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Why do frog and toad forelimbs suddenly (but asynchronously) appear every time metamorphosis is near?

1   Why do frog and toad forelimbs suddenly (but asynchronously) appear every time

2   metamorphosis is near?

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13   Short title: Why do forelimbs suddenly appear, every time metamorphosis is near?

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24 **Summary**

25 **1.** The internal development and emergence of the forelimbs at metamorphosis is a defining

26 feature of anuran amphibians (frogs and toads). However, although forelimb emergence is

27 considered sudden, it is rarely synchronous. Any asynchrony may or may not exacerbate the

28 increased drag that is predicted to occur with the emergence of the forelimbs at metamorphic

29 climax.

30 **2.** Despite the impact forelimb emergence is hypothesized to have on individual survival and

31 life history evolution, the degree of asynchrony between forelimb emergence, and any

32 consequences of such asymmetry, have not been investigated. The asynchrony in forelimb

33 emergence also provides an opportunity to test the currently held evolutionary basis for the

34 internal development and sudden emergence of the forelimbs in anurans.

35 **3.** Using a diverse range of anuran taxonomic groups we measured the time between, and

36 pattern of, emergence of the forelimbs across a range of species. To examine the

37 evolutionary impacts of forelimb emergence we assessed locomotory performance when

38 individuals had zero, one or two forelimbs emerged.

39 **4.** The duration of time between the emergence of the two forelimbs was longer and more

40 variable than predicted. Furthermore, no species suffered impaired burst speeds nor was their

41 angle of escape affected as the forelimbs emerged asynchronously. In fact, burst swimming

42 speed was faster after the emergence of one and two forelimbs than prior to their emergence.

43 **5.** Fundamentally, our results call into question the proposition, long accepted, that internal

44 forelimb development is associated with locomotion and reducing drag during

45 metamorphosis. This does not appear to be the case and we suggest that anatomical or

46 developmental constraints or advantages may be responsible.

47

Introduction

Tetrapod forelimb development is highly diverse (Polly 2007), yet some larval anuran amphibians (the tadpoles of frogs and toads) are unique in having delayed development of the forelimbs relative to the hind limbs (Bininda-Emonds *et al.* 2007), internal development of the forelimb, and sudden eruption of the well-developed limb through the outer body layer. In fact, the sudden emergence of the developed forelimbs is a distinguishing developmental characteristic of the group and represents a significant life history event, concluding larval development, and is often used to demarcate the onset of the rapid transition to the adult form (metamorphic climax) (Gosner 1960; Walsh 2010). During metamorphosis, the eruption of the forelimbs, in particular, has been viewed as critical since it noticeably and immediately changes the shape of the tadpole.

In anurans, the forelimbs commonly develop within the branchial chamber – a space containing the gills – enclosed laterally by the operculum (analogous to the gill covers of bony fishes) and that links to the exterior via an opening called the spiracle. Less commonly, as in the pipids (clawed frogs, e.g. *Xenopus laevis*), the forelimbs develop within brachial sacs, structures that are separate from and posterior to the opercular cavity (Newth 1948). The position of the spiracle or spiracles differs among taxonomic groups and has been linked to forelimb emergence (e.g. Borkhvardt & Malashichev 1997). In tadpoles where the spiracle is lateral, the forelimb on the spiracular side emerges via enlargement of the spiracle. On the other side, and in tadpoles where the spiracle is central or the forelimbs are not enclosed by the opercular cavity, the forelimb emerges through a new perforation in the overlying tissue (Newth 1949). Formation or expansion of the openings for the forelimbs occurs through thinning of the overlying tissues in response to rising thyroid hormone levels, and mechanical pressure from the underlying limb (Helff 1926; Helff 1939; Newth 1949).

