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Behavioural consistency and life history in *Rana dalmatina*

tadpoles

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Summary

Evolutionary behavioural ecology has recently turned towards understanding the causes and consequences of behavioural consistency, manifesting either as animal personality (consistency in a single behaviour) or behavioural syndrome (consistency across more behaviours). Behavioural type (mean individual behaviour) has been linked to life-history strategies, leading to the emergence of the integrated pace-of-life syndrome (POLS) theory. Using *Rana dalmatina* tadpoles as models, we tested if behavioural consistency and POLS could be detected during early ontogenesis of amphibians. We targeted two ontogenetic stages and measured activity, exploration and risk-taking in a common garden experiment. Besides individual behavioural type, we also analysed intra-individual behavioural variation. Activity was consistent in all tadpoles, exploration only became consistent with advancing age, and risk-taking only became consistent in tadpoles that were tested, and thus disturbed, earlier. Only previously tested tadpoles showed trends indicative of behavioural syndromes. We found an activity – age at metamorphosis POLS in the previously untested tadpoles irrespective of age. Relative growth rate correlated positively with the intra-individual variation of activity of the previously untested older tadpoles. In the previously tested older tadpoles, intra-individual variation of exploration correlated negatively and intra-individual variation of risk-taking positively with relative growth rate. We provide evidence for behavioural consistency and POLS in predator- and conspecific naive tadpoles. Intra-individual behavioural variation was also correlated to life history, suggesting its relevance for POLS theory. The strong effect of moderate disturbance related to standard behavioural testing on later behaviour draws attention to the pitfalls embedded in repeated testing.

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Introduction

One of the recent goals in evolutionary behavioural ecology is to understand the proximate

and ultimate mechanisms resulting in individual behavioural consistency. We simply refer

to "behavioural consistency" by meaning individual consistency that raises systematic

differences between individuals in their mean behaviour (Sih et al. 2004, 2012; Bell 2007;

Kortet et al. 2010, Wolf and Weissing 2012). Animal personality, behavioural syndrome,

temperament, etc. are often used interchangeably as synonyms in the behavioural

consistency literature. However, it has been suggested that animal personality and

behavioural syndromes should refer to different patterns for the sake of consistent

experimental design and analysis (Garamszegi and Herczeg 2012; Jandt et al. 2014) and we

follow this practice here. Since the terminology used in the behavioural consistency field

can be confusing, we provide definitions of the key terms used in this paper in Box 1.

Researchers usually test first for the presence of personality (repeatability of single

behaviours) and behavioural syndromes (correlations between repeatable behaviours) based

on a group of individuals. Upon proving the presence of personality or a syndrome, they

focus on the analysis of the individual behavioural types (mean behaviour) observed in the

studied populations or species. However, this approach is problematic, as the intra-

individual variation in behaviour remains totally neglected, meaning that an important

component of individual behaviour is lost. Recent developments allow circumventing this

issue by providing new approaches to quantify intra-individual behavioural variation both

in animal personality and behavioural syndromes (Herczeg and Garamszegi 2012; Stamps

et al. 2012; Dingemanse and Dochtermann 2013). By adopting this view, an individual is

characterised simultaneously by its behavioural type and behavioural variation, grasping

two potentially independent aspects of its behaviour. Whenever environmentally induced

71 behavioural shifts (individual behavioural plasticity; Dingemanse et al. 2010) is controlled

72 for, and measurement error is distributed evenly among the studied individuals, the

73 remaining differences in behavioural variation should represent how precisely individuals

74 express their behavioural type. Hereafter, we use the term 'intra-individual behavioural

75 variation' to describe this behavioural component following Stamps et al. (2012).

76

77 Studies on animal personalities and behavioural syndromes are accumulating at a fast pace

78 (e.g. Smith and Blumstein 2008; Bell et al. 2009; Garamszegi et al. 2012, 2013). Réale et

79 al. (2010) integrated behaviour in pace-of-life syndromes (POLS) describing life-history

80 strategies along a fast – slow lifestyle continuum, aiming to explain the adaptive nature of

81 behavioural consistency. According to the POLS hypothesis, behavioural types may couple

82 with life history characteristics, as certain combinations of life history and behaviour can

83 be more adaptive in particular situations than others, but various combinations may

84 eventually yield equal expected life-time fitness in a heterogeneous environment (Biro et

85 al. 2006; Réale et al. 2010; Wolf and Weissing 2010). In this view, behavioural consistency

86 arises from variation of life-history strategies between individuals in a population (Stamps

87 2007; Wolf et al. 2007; Careau and Garland 2012). Individuals following the fast pace-of-

88 life strategy are expected to be more active, risk-taking, aggressive and to explore more

89 superficially. They are also expected to mature earlier, have faster metabolism and weaker

90 immune system (i.e. "live fast, die young"). On the other hand, slow pace-of-life

91 individuals are expected to be risk-avoiding, less active, thoroughly exploring and less

92 aggressive, while having longer life span, longer developmental time and more efficient

93 immune responses (Réale et al. 2010).

