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Turning into frogs: asymmetry in forelimb emergence and escape direction in metamorphosing anurans

Citation for published version:

Walsh, P, Zechini, L, Waddell, E, Burns, T & Downie, R 2016, 'Turning into frogs: asymmetry in forelimb emergence and escape direction in metamorphosing anurans: Lateralised forelimb emergence and turning', *Laterality*, pp. 1-13. <https://doi.org/10.1080/1357650X.2016.1235175>

Digital Object Identifier (DOI):

[10.1080/1357650X.2016.1235175](https://doi.org/10.1080/1357650X.2016.1235175)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Peer reviewed version

Published In:

Laterality

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1 Turning into frogs: asymmetry in forelimb emergence and escape direction in metamorphosing
2 anurans

3
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7 Running head: Lateralised forelimb emergence and turning

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21 **Acknowledgements**

22 We thank Colin Dunlop (*X. borealis* and *X. tropicalis*), Gethin Evans, Adam Bland (*B.*
23 *orientalis*), and Isobel Maynard (*X. laevis*) for the captive-bred specimens and Diana Samuel for
24 use of the high speed camera. This work was supported by the Glasgow Natural History Society.

25

26 Word count:5234

27 **ABSTRACT**

28 There is considerable debate about the pattern and origin of laterality in forelimb emergence and
29 turning behaviour within amphibians, with the latter being poorly investigated in tadpoles around
30 metamorphic climax. Using six species of metamorphosing anurans, we investigated the effect of
31 asymmetrical spiracle location, and disturbance at the time of forelimb emergence, on the pattern
32 of forelimb emergence. Turning behaviour was observed to assess whether motor lateralisation
33 occurred in non-neobatrachian anurans and was linked to patterns of forelimb emergence. Biases
34 in forelimb emergence differed among species, supporting the hypothesis that asymmetrical
35 spiracle position results in the same asymmetry in forelimb emergence. However, this pattern
36 only occurred when individuals were undisturbed. Therefore, context at the time of the
37 emergence of the forelimbs may be important, and might explain some discrepancies in the
38 literature. Turning biases, unconnected to forelimb emergence, were found in Pipidae and
39 Bombinatoridae, confirming the basal origin of lateralised behaviour among anurans. Turning
40 direction in our metamorphs differed from the left-ward bias commonly observed in tadpoles, but
41 may be analogous to the prevalent right-"handedness" among adult anurans. Therefore, the
42 transitions occurring during metamorphosis may affect lateralised behaviour and metamorphosis
43 may be fruitful for understanding the development of lateralisation.

44

45 Key words: handedness, laterality, forelimb emergence, turning, metamorphic climax

46

47 **Introduction**

48 Behavioural lateralisation, or the favouring of one side of the body in a bilateral organism (so
49 called "handedness"), was once thought to be unique to humans and linked to the development of
50 quintessential human traits (e.g. language: Broca, 1865). However, laterality and morphological
51 asymmetries are now recognised in many non-human animals, including arthropods, fish,
52 amphibians, birds, and mammals (Bradshaw & Rogers, 1993; Bisazza *et al.*, 1998; Vallortigara &
53 Rogers, 2005; Vallortigara *et al.*, 2011; Ströckens *et al.*, 2013; Versace & Vallortigara, 2015).
54 While there is growing evidence that both lateralised behaviours and some morphological
55 asymmetries may be beneficial (Rogers *et al.*, 2004; Walsh *et al.*, 2011; Blackiston & Levin,
56 2013), the developmental and evolutionary origins of these lateral differences and what, if
57 anything, links physical asymmetries with behavioural lateralisation are still poorly understood
58 (Versace & Vallortigara, 2015).

59 Amphibians, particularly anurans, have emerged as a key group in which investigations of
60 lateralised behaviour and morphological asymmetries are being conducted (reviewed in Rogers,
61 2002; Wassersug & Yamashita, 2002; Malashichev & Wassersug, 2004). These investigations
62 have largely, but not exclusively, focused on three aspects: the lateralised behaviour of turning
63 preference in anuran larvae (Oseen *et al.*, 2001; Wassersug & Yamashita, 2002); the
64 asymmetrical emergence of the forelimbs at metamorphosis (Malashichev & Nikitina, 2002;
65 Malashichev, 2002; Zechini *et al.*, 2015); and forelimb preference in juveniles and adults
66 (reviewed in Ströckens *et al.*, 2013).