74   Understanding of the evolutionary processes responsible for the internal development

75   and sudden emergence of the forelimbs is poor. Wassersug (1989) argued that the forelimbs

76   develop internally and emerge suddenly because of the cost of drag (Wassersug 1989;

77   Dudley, King & Wassersug 1991) increasing predation risk (Wassersug & Sperry 1977),

78   absence of need for lateral appendages for turning (achieved by the tail), and the need for

79   morphologically distinct forelimbs at the time of metamorphosis. However, although the two

80   forelimbs emerge abruptly and fully-formed, their emergence is asymmetric (Malashichev

81   2002). Furthermore, to date the temporal separation between emergence of the two forelimbs

82   has not been quantified, and the locomotory performance impacts of forelimb emergence

83   asymmetry have not been assessed. Here we quantify this asymmetry in a taxonomically

84   diverse set of six anuran species, and test the widely assumed, but largely unexplored,

85   hypothesis that internal development and sudden emergence of anuran forelimbs in anurans

86   occurs in order to minimise reduction in locomotory performance.

87   We explored the impact of limb emergence on burst swimming speed – a key trait in

88   larval escape from predators (Van Buskirk & McCollum 2000). As tadpoles progress from

89   no forelimbs emerged, to one emerged, to both emerged, the impact of drag could have two

90   potential outcomes. The "limb drag" hypothesis proposes that drag from the limbs would be

91   cumulative, such that burst swimming speed would decrease linearly with number of

92   emerged limbs, but would not necessarily impact turning performance (Fig. 1). There would

93   also be limited selective pressure to minimize the period of asynchrony between the

94   forelimbs emerging, since in terms of locomotion, having one emerged limb is half as bad as

95   having both emerged. The "locomotion disruption" hypothesis proposes that drag from the

96   limbs would disrupt the normal swimming of the tadpole, in a manner analogous to dragging

97   an oar on only one side of a boat. Therefore it is predicted that burst speed would sharply

98   decrease after one forelimb has emerged, but with a smaller incremental decline (or

99   potentially a recovery) when both forelimbs are exposed (Fig. 1). This second hypothesis

100 further predicts that turning behaviour would be biased, and show an increased acuteness in

101 the angle of escape, in the direction of the first limb to emerge (Fig. 1). Under this scenario,

102 we would also expect that the period between forelimbs emerging would be short and

103 relatively invariable. Both hypotheses predict that burst swimming performance would be

104 best without any emerged forelimbs (Wassersug & Sperry 1977).

105

106 **Materials and methods**

107 **Study species**

108 We quantified limb emergence asymmetry and burst swimming in six anuran species. The

109 common frog *Rana temporaria* (Ranidae) and common toad *Bufo bufo* (Bufonidae) are found

110 throughout Europe. Both have aquatic tadpoles and terrestrial adults and a single spiracle on

111 the left side of the body (Helff 1939; McDiarmid & Altig 1999). The oriental fire-bellied

112 toad *Bombina orientalis* (Bombinatoridae) is a primarily aquatic species from central Eastern

113 Asia whose tadpoles have a single spiracle on the ventral midline (McDiarmid & Altig 1999).

114 The three species of clawed frogs (Pipidae: *Xenopus laevis*, *Xenopus borealis*, *Silurana*

115 *tropicalis*) are from sub-Saharan Africa and remain fully aquatic as adults. The tadpoles all

116 have a pair of symmetrically placed lateral spiracles (McDiarmid & Altig 1999).

117

118 **Animals and rearing conditions**

119 Wild *Rana temporaria* and *Bufo bufo* eggs were collected near Glasgow, Scotland. *Xenopus*

120 *laevis* (University of St. Andrews, Scotland), *Xenopus borealis*, and *Silurana tropicalis* (The

121 Fish Hut, Lanarkshire, Scotland) were obtained as fertilised eggs from captive-bred stock.

122 *Bombina orientalis* were obtained as late stage tadpoles from a private breeder. All tadpoles

123 apart from *B. orientalis* were reared similarly. Tadpoles were reared in aerated, de-

124   chlorinated copper-free water at $21 \pm 3°C$ and a 12:12 L:D photoperiod. In 2008, in an

125   attempt to space the timing of metamorphosis by varying development rates, tadpoles were

126   allocated to tanks at different densities, ranging from one to 10 individuals per litre of water.