94

95   Even though the POLS hypothesis seems logical, the number of studies investigating it are

96   few, with mixed results. For instance, Careau et al (2011) supported the hypothesis by

97   finding a link between exploration and metabolic rate in deer mice *(Perumyscus*

98   *maniculatus)*. In field crickets (*Gryllus integer*), a boldness – immune response correlation

99   supported POLS, but no link between boldness and timing of maturation was found

100   (Niemelä et al. 2012a). David et al. (2012) found a connection between feeding motivation

101   and the degree of proactivity in zebra finches (*Taeniopygia guttata*) supporting POLS. In

102   brown trout (*Salmo trutta*), the positive aggression – mortality correlation supported POLS,

103   but the negative activity – mortality correlation contradicted it (Adriaenssens and Johnsson

104   2013). We must also note that even in the supportive literature, many of the predicted

105   POLS associations have not been detected. Further, it is possible that not only behavioural

106   types, but intra-individual behavioural variation is also inlcuded in POLS. Considering that

107   the fast pace-of-life strategy relies on fast growth and early reproduction, and that a fixed

108   behavioural srategy is less energy demanding (no need for costly cognitive abilities;

109   Coppens 2010; Niemelä et al. 2012b), we hypothesize that fast-paced individuals that

110   perform better in predictable, stable environments are characterised by low intra-individual

111   behavioural variation.

112

113   Environmental effects hamper evolutionary conclusions drawn from phenotypic data

114   collected in the wild (e.g. Kuparinen and Merilä 2007; Gienapp et al. 2008; Teplitsky et al.

115   2008; Merilä 2009). Accordingly, several studies emphasized the importance of experience

116   during early ontogeny on personality expressed later in life (Dingemanse et al. 2009; Rodel

117   and Monclus 2011; Butler et al. 2012). Further, if the influence of early experience is

118   manifested in multiple traits, it can not only affect the mean expression of these traits, but

119   also their correlations. Therefore, exposure to different environmental factors during the

120 early phase of life can have consequences for behavioural syndromes and POLS measured

121 at later phase. Such environmental effects can stem from experimental manipulations. For

122 instance, when behaviour is tested multiple times along ontogeny, tests and handling

123 including novel stimuli or stress can directly alter the later behaviour of the same

124 individual. This potential confounding effect has rarely been addressed experimentally (but

125 see Ruiz-Gomez et al. 2008; Stamps and Groothuis 2010).

126

127 The primary goal of the present study was to test for behavioural consistency and POLS at

128 different ontogenetic stages using agile frog (*Rana dalmatina*) tadpoles as a model.

129 Amphibian larvae in general are excellent candidates for studies on behavioural

130 consistency (Sih et al. 2003; Wilson and Krause 2012). We also aimed to test whether the

131 disturbance connected to standard behavioural testing affected behaviour later during

132 ontogenesis. To have a full grasp on individual behavioural variation, we focused not only

133 on behavioural type, but on intra-individual behavioural variation. We reared *R. dalmatina*

134 tadpoles individually in a standardised common garden experiment providing food *ad*

135 *libitum*. This approach excluded the effects of previous experience with predators or

136 conspecifics, and energetic constraints on the behaviour and life-history characteristics of

137 focal tadpoles. Therefore, as environmental variation was negligible and no systematic

138 variation in measurement error could be expected, the behavioural variation expressed by

139 an individual represented intra-individual behavioural variation. Half of the tadpoles were

140 tested at two ontogenetic stages, while the other half only at the later stage. In particular,

141 we tested for (i) presence of animal personality and behavioural syndromes at different

142 ontogenetic stages of *R. dalmatina*, (ii) correlations of individual behavioural type or intra-

143 individual behavioural variation with age and size at metamorphosis, and (iii) an effect of

144 experimental manipulations, including stress, on later behavioural consistency and POLS.

145

Materials and Methods

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Field sampling and rearing

149

We collected *R. dalmatina* eggs from a pond on the Island of Szentendre, near

Szigetmonostor (47°40'40.77" N, 19°5'31.47" E) where both invertebrate and vertebrate

aquatic predators are present (e.g. Aeshnid dragonfly larvae, Dysticid water beetle larvae,

different fishes). We sampled 80 freshly laid clutches between 17 and 20-Mar-2011. Thirty

randomly selected eggs were taken from each clutch and kept in separate plastic containers

(8 litre volume; $34 \times 23 \times 16$ cm) holding two litres of reconstituted soft water (RSW,

APHA 1985) at 19 °C and a 12:12 light-dark photoperiod. Another 10-10 random eggs

from each clutch were photographed (Fujitsu s7000 digital camera; pictures taken from a

standard distance and angle using a size standard for each image). Later, we used the mean

egg diameter per clutch (measured with the freeware Image Tool v. 3.0, UTHSCSA 2002)

as a proxy for maternal investment (Laugen et al. 2002).

161

After hatching, one randomly selected healthy tadpole was left in each rearing container.

Hence, we had 80 individuals, each from a different clutch, included in the experiment.

This way, we could maximize the genetic variation between the studied individuals,

gathering a good representation of the original population. We note that the analysis of full-

sib families collected in the wild would have not been useful for quantitative genetic

inference, so we chose to maximise the number of families included by not having within-

family replicates. Remaining tadpoles were released at the site of collection. Experimental

tadpoles were fed with chopped and slightly boiled spinach *ad libitum*, with food refilled

170 two hours before the end of the daily light period. Water was changed every four days.