67 Anuran amphibian forelimbs develop within the opercular cavity (or, in pipids, in separate
68 brachial sacs) and, once well developed, emerge asymmetrically (Malashichev, 2002), through
69 openings in the overlying tissue. The mechanisms causing the openings are: 1) rising thyroid
70 hormone levels, leading to thinning and degeneration of the overlying tissues; and/or 2)

71 mechanical pressure from the underlying limb (Braus, 1906; Helff, 1926; Helff, 1939; Newth,
72 1949). Species level patterns in the order of forelimb emergence are often observed (e.g. left-bias
73 in several ranids: Speidel, 1925; Helff, 1926; Malashichev, 2002; right-bias in *Bufo bufo*:
74 Malashichev, 2002; or no bias in *Bombina bombina*: Malashichev, 2002) and have been linked to
75 the position and numbers of spiracles. In tadpoles, spiracles can vary from a single midline or
76 lateral (sinistral) spiracle to paired lateral spiracles, with forelimb emergence biases occurring
77 where a single lateral spiracle leads to the forelimb on that side emerging first (Speidel, 1925;
78 Borkhvardt & Ivanhintsova, 1994; Borkhvardt & Malashichev, 1997). However, there are
79 inconsistencies among studies, even within the same species (e.g. *Rana pipiens*: left bias Helff,
80 1926; Dickerson, 1969; right bias Rugh, 1977).

81 Similarly, turning behaviour in tadpoles has been demonstrated to often have a distinct
82 "handedness", with tadpoles predominantly making left turns particularly when startled
83 (Wassersug & Yamashita, 2002). However, this is not consistent across, nor within, species
84 (Yamashita et al., 2000; Oseen et al., 2001; Rogers, 2002). Most incidences of species level bias
85 have been observed in neobatrachian species (e.g. ranids, bufonids and hylids), with those that
86 diverged earlier in the anuran lineage (e.g. bombinatorids and pipids: Frost et al., 2006) possibly
87 not exhibiting a preference in turning direction (Yamashita et al., 2000; Oseen et al., 2001).
88 Furthermore, across species, the apparent left turning bias, if present, appears to diminish as
89 tadpoles develop, with the strength of the left bias strongest in early stage tadpoles (Wassersug &
90 Yamashita, 2002). However, the number of well-developed tadpole species (post-Gosner (1960)
91 stage 39) investigated has so far been limited. The apparent leftward tadpole bias differs from a
92 prevalent right forelimb preference in adult anuran amphibians (Rogers, 2002; Ströckens et al.,
93 2013).

94 In this study we investigated the directional bias in forelimb emergence and turning
95 behaviour in a taxonomically diverse set of larval anuran amphibian species at late developmental
96 stages. The results provide insight into the current ambiguity surrounding the potential link
97 between morphological and behavioural lateralisation in the emergence of forelimbs and turning
98 behaviour in late stage larval anurans.

99

100 **Method**

101 The study species (Table 1), rearing conditions and experimental procedures were the same as
102 presented in Zechini *et al.* (2015). To summarise the procedures in brief: all species, except
103 *Bombina orientalis* (acquired at Gosner stage 37) were acquired as eggs from up to two pairings
104 (Table 1), and then reared in the laboratory at varying densities in aerated, dechlorinated copper-
105 free water at 21 ± 3 °C, on a 12:12 L:D photoperiod, and fed *ad libitum* daily. When individuals
106 reached the stage prior to the onset of metamorphic climax (Walsh, 2010), where forelimbs were
107 noticeable under the skin but had not yet emerged (Gosner (1960) stage 41; Nieuwkoop & Faber
108 (1994) (NF) stage 57), they were isolated for inclusion in the study.

109 As part of a larger study, some individuals were separated to observe the duration
110 between the emergence of the forelimbs, and others were used to test turning direction and the
111 locomotory impacts of asymmetric forelimb emergence. Therefore, individuals, for inclusion in
112 this study, were subjected to two treatments: one where they were left undisturbed prior to the
113 emergence of the forelimbs; and the other where they were subjected to incidences of startling
114 prior to either forelimb emerging.

115 Assessing the lateral bias in turning behaviour was performed in a swimming arena, 30
116 cm in diameter and underlain with 1 cm grid paper, using a FASTCAM-PCI high-speed camera
117 (filmed at 250 frames per second) to capture footage of the tadpole's escape response after being

118 startled. Startling for each recording was done by a consistent discharge of air from a 1 ml Gilson
119 pipette to the rear of the animal (Van Buskirk & McCollum, 2000) using the grid paper to line up
120 the tadpole and the pipette. Each individual was recorded five times, with a 1 minute interval
121 between each recording. Turning direction was assessed as the direction that the body was flexed
122 during a c-start, which generally occurred 30 ms after startling. Turning bias was assessed in the
123 same individuals: 1) just prior to either forelimb emerging (Gosner stage 41; NF stage 57); 2)
124 after one forelimb had emerged; and 3) with both forelimbs emerged (Gosner stage 42; NF stage
125 58). The final assessment was performed as soon as both forelimbs emerged (within 12 hours),
126 so that all observations on an individual were done within a few days and were conducted prior to
127 tail re-absorption. Twenty-five *R. temporaria*, 25 *B. bufo*, 16 *X. laevis* and 30 *B. orientalis* were
128 assessed for turning bias. All individuals experienced disturbance, resulting from repeated
129 movement to and from the testing arena and the assessment of turning bias itself, at the time
130 when the forelimbs were emerging.