127   The majority of tadpoles used were reared at 10 tadpoles (57.7%) or 5 tadpoles (36.5%) per

128   litre of water, the remaining 5.7% were reared at 1 tadpole or 2.5 tadpoles per litre. Density

129   did not affect the body size of individuals used in the study (SVL: $F_{3,51} = 0.34$, $P = 0.80$;

130   mass: $F_{3,51} = 0.15$, $P = 0.93$). In 2011, all individuals were reared at the same density of

131   approximately 5 tadpoles per litre. Water was changed as required and tadpoles were fed

132   daily *ad libitum*; *R. temporaria*, *B. bufo* and *B. orientalis* were fed on commercial fish flakes

133   (Aquarian Tropical Flake Food, Mars Fishcare, Inc.) and the three pipids were fed on Tetra

134   Wafer Mix (Tetra, Melle, Germany) algal pellets.

135   Tadpoles were observed daily until the first tadpoles approached metamorphic climax;

136   thereafter tanks were inspected two to four times daily for individuals that had reached the

137   stage prior to forelimb emergence, which were removed. These individuals were not

138   provided with food, because tadpoles do not feed during metamorphic climax and this was

139   usually reached within 48 hours. The study was conducted across two years, 2008 and 2011.

140   In 2008 all individuals (13 *X. laevis*, 22 *X. borealis* and 17 *S. tropicalis*) found before

141   any limb emergence were used to examine emergence asynchrony (Observational Study; see

142   below). In 2011, individuals that were found before one forelimb had emerged were

143   randomly allocated to either the Observational study or the Experimental study (see below).

144   No tadpoles were used in both studies. The Observational study used 17 *R. temporaria* and

145   20 *B. bufo* tadpoles; the Experimental study used 25 *R. temporaria*, 25 *B. bufo*, 16 *X. laevis*

146   and 16 *B. orientalis*. Snout-vent length (SVL; ± 0.1 mm) for all individuals was measured

147   using a single digital photographic frame taken with a Photron FASTCA-PCI camera and

148   Motion Tools software (Photron USA, Inc., San Diego, California, United States).

149

Observational study: Variation in forelimb asynchrony

Tadpoles were placed individually in a 10 cm diameter glass dish filled *c.* 1 cm deep,

sufficient to cover the body and allow free swimming but restricting tadpoles to a horizontal

posture. Water was kept at 22°C (± 2°C). Data were recorded using filming under

continuous light, using a miniature charge-coupled device (CCD) CCTV camera placed

directly above the observation dish, such that the entire dish was in view. After both

forelimbs emerged, individuals were allowed to complete metamorphosis and returned to the

site or location from where they were obtained. Examination of video footage allowed

determination of the interval between first and second forelimb emergence to a resolution of

±1 min.

Experimental study: Burst speed and direction of escape

Burst swimming speed and escape direction were quantified at three developmental points: 1)

just prior to the emergence of either forelimb; 2) with one forelimb emerged; and 3) within 12

hours after the second forelimb had emerged and before significant reduction in tail length.

At each stage, tadpole burst swimming speed and direction were measured five times with *c.*

1 min interval between each. The side that the first forelimb emerged from and SVL,

following the methods above, were recorded.

Tadpoles were placed individually in the centre of a 30 cm swimming arena, as

described above and allowed to settle for 2 minutes. Burst swimming was initiated by a

consistent discharge of air from a 1 ml Gilson pipette to the rear of the animal (Van Buskirk

& McCollum 2000). All trials were recorded in the same room, under the same conditions,

that tadpoles experienced during rearing using a Photron FASTCAM-PCI high-speed camera

173 placed 50 cm directly over the arena. Laminated grid-paper was placed under the arena to

174 allow calibration of distances in video analysis.