171 Everything that came in contact with the tadpoles was thoroughly rinsed to ensure that all

172 individuals remained naïve regarding the presence of conspecifics. Each of the containers

173 was placed into white polystyrene cells to facilitate the recording of movements and to

174 prevent visual contact between adjacent cells.

175

176 We followed up the development of every individual on daily basis. We were particularly

177 interested in stage 32-36 (Gosner 1960, early stages of toe development), when we

178 performed the second round of behavioural assays (the first assays were done based on

179 tadpole age, see below) and in stage 42 (emergence of forelimbs) when we evaluated age

180 and mass at metamorphosis. When a tadpole approached stage 42, we started to monitor it

181 every two hours to be able to record age and mass at metamorphosis with high accuracy.

182 We randomly assigned tadpoles into two groups. The first group's behaviour was assessed

183 two times, first at the age of 11 days (after the onset of the free swimming stage; hereafter

184 '11-day-old') and second at stage 32-36 (hereafter 'pre-tested stage 32-36'), while the

185 second group was only assessed at stage 32-36 (hereafter 'naïve stage 32-36'). This way,

186 we could evaluate the effect of behavioural measurements performed 11 days after

187 hatching on the behaviour at stage 32-36. With other words, we could evaluate tadpole

188 behaviour at stage 32-36 independently of the potential effects of previous behavioural

189 tests.Taken together, we measured behaviour at two ontogenetic stages and recorded age

190 and mass at metamorphosis in a third ontogenetic stage.

191

192 *Behavioural assays*

193

194 We assessed three different behaviours (following Réale et al. 2007, Garamszegi et al.

195 2013): activity, novel area exploration and risk-taking. One measurement period lasted for

196 three days, during which all three behaviours were assessed daily on each individual

197 separately. We started with activity (movement rate in the familiar environment) as this

198 was estimated without disturbance, followed by measuring exploration and risk-taking in

199 random order, as the latter can be seen as invasive processes including handling and novel

200 stimuli (see details below). Between the invasive behavioural tests, we let the individuals

201 rest in their familiar rearing containers for at least two hours. We recorded the tadpoles'

202 behaviour with webcams using the open source Dorgem software (Fesevur,

203 http://dorgem.sourceforge.net/). Upon the completion of all tests, individuals were released

204 back to their pond of origin.

205

206 *Activity*

207

208 Activity in a familiar environment was measured in the rearing container without

209 disturbance, two hours after the beginning of the light period. Activity recordings lasted for

210 30 min, resulting in ca.1800 images (with 1 frame / second sampling). We measured

211 activity (movement frequency) by dividing the number of images where the sampled

212 individual changed its position compared to the previous image by the total number of

213 images.

214

215 *Exploration*

216

217 Exploration in a novel environment was recorded in four 36 litre plastic containers (for

218 details see Fig. 1). These containers had opaque grey walls and their bottom was divided

219 into 40 equal sized rectangles. In each container, there were four smaller ones filled with

220 gravel functioning as obstacles, placed in a way to prevent the overview of the whole area.

221 For each trial, we filled the arenas with four litres of RSW. Individuals were initially placed

222 behind a three-sided veil. After 5 minutes of acclimation, the veil was lifted and the

223 individual's movements were recorded for 25 minutes. Exploration was quantified as the

224 number of rectangles visited at least once divided by the number of available rectangles.

225 We deemed a rectangle visited if an individual had crossed the line separating two adjacent

226 rectangles at least with its full body without the tail. Containers were thoroughly washed

227 between trials.

228

229 *Risk-taking*

230

231 Risk-taking was measured in the rearing containers by using a threat stimulus. We used a

232 55 cm long plastic tube with handles mounted on the sides. In the tube, an 11 cm long and

233 6 mm wide metallic rod was suspended. The metallic rod could be released so that it fell

234 through the plastic tube but came to a halt at 11 cm below the lower opening of the tube.

235 We provided a threat stimulus by placing this device over the container of the tadpole and

236 letting the rod fall next to the focal tadpole. This was carried out always by TJU. Aiming

237 the device was done by eye. We could not fully exclude variation in the distance between

238 the tadpoles and the threat stimulus, but we are confident that any bias introduced by this

239 variation was minor and randomly distributed among the test animals. Tadpoles responded

240 to the stimulus by quickly swimming away and freezing (immobility). Their behaviour was

241 recorded for 15 minutes after the threat stimulus. To quantify risk-taking, we measured the

242 latency to restart activity. This measure included the time spent swimming away and the

243 time spent freezing; the former typically lasting only for a few seconds. If an individual

244   remained inactive for more than 15 minutes, we stopped the observation and assigned the

245   maximum score (900 seconds) to the individual.

