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2 **Size-dependent tradeoffs in aggressive behavior towards kin**

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18 *Short title:* Tradeoffs in larval aggression

19

20 **Abstract.**

21 Aggression between juveniles can be unexpected, as their primary motivation is to survive until their
22 reproductive stage. However, instances of aggression, which may escalate to cannibalism, can be vital
23 for survival, although the factors (e.g. genetic or environmental) leading to cannibalism vary across

24 taxa. While cannibalism can greatly accelerate individual growth, it may also reduce inclusive fitness
25 when kin are consumed. As a solution to this problem, some cannibals demonstrate kin discrimination
26 and preferentially attack unrelated individuals. Here, we used both experimental and modeling
27 approaches to consider how physical traits (e.g. size in relation to opponent) and genetic relatedness
28 mediate aggressive behavior in dyads of cannibalistic *Dendrobates tinctorius* tadpoles. We paired
29 sibling, half-sibling, and non-sibling tadpoles of different sizes together in an arena and recorded their
30 aggression and activity. We found that the interaction between size and relatedness predicts aggressive
31 behavior: large non-siblings are significantly more aggressive than large siblings. Unexpectedly,
32 although siblings tended to attack less overall, in size mismatched pairs they attacked faster than in
33 non-sibling treatments. Ultimately, it appears that larval aggression reflects a balance between
34 relatedness and size where individuals trade-off their own fitness with that of their relatives.
35

36 **Introduction**

37 Cannibalism has often been understood to be a response to stressful environmental conditions (e.g.
38 low-food, high competition) that is modulated by physical (e.g. size, condition) and genetic variables.
39 It was not until the late-twentieth century that the behavior was critically evaluated and one-off
40 observations and anecdotes (Jenkins and Carpenter, 1946; Merritt Hawkes, 1920) were synthesized
41 into a review about the trends and occurrence of cannibalism (Fox, 1975). More recent investigations
42 of cannibalism have recorded and tested its manifestation across a wide variety of clades,
43 environmental states, and life history stages (Van Allen et al., 2017; Barkae et al., 2014; Van den
44 Beuken et al., 2019; Cooper et al., 2015; Schulte and Mayer, 2017). Aggression, a behavior often
45 tested as the precursor to cannibalistic behavior, can occur in resource-abundant, low-density
46 environments (Fox, 1975; Mock et al., 1987; Summers and Symula, 2001), and the effect of commonly

47 tested variables (i.e. starvation, competition) on eliciting cannibalistic behavior does not follow a
48 consistent trend on either class-wide or even family-specific levels. Ultimately, for a behavior
49 expressed in almost every clade in the animal kingdom, it remains difficult to tease apart the
50 evolutionary motivation behind cannibalism.

51 Aggression in cannibals can be difficult to interpret, as the motivation is not always hunger-driven,
52 and death is not always the end-state of aggressive interactions. For example, cannibalistic lizards
53 (*Podarcis gaigeae*) in Greece show local variation in aggression, where males are more aggressive in
54 high-density (but high-resource) islands. It appears that aggression by males leads to the consumption
55 of conspecific tails, which although is not always deadly, serves as a high-fat meal that decreases the
56 sexual quality of male competitors (Cooper et al., 2015). Aggression between juveniles complicates
57 matters further, as individuals are not competing for mates and usually do not hold territories. For the
58 most part, juvenile aggression and cannibalism are justified by competition for immediate nutritional
59 resources (larval flounders: Dou et al., 2000; earwig nymphs: Dobler and Kölliker, 2011), or parental
60 care (vulture chicks: Margalida et al., 2004) which may be survival mechanisms to compensate for
61 fluctuating environmental conditions (Mock et al., 1987).