175   Filming was carried out at 250 frames per second (fps) for up to 5 s of swimming in

176 each trial. As elsewhere (Watkins 1996; Dayton *et al.* 2005; Wilson, Kraft & Van Damme

177 2005; Walsh, Downie & Monaghan 2008a; Walsh, Downie & Monaghan 2008b), the first

178 300 ms following initial movement were used to estimate burst speed (Fig. 2), calculated as

179 the distance travelled (cm) per second. The angle of escape was calculated by determining

180 the grid coordinates of the snout and the vent in the frame prior to initial movement and the

181 grid coordinates of the same two points in the last frame. From this the orientation of the

182 individual before and after moving and the change in angle from the starting position was

183 calculated, using the general formula: $\text{Angle} = 2 * \arctan\left(\frac{y}{\sqrt{x^2 + y^2} + x}\right)$, where y is the

184 distance the snout moved along the y-axis and x is the distance the snout moved along the x-

185 axis. This was then subtracted or added, depending on the orientation of the tadpole, from

186 the starting angle of orientation of the tadpole determined from the same formula, but where y

187 is the distance on the y-axis between the snout and vent and x is the distance on the x-axis

188 between the snout and vent. The direction of the turn was also recorded as either left or right.

189

190 **Data analysis**

191 All analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, Il, U.S.A.). A general

192 linear model (GLM) was used to examine burst speed, with SVL as a covariate, species as a

193 factor, tadpole ID as a random factor and the number of forelimbs emerged (either zero, one

194 or two) and trial as repeated measures. Escape direction was analysed with generalized

195 estimating equations (GEE), using a binary logistic model. Species was included as a factor,

196 trial and number of emerged forelimbs as repeated measures, and the first forelimb to emerge

197 (either left or right) as a covariate. To analyse the angle of escape a linear mixed model

198   (LMM) was used, with species as a fixed factor, trial and the number of emerged forelimbs as

199   repeated measures, tadpole ID as a random factor, and the first forelimb to emerge as a

200   covariate. All significant interactions were included in all models.

201

202   **Results**

203   **Forelimb emergence**

204   The time taken between the emergence of the first and second forelimb was highly variable in

205   all five species investigated (Table 1). Within the 1 minute resolution of the study, there was

206   no case of simultaneous forelimb eruption. The period between emergence of the forelimbs

207   did not differ amongst the five species ($F_{4,80} = 0.39$, p $= 0.82$), the density the individuals

208   were reared at ($F_{3,80} = 0.30$, p $= 0.47$), nor did it depend on which forelimb emerged first

209   ($F_{1,80} = 1.24$, p $= 0.27$).

210

211   **Locomotory performance**

212   In contrast to the predictions of our two hypotheses, all four species tested for burst speed

213   swam *slowest* prior to the emergence of either forelimb ($F_{2,1179.86} = 8.60$, P < 0.001; Fig. 3).

214   The four species differed in their overall burst speed ($F_{3,103.09} = 13.46$, P < 0.001). Of the

215   four species, *R. temporaria* exhibited the fastest increase in burst speed with the emergence

216   of the first forelimb ($F_{6,1170.02} = 32.12$, P < 0.001) such that they swam fastest with only one

217   forelimb emerged (post-hoc P < 0.001; Fig. 3) compared to when no and both forelimbs were

218   exposed. SVL, which decreased as the forelimbs emerged (Fig. 4), did not consistently affect

219   burst speed ($F_{1,318.86} = 0.47$, P $= 0.49$). However, there was a significant interaction between

220   SVL and the number of forelimbs emerged ($F_{2,1180.91} = 6.78$, P < 0.005), with burst speed

221   getting faster as SVL increased when one or both forelimbs emerged, but not when neither

10

222 forelimb had emerged. Trial did not significantly affect burst speed ($F_{4,1132.92}$ = 2.32, P =

223 0.06).

224   Individuals from all four species tested had a right-bias in turning after being startled

225 (χ^2_1 = 56.21, p < 0.0001; Fig. 5). Species differed in the magnitude of this bias (Wald χ^2_3 =

226 11.81, p = 0.008) consistently over the five trials (Wald χ^2_4 = 1.59, p = 0.81). Within species,

227 the strength of rightwards bias decreased as tadpoles progressed from no forelimbs to both

228 forelimbs emerged (Wald χ^2_2 = 12.09, p = 0.002). However, contrary to the locomotion

229 disruption hypothesis, the first forelimb to emerge did not affect the direction that individuals

230 turned on escaping (Wald χ^2_1 = 0.56, p = 0.46).