246

247   *Statistical analyses*

248   We only included individuals in the analyses that have reached Gosner stage 42 and had

249   complete behavioural data. In thirteen individuals, behavioural data were lost due to

250   camera malfunction, 12 individuals were excluded due to abnormal development, five

251   individuals got stressed/injured during handling (by e.g. jumping out of the holding net)

252   and 10 individuals died from unknown reasons. We had 19 individuals in the group that

253   was assessed twice during ontogeny, providing the 11-day-old and pre-tested stage 32-36

254   data, and 21 individuals in the group that was assessed only once at the later developmental

255   stage providing the naïve stage 32-36 data. In our analyses, we treated the three data

256   batches separately, because of the imbalanced design and the different patterns regarding

257   the presence/absence of personalities and syndromes between the batches (see Results).

258

259   To assess if tadpoles exhibited personality, we estimated the repeatability of the different

260   behaviours by comparing the between-individual component of variation to the total

261   variation based on the three measurements of every individual. We used an Analysis of

262   Variance based approach following Becker (1985), which generally gives a reliable

263   estimate (Nakagawa and Schielzeth 2010). We also ran General Linear Mixed Models

264   (GLMMs) with individual as random factor and the behavioural variable of interest as the

265   dependent variable to calculate repeatabilities, and we got almost identical repeatability

266   estimates (data not shown). To test directly whether behavioural consistency changed along

267   ontogeny after the disturbance involved with behavioural testing at the early stage, we ran

268   GLMMs with the given behaviour as the dependent variable, developmental stage (11 days

269 old *vs*. Gosner stage 32-36) as a fixed and individual as a random effect. The main interest

270 here was in the individual $\times$ developmental stage interaction entered in the model as a

271 random effect, which would indicate that the individual effect differs between ontogenetic

272 stages when testing for behavioural consistency.

273

274 We tested for behavioural syndromes using Spearman rank correlations between

275 behavioural types using repeatable behaviours only. We also included the intra-individual

276 variation of the different behaviours in the correlations, irrespective whether the behaviour

277 was repeatable or not, as this variable might be informative even in the absence of

278 significant individual variation in behavioural types. This was necessary since complex

279 behavioural strategies, like different behavioural types being expressed with different level

280 of variation or intra-individual variation of functionally different behaviours being non-

281 independent, could also be present. For the calculation of behavioural type and variation,

282 see below. In the case of the group where behaviour was assessed at two ontogenetic

283 stages, we tested for correlations between behavioural types also across these stages.

284

285 To control for the statistical problems arising from the large number of non-independent

286 tests, we applied False Discovery Rate correction (Benjamini and Hochberg 1995), which

287 is thought to perform best among Bonferroni-type corrections (as suggested by García 2004

288 and Verhoeven et al. 2005). In the variable that describes risk-taking, individuals that

289 remained immobile during the whole observation period of 15 min received the maximum

290 score of 900 sec. Individuals receiving this score more than once would falsely increase

291 repeatability, hence they were excluded from repeatability calculations (N = 0 in 11-day-

292 old tadpoles; N = 1 in pre-tested stage 32-26 tadpoles; N = 3 in naïve stage 32-36 tadpoles).

293 This was a typical "right censoring" effect often observed in latency variables, as most

294 researchers are not able to sample individuals beyond a given threshold (Stamps et al.

295 2012), and there is no unbiased method of repeatability calculations avoiding right

296 censoring without using a much longer observational period or using different tests to

297 record the same behaviour (Carter et al. 2013). In a follow-up experiment, we doubled the

298 observation period, and we still could not avoid this effect.

299

300 In the subsequent analyses, individual behaviour was characterised by two variables: (i)

301 behavioural type and (ii) intra-individual behavioural variation. When significant

302 repeatability indicated the presence of personality regarding the given behaviour,

303 behavioural type was represented by the mean behaviour. We calculated intra-individual

304 behavioural variation as the standard deviation of the subsequent three behavioural

305 measures. Those individuals (see above) that received 900 sec score more than once in the

306 risk-taking trials were not used in analyses using intra-individual behavioural variation

307 variables. As we found no strong support for behavioural syndromes (see Results), we did

308 not quantify complex behavioural types.

309

310 We applied General Linear Models (GLMs) to test for relationships between variables

311 describing individual behaviours and age and mass at metamorphosis in the different

312 groups to test for the presence of POLS. Age and mass at metamorphosis were not

313 independent ($r = 0.414$, N = 40, $P = 0.008$). Therefore, we analysed (1) age at

314 metamorphosis and (2) mass at metamorphosis corrected for age by including age at

315 metamorphosis as a covariate in the latter models. This way, we could analyse relative

316 growth rate irrespective of the timing of metamorphosis. We also added mean egg size to

317 our models as a proxy for maternal effects. The starting models were built with all

318 explanatory variables and then we applied backward stepwise model simplification based

319   on the *P* values (only effects with $P < 0.05$ were kept in the final models; Grafen and Hails

320   2002) to avoid potential problems due to the inclusion of non-significant terms (Engqvist

321   2005). This method is generally considered conservative (Murtaugh 2009; Hegyi and

322   Garamszegi 2011). We found no significant deviations from normality in model residuals

323   applying Kolmogorov-Smirnov tests with Lilliefors correction.