131 For those observed for the duration between the emergence of the forelimbs (Zechini *et*
132 *al.*, 2015), 17 *R. temporaria*, 20 *B. bufo*, 20 *X. laevis*, 49 *X. borealis*, 39 *X. tropicalis* and 22 *B.*
133 *orientalis* tadpoles were used. All individuals found prior to either forelimb emerging were held
134 under the same conditions, and the first forelimb to emerge was recorded, while only a subsample
135 of these were filmed and reported in Zechini *et al.* (2015) due to limited numbers of cameras.
136 Unlike individuals assessed for turning bias, all of these individuals were in isolated conditions
137 and not disturbed, even for feeding, until both forelimbs emerged and they were removed from
138 the study.

139 Chi-squared tests were used to determine whether each species had a bias in which
140 forelimb emerged first, while binary logistic regression was used to analyse whether the first
141 forelimb to emerge differed between the undisturbed and startled tadpoles. To determine whether

142 species were biased in their direction of turning when startled, each species at zero, one and both
143 forelimbs emerged, was analysed separately using Repeated G-tests for goodness of fit. Due to
144 the high frequency of heterogeneity, species level biases were confirmed using a modified
145 version of the laterality index for each individual as described by Bisazza et al. (2000), so that:

$$146 \quad \text{Laterality Index} = \left(\frac{\text{Turns to the right} - \text{Turns to the left}}{\text{Turns to the right} + \text{Turns to the left}} \right)$$

147 A general linear mixed model (GLMM) was used to examine the Laterality Index scores, with
148 species and the number of forelimbs emerged as fixed factors, ID as a random effect and the first
149 forelimb to emerge (either the left or right) as a covariate. Only significant interactions were
150 retained in the model. Bias in the laterality index was analysed using one-sample t-tests, for each
151 species and at each stage of forelimb emergence.

152

153 **Results**

154 *Forelimb emergence bias*

155 Of the six species in our study, four (*Xenopus laevis*, *X. borealis*, *X. tropicalis* and *Bombina*
156 *orientalis*) did not differ in which forelimb emerged first, regardless of whether they were startled
157 prior to forelimb emergence or not (Table 2). *Xenopus laevis* and *B. orientalis* had individuals
158 subjected to both treatments, and there was no difference in which forelimb emerged first
159 between the two observational groups (*X. laevis*: Wald = 2.74, df = 1, p = 0.10; *B. orientalis*:
160 Wald = 2.30, df = 1, p = 0.13).

161 In both *R. temporaria* and *B. bufo*, the left forelimb emerged first more frequently when
162 they were observed for the duration between forelimb emergences, but not when they were
163 assessed for turning direction (Table 2). In *R. temporaria* (Wald = 4.65, df = 1, p = 0.031), but

164 not *B. bufo* (Wald = 2.76, df = 1, p = 0.10), there was a significant difference in forelimb
165 emergence bias between the two observational groups (Table 2).

166

167 *Tadpole turning bias*

168 Species differed in their laterality index scores ($F_{3,274.62} = 4.19$, p = 0.006; Table 3). Laterality
169 index scores were closest to 0 (no bias) when both forelimbs had emerged, compared to either no
170 or one forelimb emerged ($F_{2,199.64} = 8.50$, p < 0.001). However, the forelimb to emerge first did
171 not affect the laterality index score ($F_{1,274.62} = 0.09$, p = 0.76).

172 *Bufo bufo* was the only species assessed for the direction of turning that did not show a
173 directional bias at any stage in the progression from neither to both forelimbs emerged (Table 3),
174 and individuals were homogeneous in not showing a directional bias (Table 3). In contrast,
175 *Xenopus laevis* exhibited a significant right bias in turning at each stage (Table 3). However,
176 there was greater heterogeneity at no and one forelimb emerged (Table 3), with some individuals
177 showing a very strong right bias while in others the right bias was less strong or individuals had a
178 left bias.

179 *Rana temporaria* exhibited a significant right bias only at stage 41, when no forelimbs
180 had emerged (Table 3), but there was a difference among individuals in the strength of their
181 rightward bias (Table 3). When *R. temporaria* had one or both forelimbs exposed, they did not
182 show a significant bias in either direction (Table 3). However, when one forelimb was exposed,
183 there was significant heterogeneity indicating that some individuals did show a significant
184 directional bias, but overall most individuals did not.

185 *Bombina orientalis* showed a consistent right bias when neither and one of the forelimbs
186 had emerged (Table 3), but there was significant variation in the strength of the bias when one
187 forelimb was emerged. After both forelimbs had emerged, the right bias diminished (Table 3).