231   There was no overall change in the angle of escape with the progression through

232 forelimb emergence ($F_{2,\ 785.66}$ = 2.66, p = 0.07). Species differed in their responses ($F_{3,\ 77.23}$ =

233 4.69, p = 0.005) and responded differently when either no, one, or both forelimbs had

234 emerged ($F_{6,\ 752.78}$ = 4.92, p < 0.0001; Fig. 6). Individuals appeared to escape at a steeper

235 angle, regardless of direction, when the left forelimb, rather than the right, was the first to

236 emerge but this was marginally non-significant ($F_{1,\ 75.86}$ = 3.85, p = 0.05). The trial did not

237 affect the angle of escape ($F_{4,416.81}$ = 1.26, p = 0.29).

238

239 **Discussion**

240 Our results do not support either the limb drag or locomotion disruption hypothesis, or the

241 general idea that external forelimbs influence swimming performance and hence predation

242 risk (Wassersug & Sperry 1977; Wassersug 1989). We observed no decrease in burst speed

243 during the progression of forelimb emergence (counter to both hypotheses), no direct

244 predicted bias or impairment of turning during escape (counter to the locomotion disruption

245 hypothesis), and a long and highly variable period between the emergence of the forelimbs

246 (counter to the locomotion disruption hypothesis). Our results thus re-awaken the debate

247 over why anuran forelimbs develop internally and erupt suddenly at the start of metamorphic

248 climax.

249   There are several possible explanations for our findings. Firstly, it is possible that

250 limb emergence does influence burst speed and ease of turning, but that we did not observe

251 any effects due to compensation for increased or asymmetrical drag forces by the

252 metamorphs (Schmidt-Nielsen 1972; Webb 1988; Hildebrand & Goslow Jr. 2001; Azizi &

253 Landberg 2002; Landberg & Azizi 2010). Evidence for such investment would imply that

254 there is a direct cost to impaired swimming performance. Utilising more energy to maintain

255 burst performance during forelimb emergence could deplete energy stored for

256 metamorphosis, affecting survival in subsequent encounters and size on completion of

257 metamorphosis with associated knock-on effects (Altwegg & Reyer 2003; Chelgren *et al.*

258 2006).

259   Similarly, we may not have observed a decrease in burst speed because burst speed is

260 based on physiological capacity and motivation (Losos, Creer & Schulte 2002), and our

261 methodology might not have provoked a consistent level of motivation across different

262 stages. While motivation may differ across the different stages examined in this study, the

263 maximum burst speeds for *Xenopus laevis* (18.9 - 27.7 cm s^{-1}) from our current study are

264 comparable with previous studies on this species (Wilson, James & Johnston 2000: *c.* 30 cm

265 s^{-1}; Walsh *et al.* 2008a: 19.6 - 24.6 cm s^{-1}). Directly comparable results are not available for

266 the other species, yet *Bombina orientalis* from our study were substantially faster (21.8-27.0

267 cm s^{-1}) than early stage (Gosner stage 20-21) *B. orientalis* tadpoles (Kaplan & Phillips 2006:

268 4.9 - 5.5 cm s^{-1}), as would be expected. Therefore, our methodology does appear to elicit an

269 appropriate response.

270   Alternatively, the drag force imposed by exposed forelimbs, calculated by Dudley *et*

271 *al.* (1991) to be less than that presented by the externally developing hind limbs, may not

272   represent a significant impediment to locomotion. The forelimbs, after erupting, can be held

273   close to the body during swimming, rather than in a protracted position (Dudley *et al.* 1991),

274   lessening their drag. Furthermore, forelimb emergence should not only be regarded as a

275   potential cost, since it is central to the opportunity, unavailable to limbless tadpoles, of

276   moving on land and flexibility in the timing of the transition onto land appears beneficial

277   (Downie, Bryce & Smith 2004; Touchon *et al.* 2013).