324

325   Pre-tested stage 32-36 tadpoles showed correlations between behavioural types and intra-

326   individual behavioural variation (see Results). In this case, the original variables should not

327   be put into the same GLM as explanatory variables to avoid multicollinearity. Therefore,

328   we ran a Principal Component Analysis (PCA) on the behavioural variables of this group,

329   and after entering the individual variables separately, we also ran our models with the new,

330   by definition independent, unrotated PCs. The PCA resulted in two PCs with Eigenvalues

331   higher than one (Table 1). The first PC explained 48.6 % of the total variation and

332   described relationships with all variables but exploration (Table 1). The second PC

333   explained 17.6% of the total variation and described mainly variation in exploration (Table

334   1). In our GLM results, we also report effect sizes (partial eta squared, η^2) besides

335   significance. All analyses were performed with PASW Statistics 18 (PASW Inc., Chicago,

336   IL, USA).

337

338   **Results**

339

340   *Personality and behavioural syndromes*

341

342   Only activity was repeatable in all three studied groups. Exploration was repeatable at both

343   older tadpole groups. Risk-taking was only repeatable in the pre-tested stage 32-36 tadpole

344   group (Table 2). Hence, these behaviours can be considered as the ones describing

345   personality in the different tadpole groups. Our GLMMs indicated that behavioural

346   consistency changed along ontogeny following disturbance (individual $\times$ developmental

347   stage interaction; activity: $\chi^2 = 3.49$, $P = 0.031$; exploration: $\chi^2 = 2.45$, $P = 0.059$; risk-

348   taking: $\chi^2 = 6.29$, $P = 0.006$). Consistency of activity got weaker, while consistency of

349   exploration and risk-taking emerged only at the later stage (see Table 2). The mean

350   behavioural type changed only regarding exploration (activity: $F_{1,18} = 0.11$, $P = 0.92$;

351   exploration: $F_{1,18} = 17.13$, $P < 0.001$; risk-taking: $F_{1,18} = 0.24$, $P = 0.63$): older tadpoles

352   explored larger areas (data not shown). We note that here we cannot separate age effect

353   from size effect, because older tadpoles were also larger and exploration was tested in

354   similarly sized arenas. However, this should not influence estimates of behavioural

355   consistency or any of the following results.

356

357   We found no correlations between behavioural types and intra-individual behavioural

358   variation in 11-day-old or naïve stage 32-36 tadpoles, but various correlations emerged in

359   the pre-tested stage 32-36 group (for details please see Table 3). There were strong trends

360   for an activity –risk-taking correlation in the pre-tested stage 32-36 tadpoles ($r_S = 0.53$, N =

361   18, $P = 0.023$), and for a correlation between the activity of 11-day-old and exploration of

362   naïve stage 32-36 tadpoles ($r_S = -0.59$, N = 18, $P = 0.009$), but these correlations fell out

363   after the False Discovery Rate correction. The significance of these, otherwise strong,

364   effects (the mean effect size of behavioural correlations was found to be approximately 0.2

365   in a meta-analysis; Garamszegi et al. 2012) might have been higher upon higher sample

366   size. Hence, it is possible that a behavioural syndrome emerged in the pre-tested stage 32-

367   36 group and there was also an ontogenetic syndrome between different behaviours, but the

368   significance of these correlations is sensitive to the large number of tests we ran. For all

369   correlations, see Supplementary Table 1. PC1 from our PCA contained both activity and

370   risk-taking with high and positive loadings, further emphasizing the possibility for a valid

371   activity – risk-taking syndrome in this group (Table 1).

372

373   *Pace-of-Life Syndrome*

374

375   Activity of both 11-day-old and naïve stage 32-36 tadpoles showed negative relationship

376   with age at metamorphosis (11-day-old: $F_{1,17} = 19.23$, $P < 0.001$, $\eta^2 = 0.53$; naïve stage 32-

377   36: $F_{1,16} = 7.16$, $P = 0.017$, $\eta^2 = 0.31$; Fig. 2). The intra-individual variation of activity in

378   naïve stage 32-36 tadpoles showed a negative correlation with relative mass at

379   metamorphosis ($F_{1,15} = 6.69$, $P = 0.021$, $\eta^2 = 0.31$; Fig. 3a). This implies that more active

380   individuals metamorphosed earlier and less variable individuals reached higher mass

381   relative to their age.

382

383   In the pre-tested stage 32-36 group, the intra-individual variation of exploration showed a

384   negative relationship with relative mass at metamorphosis ($F_{1,14} = 5.98$, $P = 0.028$, $\eta^2 = 0.3$;

385   Fig. 3b), while the intra-individual variation of risk-taking showed a positive relationship

386   with relative mass at metamorphosis ($F_{1,14} = 5.14$, $P = 0.04$, $\eta^2 = 0.27$; Fig. 3c). In other

387   words, individuals expressing low variation in exploration or high variation in risk-taking

388   gained more mass during the tadpole stage. The effect sizes obtained from the above tests

389   can be seen as strong (Cohen 1988). No other explanatory variable had significant effect on

390   age or mass at metamorphosis (all $P > 0.07$, for more details see Supplementary Table 2).

391

392   **Discussion**

393

394 We found strong support for personalities and POLS, but only a marginally significant

395 trend for behavioural syndromes in naïve *Rana dalmatina* tadpoles. Besides behavioural

396 type, intra-individual behavioural variation was also linked to life history. We also found

397 strong effects of the minor manipulation connected to our behavioural assays on all levels

398 of behavioural patterns recorded approximately 30 days after the first manipulation.