63 Theoretical justifications predicting cannibalism are often built around physical qualities. Across the
64 animal kingdom, many studies have found that cannibals are most often larger than their prey (Barkae
65 et al., 2014; Claessen et al., 2004; Ibáñez and Keyl, 2010; Rojas, 2014) although exceptions exist when
66 larger individuals are weakened (Richardson et al., 2010). Kinship between individuals has also been
67 used to characterize cannibalistic interactions. This has been shown to be an important factor in several
68 cannibalistic species who demonstrate kin discrimination and avoid eating kin (salamanders: Pfennig
69 et al., 1994, bulb mites: Van den Beuken et al., 2019, toad tadpoles: Pfennig and Frankino, 1997),

71 though this is far from a rule, as similar range of cannibals do consume their kin without avoidance
72 (moths: Boots, 2000, poison frog tadpoles: Gray et al., 2009). Despite kinship and physical
73 attributes driving much of the discussion on the evolution and maintenance of cannibalism, few
74 studies have incorporated both in predicting cannibalism outside of oophagy or sexually competitive
75 contexts. Moreover, such studies have so far been limited to invertebrates: in European earwigs,
76 kinship and weight asymmetry affected cannibalistic behaviour both separately and in an interaction,
77 such that weight asymmetry effects were stronger among unrelated individuals (Dobler and Kölliker,
78 2011). In both desert (Bilde and Lubin, 2001) and wolf spiders (Roberts et al., 2003), kinship but not
79 weight asymmetry affected cannibalism. While fascinating in their own right, these studies hardly
80 provide a basis for generalising to distant clades such as vertebrates.

81 *Dendrobates tinctorius* is a species of Neotropical poison frog whose larvae are aggressive cannibals
82 (Rojas, 2014). Tadpoles are often deposited by their fathers in ephemeral pools of water, where they
83 are left to develop until metamorphosis (Rojas and Pašukonis, 2019). Although tadpoles are most often
84 transported singly, the ephemeral pools in which they are deposited can have multiple tadpoles of
85 various developmental stages (Rojas and Pašukonis, 2019) and degrees of relatedness (B. Rojas & E.
86 Ringler, unpublished data). In these environments, cannibalism is common (Rojas, 2014, 2015), yet
87 the escalation of aggression to cannibalism in this species has not been explicitly tested. Closely related
88 poison frogs have linked aggressive behavior and cannibalism (Gray et al., 2009; Summers and
89 Symula, 2001), though exceptions exist in obligate egg-feeders with parental care (Dugas et al., 2016).
90 For *D. tinctorius*, the costs of aggression are direct, as consuming kin reduces inclusive fitness and the
91 potential for injury (even with a small counterpart) is high; the potential benefits, on the other hand,
92 are complex, as by shortening time to metamorphosis and increasing physical size thereafter,
93 individuals may be able to escape precarious conditions and improve fitness prospects.

94 In this study we tested physical and genetic variables to better understand the basis of aggression in a
95 cannibalistic species. We conducted behavioral assays between pairs of *Dendrobates tinctorius*
96 tadpoles, and measured aggression and activity in response to size differences and relatedness. These
97 tests will allow us to evaluate the importance of physical characteristics with respect to genetic
98 relatedness as predictors of aggression. Experiments were supplemented with theoretical models based
99 on inclusive fitness theory to study the predictors of aggression in this species; together, these
100 experiments and models contribute to our understanding of how cannibalism is shaped by the costs
101 and benefits of relatedness and aggression in animals.

102 **Methods**

103 *Study species*

104 *Dendrobates tinctorius* is a species of Neotropical poison frog with elaborate parental care. Males
105 attend small terrestrial clutches and transport newly-hatched tadpoles, one or two at a time, to pools
106 of water where they are left until metamorphosis. Males carrying more than one tadpole at once can
107 be seen either depositing both tadpoles in the same pool or distributing tadpoles between pools (Rojas
108 and Pašukonis, 2019). Tadpoles of this species are omnivorous and frequently demonstrate
109 cannibalistic behaviour (Rojas, 2014, 2015); despite this, it is not unusual to see tadpoles of various
110 stages coexisting within the same pool in the wild (Rojas and Pašukonis, 2019).