278   To be effective, the degree and direction of turning when escaping a predator should

279   be somewhat unpredictable (Domenici, Blagburn & Bacon 2011), but could be biased by the

280   presence of a single exposed forelimb. However, having only one forelimb emerged did not

281   bias the direction that individuals turned (i.e. individuals with only the left forelimb emerged

282   did not predominantly turn left, as expected under significant drag). Similarly, forelimb

283   asynchrony did not appear to impair their ability to turn, nor did it exaggerate or dampen the

284   angle of escape. Interestingly, we also did not observe a change in the angle of escape when

285   both forelimbs were present as might be expected from having lateral appendages available

286   for steering (Wassersug 1989).

287   Though our hypotheses make only qualitative predictions about the extent of limb

288   emergence asynchrony (that it should be minimal), we saw relatively substantial differences

289   in emergence times of the two limbs. It has been argued that the fully aquatic life history of

290   pipid clawed frogs may limit any costs of drag caused by emergence of forelimbs (Walsh *et*

291   *al.* 2008a). The pipid species did indeed demonstrate the greatest degree of variability (CoV

292   > 90%). However, species with terrestrial adults – the frog *R. temporaria* and the toad *B.*

293   *bufo*, whose tadpoles more closely resemble the generalised form considered by Wassersug

294   (1989) and Dudley *et al.* (1991), also showed high variability (CoV ≥ 75%). One suggestion

295   is that any cost of forelimb emergence asymmetry might be avoided by behavioural

296   modification during metamorphic climax (Touchon *et al.* 2013), limiting selection for

297 synchronised emergence. Anurans do not feed during metamorphosis and may alter their

298 behaviour or choices of microhabitat to remain sheltered from predators (Ydenberg & Dill

299 1986; Lima & Dill 1990; Skelly 1994; Downie *et al.* 2004), rather than relying on escape

300 responses. Examining the behaviour of metamorphosing individuals for these species - in

301 particular, activity levels, microhabitat selection, and when during metamorphic climax

302 individuals make the transition between habitats - would be required to explore any impact of

303 forelimb synchronisation (Touchon *et al.* 2013).

304   However, more significantly, not only did all species not show a decrease in burst

305 speed, they actually swam faster after the emergence of one forelimb than before, regardless

306 of whether their adults are predominantly aquatic (*X. laevis* and *B. orientalis*) or terrestrial (*R.*

307 *temporaria* and *B. bufo*). Similarly, with the exception of one study (Wassersug & Sperry

308 1977), locomotory impairment during metamorphic climax has not been observed at all

309 (Watkins 1997; Walsh *et al.* 2008a; Walsh *et al.* 2008b) or occurs only after forelimb

310 emergence, associated with re-absorption of the tail (e.g. post-Gosner (Gosner 1960) tadpole

311 development stage 43: (Huey 1980; Brown & Taylor 1995)). Overall, these results seriously

312 question the proposition that locomotory impairment from the exposed forelimb explains

313 their internal development and sudden eruption (Wassersug 1989). Therefore, another

314 explanation is needed.

315   One possibility is that the internal development and sudden eruption may represent a

316 simple developmental constraint (Smith *et al.* 1985) of the lineage. For example, in the

317 common coqui *Eleutherodactylus coqui*, which develops directly into its adult form within an

318 egg, the forelimbs are enclosed by the opercular fold and emerge from this tissue via

319 perforations similar to species that undergo a metamorphosis (Callery & Elinson 2000).

320 However, there are no data on this phenomenon in other direct developing frogs.

321 Alternatively, it may represent an anatomical constraint, for example, of having the forelimbs

322 developing outside the body in the vicinity of the branchial chamber. Comparative studies

323 with urodele amphibians (newts and salamanders), whose tadpoles commonly have external

324 gills and develop forelimbs externally, may prove useful. Thirdly, selection for the delayed,

325 internal development and sudden eruption of the forelimbs could occur during different life

326 stages not examined in this study. Similarly, the selection for internal development of the

327 forelimbs may not relate to heightened predation risk caused by drag from the exposed limb,