399

400 *Personality and behavioural syndromes*

401

402 Behavioural traits typically show low to moderate repeatability (0.37-0.47) with wild-

403 caught individuals showing higher consistency than laboratory-reared ones (Bell et al.

404 2009). Our results generally concur with this trend. We found that while certain behaviours

405 are consistent throughout ontogeny (activity), others only become consistent during

406 development (exploration) or as a response to an environmental stimulus (risk-taking).

407 Hence, the developmental component in emerging personalities is an important one (e.g.

408 Groothuis and Trillmich 2011; Trillmich and Hudson 2011). Wilson and Krause (2012)

409 showed that in the marsh frog (*Pelophylax ridibundus*) activity and exploration was

410 consistent even over metamorphosis. However, Wilson and Krause (2012) used wild-

411 caught tadpoles and thus the circumstances resulting in the emergence of behavioural

412 consistency were not controlled for. In our study on *R. dalmatina*, ontogenetic consistency

413 appears to be weak at best within the tadpole stage. This implies strong time scale

414 dependency in both the presence/absence of personality, and in the actual behavioural type

415 in predator- and conspecific-naïve tadpoles. Besides aging, individual experience during

416 development seems also crucial in emerging personalities, as in our case risk-taking

417 became consistent only in individuals that were previously subjected to potentially stressful

418 situations. This draws attention to the fact that the interpretation of patterns observed in

419   wild-caught individuals is not straightforward (see e.g. Merilä 2009) and also adds to the

420   growing literature emphasizing that individual experience during ontogeny is an important

421   component to consider in behavioural studies (Stamps and Groothuis 2010).

422

423   Correlations among behavioural traits occur in many taxa in the wild (Garamszegi et al.

424   2012, 2013). However, studies based on laboratory-reared predator- and conspecific-naïve

425   individuals are scarce, and typically report on the lack of behavioural syndromes (Herczeg

426   et al. 2009, present study; but see Riechert and Hedrick 1993). After we applied the False

427   Discovery Rate method, we could only find a fairly strong but non-significant trend for an

428   activity – risk-taking syndrome in the pre-tested group experiencing some disturbance in an

429   earlier stage. Sweeney et al. (2013) compared wild-reared spiders with laboratory-reared

430   ones through multiple ontogenetic stages, and found syndromes only in the older wild-

431   reared spiders, suggesting that behavioural syndromes are manifestations of

432   environmentally induced phenotypic plasticity. The same inference was also supported by

433   an elegant experiment of Bell and Sih (2007), where behavioural syndromes in three-spined

434   sticklebacks (*Gasterosteus aculeatus*) emerged as a plastic response to predation risk. On

435   the other hand, some studies showed behavioural correlations on the genetic level (e.g. van

436   der Waaij et al. 2008; Dingemanse et al. 2009; Dochtermann and Dingemanse 2013;

437   Rigterink et al. 2014). Studies separating environmental and genetic contributions to the

438   emergence of behavioural syndromes are necessary to resolve this question.

439

440   Besides individual behavioural type, the behavioural variation an individual expresses

441   should also be considered in studies focusing on behavioural consistency in general

442   (Herczeg and Garamszegi 2012; Stamps et al. 2012). In theory, this variation can have

443   three main components: (i) behavioural plasticity, which is an environmentally induced,

444 potentially adaptive shift; (ii) intra-individual behavioural variation, which is an

445 environment-independent estimate of how precisely an individual expresses its behavioural

446 type; and (iii) measurement error. Here, we analysed variables measured in standardised

447 behavioural assays of common garden reared tadpoles, hence, behavioural plasticity should

448 be close to zero, and measurement error should be low and even across individuals. Thus,

449 individual divergence in behavioural variation reflects divergence in intra-individual

450 behavioural variation in this case. An interesting pattern emerged regarding intra-individual

451 behavioural variation: several correlations were present between intra-individual

452 behavioural variation of different behaviours or between intra-individual behavioural

453 variation and behavioural type, but only in the previously tested stage 32-36 group. This

454 allows for two conclusions. First, these results provide evidence for complex behavioural

455 strategies, where different behavioural types are expressed with different variation. More

456 risk-taking individuals were less variable in their risk-taking, more active individuals were

457 also less variable in their activity, and individuals less variable in exploration were less

458 variable in risk-taking too. We are aware that there are non-biological explanations for a

459 positive correlation between the mean and variation of any variable. However, in the

460 present case high risk-taking, represented by low values, and high activity, represented by

461 high values, were both coupled with low variation. Hence, it is unlikely that the

462 correlations are mere statistical artefacts. Second, the emergence of this pattern in the late

463 stage was a result of the disturbance related to the behavioural trials at the early stage;

464 hence, it can be seen as environmentally induced. We further discuss this below.

