arena (914.4 mm).



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Figure S3: Distance jumped (mm) per observation separated according to age group. 'Line of best fit' and associated statistics for the relationship between observation and distance jumped are shown.



760
 761 Figure S4: Total phenotypic variation, or sum of the diagonal of \mathbf{P} (i.e. matrix trace), separated
 762 by age group for *K. marmoratus*. Age groups are significantly different when 95% confidence
 763 intervals are not overlapping.
 764