328 but from exposed limbs being vulnerable to, or presenting additional targets for, predators

329 (Ballengee & Sessions 2009). Finally, there may be a developmental advantage in retaining

330 the forelimbs within the body cavity as they develop. While the forelimbs seemingly have no

331 function in the developing tadpole, they are essential for the juvenile and adult frog

332 (Wassersug 1989). Therefore their protection during the larval stage and rapid development

333 approaching metamorphosis may be advantageous. We hypothesise that retaining the

334 forelimbs within the body cavity may make it easier to both elevate and regulate their

335 temperature for rapid development (Casterlin & Reynolds 1978). This would coincide with

336 anuran amphibians' peak thermal preference, which occurs as their forelimbs develop and

337 just before they emerge (Dupré & Petranka 1985), and is compatible with the finding by

338 Bininda-Emonds *et al.* (2007) that amongst the tetrapod vertebrates studied, anuran

339 amphibians are unique in having forelimb development retarded relative to hind limb

340 development.

341

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Table legends

Table 1: Mean, median, range and coefficient of variance of time taken between the emergence of first and second forelimb. All times are presented in minutes.

Figure legends

Fig. 1 Graphical representations of the predictions for the "limb disruption" (circle) and "limb drag" (square) hypotheses. Filled shapes relate to the burst speed axis and open shapes to the escape direction bias axis. The dotted lines indicate the alternative possibility that burst performance may improve with the emergence of the second limb. For the escape direction bias axis, 0.0 represent no directional bias in turning, negative values indicate turning in the opposite direction from the first limb to emerge, and positive values indicate turning in the same direction as the first exposed limb

Fig. 2 Composite image from video footage of tadpole burst swimming. Images of the tadpole are taken from just prior to initiating burst response and every 60 ms after the first sign of movement. Burst speed was measured as the distance the snout travelled from the initial position to the final position over the 300 ms

Fig. 3 Mean (±SE) absolute (a) and relative (b) burst speed of the four different species, with no forelimbs, one forelimb and both forelimbs emerged (open circle: *Rana temporaria* (N = 25); closed circle: *Bufo bufo* (N = 25); closed triangle: *Xenopus laevis* (N = 16); open triangle: *Bombina orientalis* (N = 16)). Relative burst speed is presented to allow comparisons among the different sized species used in this study

486 **Fig. 4** The mean (±SE) snout-vent length of the four different species, with no forelimbs, one

487 forelimb and both forelimbs emerged that were used for assessing burst speed (open circle:

488 *Rana temporaria*; closed circle: *Bufo bufo*; closed triangle: *Xenopus laevis*; open triangle:

489 *Bombina orientalis*. Sample sizes as in Fig. 3

490

491 **Fig. 5** The turning bias after being startled in the four different species, with no forelimbs, one

492 forelimb and both forelimbs emerged. Positive values indicate a right bias and negative values

493 a left bias, 0.0 represents no bias (open circle: *Rana temporaria*: closed circle: *Bufo bufo*; closed

494 triangle: *Xenopus laevis*; open triangle: *Bombina orientalis*). Sample sizes as in Fig. 3

495

496 **Fig. 6** The angle of escape away from swimming directly forward in a straight line, which

497 would be represented by 0°, of the four different species, with no forelimbs, one forelimb and

498 both forelimbs emerged (open circle: *Rana temporaria*: closed circle: *Bufo bufo*; closed

499 triangle: *Xenopus laevis*; open triangle: *Bombina orientalis*). Sample sizes as in Fig. 3

500

501

502   Table 1

Species	N	Mean time (± SE)	Median time	Time range	CoV
R. temporaria	17	354.1 ± 64.4	349.0	11 – 847	74.9 %
B. bufo	20	362.8 ± 68.3	246.0	10 – 913	84.2 %
X. laevis	13	544.6 ± 142.0	264.0	29 – 1433	94.0 %
X. borealis	22	280.8 ± 63.7	193.0	2 – 1348	106.4 %
S. tropicalis	17	320.2 ± 82.5	231.0	1 – 1477	106.2 %

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