465

466 *Pace-of-Life Syndromes*

467

468   The integrative POLS hypothesis predicts complex relationships between behaviour, life

469   history, immune defence and physiology (Réale et al. 2010). As the POLS framework is

470   relatively recent, studies aiming to find evidence pro or contra are scarce. So far, the results

471   are mixed (Careau et al. 2011; David et al. 2012; Niemelä et al. 2012a; Adriaenssens and

472   Johnsson 2013; Sweeny et al. 2013; Le Galliard et al. 2013). Our results add to the

473   supportive literature: tadpoles with high activity developed faster, showing support for the

474   integration of behaviour to POLS at both ontogenetic stages. The fact that in our survey

475   activity was the only trait repeatable at both ontogenetic stages in predator- and

476   conspecific-naïve tadpoles further suggests its importance in tadpole life history strategies.

477   The minor disturbance regarding testing the tadpoles at the early ontogenetic stage

478   uncoupled the POLS in this group at the later stage.

479

480   We found no POLS regarding exploration, risk-taking or mass at metamorphosis. Hence, it

481   seems that even in studies supporting POLS, only a subset of the expected correlations can

482   be found. However, we found strong links between intra-individual behavioural variation

483   and life-history: individuals with lower variation in their activity grew faster than their

484   more variable conspecifics in the naïve stage 32-36 tadpoles, and individuals with lower

485   variation in exploration and higher variation in risk-taking gained more mass in pre-tested

486   stage 32-36 tadpoles. This implies that intra-individual variation in behaviour can be linked

487   to fitness and possibly fit into the POLS framework. In our case, low intra-individual

488   behavioural variation in activity was coupled with higher growth rates in tadpoles kept in a

489   predictable environment (*ad libitum* food, predator and conspecific free), i.e. low intra-

490   individual behavioural variation seems to be an attribute of high pace-of-life individuals.

491   This makes sense if we consider that the proactive (fast-pace-of-life) strategy is beneficial

492   in stable environments (Sih et al. 2004) where low behavioural variation is also expected

493   (Coppens et al 2010; Niemelä et al. 2012b). On the other hand, in the disturbed

494   environment, low intra-individual variation in risk-taking was associated with relatively

495   low growth rate, while low intra-individual variation in exploration was associated with

496   higher growth rates. Hence, the relationships between intra-individual behavioural variation

497   and life-history are not always straightforward to explain. Further, it appears that both

498   thorough and superficial explorers could have high relative mass gain if they had exhibited

499   low intra-individual behavioural variation. This would be against the general predictions of

500   the POLS hypothesis. Integration of behavioural variation into the POLS theory is indeed a

501   fascinating possibility, but further studies will be necessary to establish a general pattern.

502

503   The importance of maternal effects on larval phenotypes has been recognized in

504   amphibians. Larvae hatching from larger eggs have higher growth rates and increased

505   survival with possible carry-over effects into juvenile frog stage and even adulthood

506   (Kaplan 1998). Laugen et al. (2002) found that egg size affected the size of offspring and

507   their growth rate positively, but only under *ad libitum* food availability in *R. temporaria*.

508   Egg size may also influence offspring personality (Andersson and Höglund 2012). In our

509   case, egg size did not influence any of the measured fitness traits directly during ontogeny.

510

511   *The effects of prior manipulation*

512

513   We found considerable differences in behaviour between the pre-tested and naïve tadpoles

514   at stage 32-36. Only the pre-tested group showed (i) repeatability in risk-taking, (ii) a

515   strong, near-significant behavioural correlation, (iii) correlation of individual behavioural

516   type and intra-individual behavioural variation, (iv) correlation between intra-individual

517   behavioural variation and relative body mass, and (v) the absence of the activity – age at

518 metamorphosis POLS. Mean behavioural type and mean behavioural variation did not

519 differ between the naïve and pre-tested groups (data not shown). The only difference

520 between these groups' development was the disturbance caused by the behavioural assays

521 (carrying tadpoles to the exploration-arena and exposing them to the simulated predator-

522 strike three times across three days) approximately one month before the differences

523 between the pre-tested and naïve stage 32-36 groups were documented. We emphasize that

524 (i) apart from the tests done at 11 days age, the rearing procedure was totally standardised

525 and (ii) the behavioural testing related disturbance can be seen as nothing extraordinary in

526 the behavioural literature. These results draw attention to two facts. First, behaviour seen in

527 the wild must have a strong environmentally induced component and cannot be used for

528 evolutionary inference *sensu stricto*. Second, it will be challenging to study ontogenetic

529 shifts in individual behaviour when the given behavioural assay includes novelty stimuli or

530 stress. These finding coupled with the important role of individual experience in the

531 emergence of complex behavioural strategies, draws attention to a serious methodological

532 problem regarding the study of behavioural consistency and suggests that current

533 methodologies based on repeated and invasive testing of individuals will have to be

534 reconsidered.

535

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549

550   **References**

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784

785

Box 1. Definitions of the key terms used in this paper.	
Term	Definition
Animal personality	Consistent between-individual differences in a single behaviour
Behavioural syndrome	Consistent between-individual differences across functionally different behaviours
Behavioural type	The mean behaviour of an individual
Individual behavioural plasticity	Individual behavioural variation induced by environmental change
Intra-individual behavioural variation	Individual behavioural variation unrelated to the environment, i.e. the precision of the expression of the behavioural type
Pace-of-life syndrome (POLS)	Consistent individual differences across behavioural, physiological and life history traits

786

787

788   Table1. Results of the Principal Component Analysis ran on the pre-tested stage 32-36

789   *Rana dalmatina* tadpole group's behavioural variables. Behavioural type is represented by

790   means and behavioural variation by standard deviations (SD).