188

189 **Discussion**

190 *Forelimb emergence bias*

191 We observed in all three pipid species and *B. orientalis* that although there was always
192 asymmetry in forelimb emergence, with variable timing between the emergence of the first and
193 second limb, there was no bias in which forelimb emerged first. This provides confirmation of
194 the results of Borkhvardt & Malashichev (1997) and Malashichev (2002), and expands them to
195 include two new species (*X. borealis* and *X. tropicalis*). We also observed that both *R.*
196 *temporaria* and *B. bufo*, when not assessed for turning direction, demonstrated a left bias in
197 forelimb emergence. These patterns of forelimb emergence bias provide support for the
198 hypothesis that forelimb emergence and spiracle position are linked with limbs able to emerge
199 more readily through a spiracle, which was proposed by Speidel (1925) and Borkhvardt &
200 Malashichev (1997), but later contested by Malashichev (2002) due to observations of strong
201 right bias in *B. bufo*. Any asymmetry in spiracle position, as in *Bufo* and *Rana*, may therefore
202 lead to asymmetrical forelimb emergence showing the same directional bias, whereas
203 symmetrical spiracles (either a single nearly midline, as in *Bombina*, or paired lateral spiracles as
204 in *Xenopus*) do not lead to a consistent bias in which forelimb emerges first.

205 Unexpectedly, *R. temporaria* and *B. bufo* did not show any lateral bias in forelimb
206 emergence when individuals were subjected to repeated startle stimuli during testing for turning
207 direction. This result suggests that under different circumstances additional factors may drive
208 forelimb emergence. When tadpoles approaching metamorphic climax are startled they often
209 erratically move their forelimbs within the opercular chamber (PTW, personal observations).
210 Therefore mechanical pressure from the elbows, which may be equal on both sides, may drive
211 emergence. Conversely, without the repeated disturbance caused by the assessment of turning

212 behaviour at the time the forelimbs were about to emerge, the perforations that form with the
213 impending onset of metamorphic climax might occur more readily on the side with the spiracle
214 (Speidel, 1925), leading to the biases we observed. That circumstances occurring around the time
215 of forelimb emergence play a role in the subsequent asymmetry (Versace & Vallortigara, 2015)
216 may also explain the often contradictory, or at least variable results that have been reported on
217 this phenomenon (Malashichev, 2002).

218 That there was such a stark difference in the lateral bias of forelimb emergence in *B. bufo*
219 between our current study and Malashichev (2002), is surprising. Unfortunately, the conditions
220 under which forelimb emergence occurred in Malashichev (2002) are not reported, so may have
221 contributed. Alternatively, given the challenges of classifying the *Bufo bufo* species group (e.g.
222 Garcia-Porta *et al.*, 2012; Arntzen *et al.*, 2013) there may be population differences in forelimb
223 emergence asymmetry that supercedes associations with spiracle placement, which would
224 warrant further investigation. However, with the exception of *B. bufo* where there is some
225 ambiguity, the species in our current study and those cited within Malashichev (2002) (Bhati,
226 1961; Borkhvardt & Ivanhintsova, 1994) all conform to the hypothesized association between
227 spiracle position and forelimb emergence. This suggests that the hypothesis may be more robust
228 than previously considered. Ultimately, confirmation would require reconciling currently
229 anomalous species (e.g. *Bufo bufo*) with this hypothesis or other hypotheses (e.g. link between
230 alternate limb locomotion to lateralisations (Malashichev, 2006)), or determine the significance
231 of context-dependent impacts on lateralisations.

232

233 *Tadpole turning bias*

234 All four species at all three stages, with the exception of *R. temporaria* and *B. bufo* with both
235 forelimbs emerged, demonstrated a weak trend towards turning right when startled, while

236 forelimb emergences were either left-biased or no bias present. Furthermore, across all species,
237 the direction of turning was not affected by whether the right or left forelimb emerged first.
238 Surprisingly, *X. laevis* and *B. orientalis*, which did not exhibit a lateral bias in the emergence of
239 their forelimbs, showed the most persistent lateralised turning response. The literature on turning
240 bias is highly equivocal, often due to different methods being used across studies (Wassersug &
241 Yamashita, 2002), but our result was unexpected for two reasons. Firstly, the most commonly
242 observed turning bias, if present, appears to be a left bias in tadpoles (Wassersug & Yamashita,
243 2002; Rogers, 2002; Malashichev & Wassersug, 2004). Secondly, species of Pipidae and
244 Bombinatoridae have previously been shown to lack any lateral bias, either in tadpole turning
245 (Wassersug *et al.*, 1999; Goree & Wassersug, 2001) or in adult forelimb use (*B. orientalis*: Goree
246 & Wassersug, 2001; *B. bombina*: Malashichev & Nikitina, 2002) or turning (*Xenopus laevis*:
247 Kostylev & Malashichev, 2007).

248 With respect to the right biased turning behaviour we observed, there is a possible
249 explanatory difference between the current study and previous work. Our study was specifically
250 focussed on late stage tadpoles just before and at the start of metamorphic climax. Most (8 out of
251 11) of the studies presented in Wassersug & Yamashita (2002) reporting a left bias did not
252 include individuals beyond Gosner stage 39. It has previously been observed that the prevalence
253 of the left bias diminishes as tadpoles develop (Wassersug *et al.*, 1999; Oseen *et al.*, 2001;
254 Malashichev & Wassersug, 2004). However, with the inclusion of our findings on late stage
255 tadpoles, the declining left bias may represent a transition from left bias to right bias with
256 development (Figure 1; Wassersug & Yamashita, 2002). This would be supported by the
257 prevalence of right limb bias, where biases occur, in adult anurans (Rogers, 2002, for exceptions
258 see *Bufo viridis*: Robins *et al.*, 1998). This could be due to the changes that occur in the transition
259 from tadpole tail driven locomotion to the inclusion of limbs in their locomotion, or neurological

260 changes in asymmetries observed during metamorphosis (Proshchina & Savel'ev, 1998).
261 However, given that the right bias diminished as one or both forelimbs emerged in two of the
262 four species that were assessed, this is not conclusive. Ultimately, greater focus on the
263 developmental progression of lateral bias across all stages is required.