791

Variable	PC1	PC2
Mean Activity	0.698	-0.501
SD Activity	-0.706	0.029
Mean Exploration	-0.234	0.711
SD Exploration	0.584	0.488
Mean Risk-taking	0.827	0.035
SD Risk-taking	0.921	0.243
Proportion of variation explained (%)	48.6	17.6
Eigenvalue	2.92	1.06

792

793

794   Table 2. Repeatability ± Standard Errors of the different behaviours of *Rana dalmatina*

795   tadpoles. The *P* value of the given General Linear Model is also provided in parenthesis.

796   Significant repeatability values are in bold font.

797

	Activity	Exploration	Risk-taking
11-day-old	**0.4 ± 0.15**	0.01 ± 0.15	0.005±0.13
N=19	($P = 0.0021$)	($P = 0.22$)	($P = 0.5$)
Naïve stage 32-36	**0.55 ± 0.12**	**0.24 ± 0.14**	0.087 ± 0.15
N=18	($P = 0.00013$)	($P = 0.032$)	($P = 0.27$)
Pre-tested stage 32-36	**0.3 ± 0.15**	**0.39 ± 0.15**	**0.24 ± 0.16**
N=18	($P = 0.015$)	($P = 0.002$)	($P = 0.048$)

798

799

800   Table 3. Multiple behavioural type – intra-individual behavioural variation correlations

801   found significant after Bonferroni correction in 'pre-tested stage 32-36' tadpole group.

802   Namely, more active individuals were less variable in their activity; more risk-taking

803   individuals were also less variable in their risk-taking, and finally, individuals less variable

804   in exploration were also less variable in risk-taking. Note that in the case of Risk-taking,

805   high numbers represent shy individuals.

806

Spearman correlations of Pre-tested stage 32-36	r_s	N	*P*
Activity – Variation of Activity	-0.63	18	0.005
Risk-taking – Variation of Risk-taking	0.81	18	<0.0001
Variation Exploration – Variation of Risk-taking	0.72	18	0.001

807

808

809

810 Figure legends

811

812 **Fig. 1**. Experimental setup to study novel area exploration. The arena dimensions were: 80

813 $\times$ 32 $\times$ 18 cm (length, width, height, respectively). Grey squares represent the fenced

814 starting area, black areas represent the obstacles (boxes filled with gravel), while the grid

815 was used to quantify movements

816

817 **Fig. 2**. An activity – age at metamorphosis Pace of Life Syndrome seen in A) 11-day-old

818 (N=19) and B) naïve stage 32-36 *Rana dalmatina* tadpoles (N=18). More active individuals
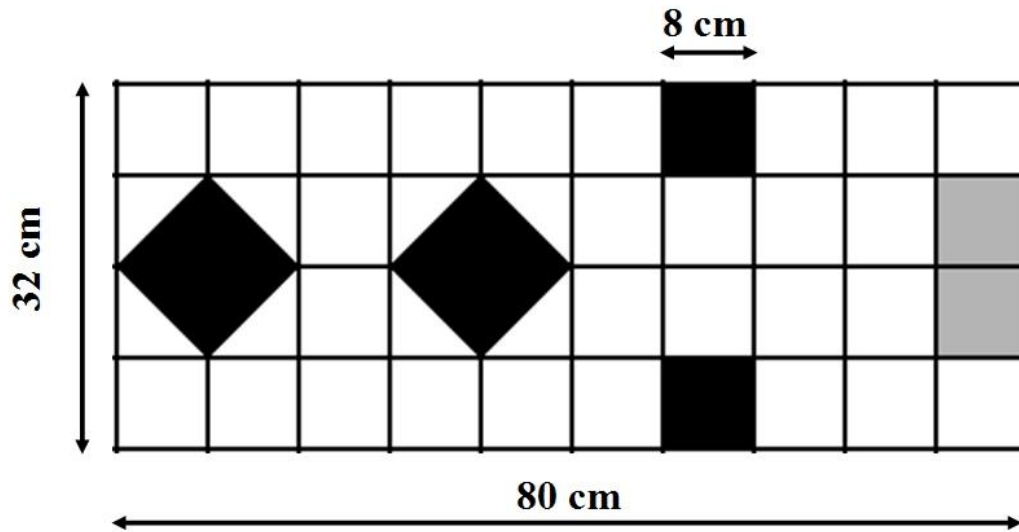
819 started metamorphosis earlier

820

821 **Fig. 3**. The relationship between intra-individual behavioural variation and relative mass at

822 metamorphosis (mass corrected for age) in *Rana dalmatina* tadpoles. A) Negative

823 correlation between variation of activity and relative mass in naïve stage 32-36 tadpoles

824 (N=18). B) Negative correlation between variation of exploration and relative mass in pre-

825 tested stage 32-36 tadpoles (N=18). C) Positive correlation between variation of risk-taking

826 and relative mass in pre-tested stage 32-36 tadpoles (N=18)

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828   Figure 1.

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836   Figure 3

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