264 Our results are the first to show a lateral turning bias in a pipid or a bombinatorid, both
265 sister groups to the neobatrachians (Frost *et al.*, 2006). This indicates that the origin of this
266 phenomenon in anurans is more ancient than previously suspected (Wassersug *et al.*, 1999; Goree
267 & Wassersug, 2001; Briggs-Gonzalez & Gonzalez, 2016) and conforms with lateralisations in
268 other features of this group (e.g. visual lateralisation in *Bombina variagata*: Bisazza *et al.*, 2002;
269 and *Xenopus laevis*: Gouchie *et al.*, 2008). While it has been argued that the late stage of the
270 *Bombina orientalis* tadpoles used by Goree & Wassersug (2001) may have contributed to the lack
271 of any apparent bias (Malashichev & Wassersug, 2004), our *Bombina orientalis* were even
272 further developed than those assessed previously. This could be explained by the transition in the
273 direction of bias mentioned earlier, but it is unclear why these species would be distinctly
274 affected. Regardless, the occurrence of lateralised behaviour in *Xenopus*, a common model
275 organism for neurological and developmental studies and amenable to manipulation experiments
276 on the direction of morphological lateralisations (Blackiston & Levin, 2013), means that there is
277 considerable scope for greater understanding of lateralisation and its origin.

278

279 **Disclosure statement**

280 No potential conflict of interest.

281 **References**

- 282 Arntzen, J. W., Recuero, E., Canestrelli, D., & Martínez-Solano, I. (2013). How complex is the
283 *Bufo bufo* species group? *Molecular Phylogenetics and Evolution*, 69, 1203–1208.
- 284 Bhati, D. P. S. (1961). Studies on the pectoral girdle and its musculature (with innervation) of
285 *Rana tigrina* Daud and *Bufo andersonii* Bouleng. *Agra University Journal of Research*
286 *(Science)*, 10, 131–135.
- 287 Bisazza, A., Cantalupo, C., Capocchiano, M., & Vallortigara, G. (2000). Population lateralisation
288 and social behaviour: a study with 16 species of fish. *Laterality*, 5, 269–284.
- 289 Bisazza, A., De Santi, A., Bonso, S., & Sovrano, V. A. (2002). Frogs and toads in front of a
290 mirror: lateralisation of response to social stimuli in tadpoles of five anuran species.
291 *Behavioural Brain Research*, 134, 417–424.
- 292 Bisazza, A., Rogers, J., & Vallortigara, G. (1998). The origins of cerebral asymmetry: a review of
293 evidence of behavioural and brain lateralization in fishes, reptiles and amphibians.
294 *Neuroscience & Biobehavioral Reviews*, 22, 411–426.
- 295 Blackiston, D. J., & Levin, M. (2013). Inversion of left–right asymmetry alters performance of
296 *Xenopus* tadpoles in nonlateralized cognitive tasks. *Animal Behaviour*, 86, 459–466.
- 297 Borkhardt, V. G., & Ivanhintsova, E. B. (1994). On the position of the epicoracoids in
298 amphibian arciferal pectoral girdles. *Russian Journal of Herpetology*, 1, 114–116.

- 299 Borkhvardt, V. G., & Malashichev, Y. B. (1997). Position of the epicoracoids in arciferal pectoral
300 girdles of the fire-bellies *Bombina* (Amphibia: Discoglossidae). *Russian Journal of*
301 *Herpetology*, 4, 28–30.
- 302 Bradshaw, J. L. & Rogers, L. J. (1993). *The evolution of lateral asymmetries, language, tool use,*
303 *and intellect*. New York: Academic Press.
- 304 Braus, H. (1906). Vordere extremität und operculum bei Bombinatorlarven. Ein beitrage zur
305 kenntnis morphogener correlation und regulation. *Morphol Jb*, 35, 509–590.
- 306 Briggs-Gonzalez, V. S., & Gonzalez, S. C. (2016). Lateralized turning biases in two neotropical
307 tadpoles. *Ethology*, 122, 582–587.
- 308 Broca, P. (1865). Du siège de la faculté du langage articulé. *Bulletins de la Société*
309 *d'Anthropologie*, 6, 377–393.
- 310 Dickerson, M. C. (1969). *The frog book: North American toads and frogs, with a study of the*
311 *habits and life histories of those of the Northeastern States*. New York: Dover
312 Publications.
- 313 Frost, D. R. (2013). Amphibian species of the world: An online reference (Version 5.6).
- 314 Frost, D. R., Grant, T., Faivovich, J., Bain, R. H., Haas, A., Haddad, C. F. B., De Sá, R. O.,
315 Channing, A., Wilkinson, M., Donnellan, S. C., Raxworthy, C. J., Campbell, J. A., Blotto,

- 316 B. L., Moler, P., Drewes, R. C., Nussbaum, R. A., Lynch, J. D., Green, D. M., & Wheeler,
317 W. C. (2006). The amphibian tree of life. *Bulletin of the American Museum of Natural*
318 *History*, 297, 1–291.
- 319 Garcia-Porta, J., Litvinchuk, S. N., Crochet, P. A., Romano, A., Geniez, P. H., Lo-Valvo, M.,
320 Lymberakis, P., & Carranza, S. (2012). Molecular phylogenetics and historical
321 biogeography of the west-palearctic common toads (*Bufo bufo* species complex).
322 *Molecular Phylogenetics and Evolution*, 63, 113–130.
- 323 Goree, B. D., & Wassersug, R. J. (2001). Are archeobatrachian anurans ambidextrous? Assessing
324 handedness in *Bombina orientalis*. *Journal of Herpetology*, 35, 538–541.
- 325 Gosner, KL. (1960). A simplified table for staging anuran embryos and larvae with notes on
326 identification. *Herpetologica*, 16, 1–12.
- 327 Gouchie, G. M., Roberts, L. F., & Wassersug, R. J. (2008). The effect of mirrors on African
328 clawed frog (*Xenopus laevis*) larval growth, development, and behavior. *Behavioral*
329 *Ecology and Sociobiology*, 62, 1821–1829.
- 330 Helff, O. M. (1926). Studies on amphibian metamorphosis: I. Formation of the opercular leg
331 perforation in anuran larvae during metamorphosis. *Journal of Experimental Zoology*, 45,
332 1–67.

- 333 Helff, O. M. (1939). Studies on amphibian metamorphosis: XVI. The development of forelimb
334 opercular perforations in *Rana temporaria* and *Bufo bufo*. *Journal of Experimental*
335 *Biology*, 16, 96–120.
- 336 Kostylev, M. A., & Malashichev, Y. B. (2007). Correlation of the shoulder girdle asymmetry
337 with the limb skeleton asymmetry in *Xenopus laevis*. *Doklady Biological Sciences*, 416,
338 374–376.
- 339 Malashichev, Y. B. (2002). Asymmetries in amphibians: A review of morphology and behaviour.
340 *Laterality*, 7, 197–217.
- 341 Malashichev, Y. B. (2006). One-sided limb preference is linked to alternating-limb locomotion in
342 anuran amphibians. *Journal of Comparative Psychology*, 120, 401–410.
- 343 Malashichev, Y. B., & Nikitina, N. G. (2002). Preferential limb use in relation to epicoracoid
344 overlap in the shoulder girdle of toads. *Laterality*, 7, 1–18.
- 345 Malashichev, Y. B., & Wassersug, R. J. (2004). Left and right in the amphibian world: which
346 way to develop and where to turn? *BioEssays*, 26, 512–522.
- 347 McDiarmid, R. W., & Altig, R. (1999). Body plan: Development and morphology. In R. W.
348 McDiarmid & R. Altig (Eds.), *Tadpoles: The biology of anuran larvae*. (pp. 24–52).
349 University of Chicago Press: Chicago.

- 350 Newth, D. R. (1949). A contribution to the study of fore-limb eruption in metamorphosing
351 *Anura*. *Proceedings of the Zoological Society of London*, 119, 643–659.
- 352 Nieuwkoop, P. D. & Faber, F. (1994). *Normal table of Xenopus laevis*. New York: Garland
353 Publishing.
- 354 Oseen, K. L., Newhook, L. K. D., & Wassersug, R. J. (2001). Turning bias in woodfrog (*Rana*
355 *sylvatica*) tadpoles. *Herpetologica*, 57, 432–437.
- 356 Proshchina, A. E., & Savel'ev, S. V. (1998). Study of amphibian brain asymmetry during normal
357 embryonic and larval development. *Izvestiia Akademii Nauk. Serii Biologicheskaja*,
358 1998, 408–411.
- 359 Robins, A., Lippolis, G., Bisazza, A., Vallortigara, G., & Rogers, L. J. (1998). Lateralized
360 agonistic responses and hindlimb use in toads. *Animal Behaviour*, 56, 875–881.
- 361 Rogers, L. J. (2002). Lateralised brain function in anurans: Comparison to lateralisation in other
362 vertebrates. *Laterality*, 7, 219–239.
- 363 Rogers, L. J., Zucca, P., & Vallortigara, G. (2004). Advantages of having a lateralized brain.
364 *Proceedings of the Royal Society of London B: Biological Sciences*, 271, S420–S422.
- 365 Rugh, R. (1977). *A guide to vertebrate development*. Minneapolis: Burgess Publishing Company.

- 366 Speidel, C. C. (1925). Endocrine glands and bilateral symmetry: Observations upon forelimb
367 eruption in frog larvae under treatment with thyroid and thymus extracts. *Biological*
368 *Bulletin*, 48, 336–346.
- 369 Ströckens, F., Güntürkün, O., & Ocklenburg, S. (2013). Limb preferences in non-human
370 vertebrates. *Laterality*, 18, 536–575.
- 371 Vallortigara, G., Chiandetti, C., & Sovrano, V. A. (2011). Brain asymmetry (animal). *Wiley*
372 *Interdisciplinary Reviews: Cognitive Science*, 2, 146–157.
- 373 Vallortigara, G., & Rogers, L. J. (2005). Survival with an asymmetrical brain: advantages and
374 disadvantages of cerebral lateralization. *Behavioral and Brain Sciences*, 28, 575–589.
- 375 Van Buskirk, J., & McCollum, S. A. (2000). Influence of tail shape on tadpole swimming
376 performance. *Journal of Experimental Biology*, 203, 2149–2158.
- 377 Versace, E., & Vallortigara, G. (2015). Forelimb preferences in human beings and other species:
378 multiple models for testing hypotheses on lateralization. *Frontiers in Psychology*, 6, 233–
- 379 Walsh, P. T. (2010). Anuran life history plasticity: A review of variable practice in determining
380 the end-point of larval development. *Amphibia-Reptilia*, 31, 157–167.

381 Walsh, P. T., Hansell, M., Borello, W. D., & Healy, S. D. (2011). Individuality in nest building:
382 Do Southern Masked weaver (*Ploceus velatus*) males vary in their nest-building
383 behaviour? *Behavioural Processes*, 88, 1–6.

384 Wassersug, R. J., Naitoh, T., & Yamashita, M. (1999). Turning bias in tadpoles. *Journal of*
385 *Herpetology*, 33, 543–548.

386 Wassersug, R. J., & Yamashita, M. (2002). Assessing and interpreting lateralised behaviours in
387 anuran larvae. *Laterality*, 7, 241–260.

388 Yamashita, M., Naitoh, T., & Wassersug, R. J. (2000). Startle response and turning bias in
389 *Microhyla* tadpoles. *Zoological Science*, 17, 185–189.

390 Zechini, L., Lilley, A., Downie, J. R., & Walsh, P. T. (2015). Why do frog and toad forelimbs
391 suddenly (but asynchronously) appear, every time metamorphosis is near? *Functional*
392 *Ecology*, 29, 816–822.

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396 Table 1. The species used in the study, detailing the number of pairs used to collect the spawn for
 397 the study, the number and location of spiracle(s), geographical region of origin, whether they are
 398 considered Neobatrachians or a sister group.

Species	Parentage of spawn	Spiracle location ¹	Geographical region of origin ²	Neobatrachia ³
<i>Rana temporaria</i>	Single pair	Single, sinistral	Europe	Yes
<i>Bufo bufo</i>	Single pair	Single, sinistral	Europe	Yes
<i>Xenopus laevis</i>	Two pairs	Two symmetrical	sub-Saharan Africa	No
<i>Xenopus borealis</i>	Single pair	Two symmetrical	sub-Saharan Africa	No
<i>Xenopus tropicalis</i> ⁴	Single pair	Two symmetrical	sub-Saharan Africa	No
<i>Bombina orientalis</i>	Two pairs	Single, virtually midline	central eastern Asia	No

399 ¹ McDiarmid & Altig, 1999; ² Frost, 2013; ³ Frost et al., 2006; ⁴ using *Xenopus tropicalis* as
 400 according to Frost, 2013

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404 Table 2. The number of individuals from each of the six species that had either the right or left
 405 forelimb emerge first, whether they were tested for turning direction or not. Chi-squared values
 406 are presented (df = 1 for all tests; * P < 0.01; ^{NS} Not significant).

	Observation: Duration of forelimb			Experiment: Assessed for turning		
	asymmetry			direction		
	Right first	Left first	χ^2	Right first	Left first	χ^2
<i>R. temporaria</i>	3	14	7.12*	13	12	0.04 ^{NS}
<i>B. bufo</i>	4	16	7.20*	11	14	0.36 ^{NS}
<i>X. laevis</i>	12	8	0.80 ^{NS}	11	5	2.25 ^{NS}
<i>X. borealis</i>	23	26	0.18 ^{NS}	-	-	-
<i>X. tropicalis</i>	23	16	1.26 ^{NS}	-	-	-
<i>B. orientalis</i>	10	12	0.18 ^{NS}	20	10	3.33 ^{NS}

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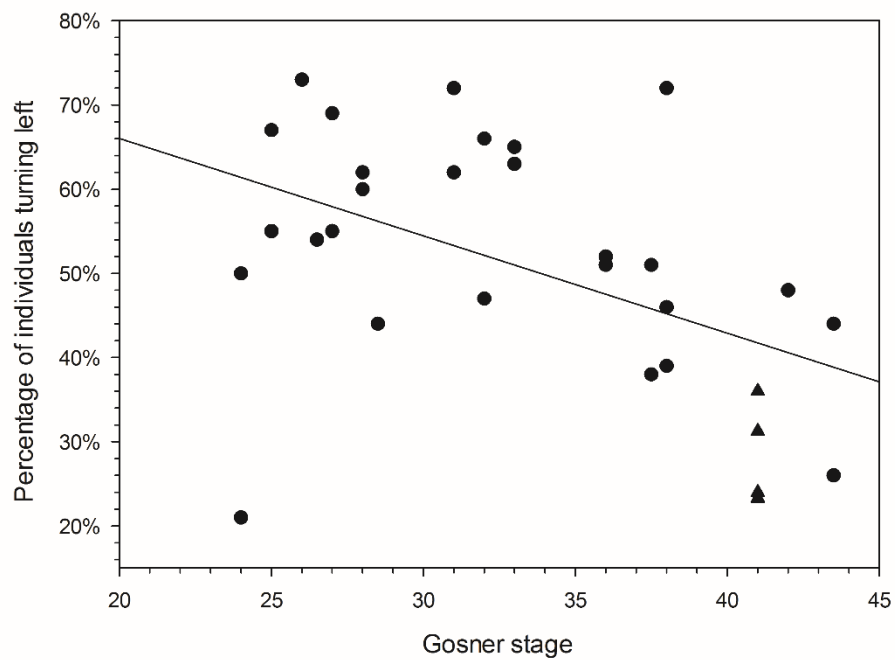
410 Table 3. Mean laterality Index (indicating right-ward turning bias) of all four species assessed for
 411 turning direction with neither, one and both forelimbs emerged. The t-statistic indicates whether
 412 the rightward-bias deviates from an index score of 0 (indicating no bias). Pooled G-value (df = 1)
 413 indicates whether the number of turns to the right for all individuals within a given category
 414 deviates from the expected 50:50 ratio of right : left turns. The Heterogeneity G-value indicates
 415 whether individuals differ in their tendency to turn right or left, significant values indicate
 416 individuals differ in the strength or direction of bias. For Heterogeneity G-values the df for *Rana*
 417 *temporaria* and *Bufo bufo* are 24, for *Xenopus laevis* 16, and *Bombina orientalis* 29. Significant
 418 individual-level biases in turning direction occurred when all five turns were in a single direction
 419 (G = 6.93, p = 0.008). Only individuals with significant biases have been shown, and are
 420 displayed as the ratio of left turning individuals to right turning individuals. (^{NS} Not significant, *
 421 < 0.05, ** < 0.01. *** < 0.005, **** < 0.0001)

			<i>R. temporaria</i>	<i>B. bufo</i>	<i>X. laevis</i>	<i>B. orientalis</i>
Neither forelimb emerged	Laterality Index	Right-bias	0.36 ± 0.10	0.04 ± 0.09	0.35 ± 0.15	0.28 ± 0.07
		t-statistic	3.49***	0.43 ^{NS}	2.41*	3.99****
	Repeated G-test	Pooled G-value	16.57****	0.20 ^{NS}	10.01***	11.92***
		Heterogeneity G-value	42.82*	30.37 ^{NS}	35.49***	26.56 ^{NS}
	Individual-level bias (L:R ratio)		0 : 5	2 : 0	0 : 5	0 : 2
One forelimb emerged	Laterality Index	Right-bias	0.07 ± 0.11	0.17 ± 0.09	0.48 ± 0.13	0.23 ± 0.10
		t-statistic	0.67 ^{NS}	1.88 ^{NS}	3.80***	2.24*
	Repeated G-test	Pooled G-value	0.65 ^{NS}	3.55 ^{NS}	18.80****	7.77**
		Heterogeneity G-value	41.83*	27.20 ^{NS}	29.98*	57.62*
	Individual-level bias (L:R ratio)		1 : 2	0 : 1	0 : 6	1 : 5
Both forelimbs emerged	Laterality Index	Right-bias	-0.14 ± 0.09	-0.04 ± 0.10	0.23 ± 0.08	0.01 ± 0.08
		t-statistic	-1.54 ^{NS}	-0.42 ^{NS}	2.92*	0.17 ^{NS}

Repeated G-test	Pooled G-value	2.32 ^{NS}	0.20 ^{NS}	5.05*	0.03 ^{NS}
	Heterogeneity G-value	26.70 ^{NS}	30.541 ^{NS}	8.52 ^{NS}	31.55 ^{NS}
Individual-level bias (L:R ratio)		1 : 0	1 : 0	0 : 0	1 : 1

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423 Figure 1: Percentage of individuals in a study that exhibited a left bias in turning direction at the
424 mean Gosner stage from data published (●) in Wassersug & Yamashita (2002) and our data from
425 stage 41 tadpoles (▲). Each point represents a species and stage class ($R^2 = 24.3\%$, $t = -3.15$, $p <$
426 0.005).



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