112
113 We used tadpoles from a breeding laboratory population of *D. tinctorius* kept at the University of
114 Jyväskylä, Finland. We maintained a paternal half-sibling design as it could be expected that paternal
115 half-siblings are more likely to co-occur as a result of fathers reusing pools after multiple transport
116 events. Tadpole dyads were assigned in response to (1) individuals needing to be visually
117 distinguishable from each other (i.e. size), and (2) the laboratory mating schedule/network, which was

118 prioritized as to not stress the animals from overbreeding. Adult pairs were each housed in a 55L
119 terrarium that contained layered expanded clay, leaf-litter, moss substrate and were equipped with a
120 shelter, logs, and live plants. Terraria were maintained at 26C ($\pm 2\text{C}$) and were automatically misted
121 with reverse osmosis water four times a day (maintaining a humidity around 95%) and lit with a 12:12
122 photoperiod. Frogs were fed live *Drosophila* fruit flies coated in vitamin supplements five times per
123 week. Tadpoles were raised singly in 10 x 6.5 x 5 cm containers which were filled with spring water,
124 and fed *ad libitum* a diet of fish food (JBL NovoVert flakes) three times a week. Adult and tadpole
125 health and water levels were checked daily.

126
127 *Behavioral trials*

128 Pairs of tadpoles of different degrees of relatedness (full sibling, half-sibling, non-sibling) were placed
129 together in an arena. Tadpoles in early larval development were used, but we established a cut-off
130 point before the toe differentiation in hind legs development to control for possible life-history effects
131 (stage 31; Gosner, 1960). Experimental tadpole weight ranged from 0.04g to 0.38g, and size
132 differences between pairs ranged from 0.03g to 0.30g. Blinding in the experiment was not possible, as
133 the set-up and experiment were conducted by the same person, but the order of trials was assigned
134 randomly. The arena was a 18.5cm by 12cm clear plastic container filled with 400 mL of spring water;
135 four quadrants were delineated on the base of the arena to provide information about tadpole activity
136 throughout the experiment. Initially, each tadpole was placed on either side of an opaque partition
137 dividing the arena; this partition kept tadpoles separated but allowed water to flow throughout the
138 container. After an acclimation period of one hour, tadpole activity (resting, swimming) of the
139 separated individuals was recorded every 15 seconds for 10 minutes.

140

141 Following the acclimation and separated observation, the barrier was removed and tadpole interactions
142 were recorded for 60 minutes. Focal behaviors (resting, swimming, biting, and chasing; see Table 1
143 for descriptions) were recorded for both tadpoles every 15 seconds. Tadpoles were visually
144 distinguishable from each other as a result of size differences. Individuals were photographed and
145 weighed before the beginning of each trial to establish initial tadpole condition, and were only used
146 once ($n_{\text{Trial}} = 15$ for each relatedness level, $n = 90$ tadpoles for the entire experiment).

147 Trials were ended prematurely if tadpoles demonstrated aggression levels that would cause severe
148 damage or death (where bites lasted for more than 2 seconds, recorded as “potential lethal attack”).
149 Although aggression was common, potential lethal attacks were rare, occurring in only 3/45 trials.
150 There were no tadpole deaths as a result of the behavioral trials, and all tadpoles were kept and reared
151 in the laboratory after the experiment. Assays were done according to the Association for the Study of
152 Animal Behaviour’s guidelines for the treatment of animals in behavioural research and teaching
153 (ASAB 2017), and with the approval of the National Animal Experiment Board at the Regional State
154 Administrative Agency for Southern Finland (ESAVI/9114/04.10.07/2014).
155

156

Behavior	Description
Resting	Non-movement under normal, healthy conditions
Swimming	Movement of the tadpole’s tail in over two consecutive oscillations without ‘intent’ (see below) with respect to the other tadpole. Sometimes tadpoles contract their muscles or displace by floating up or down the water column; these behaviors did not qualify as swimming in this experiment.

157	Bitting	A tadpole attempting or successfully grasping/clutching any body part of another. This behavior was allowed if it was isolated and its duration lasted less than 2 seconds. If the bite was followed by a “hold” by a tadpole that could result in permanent injury or death of the counterpart, the trial was terminated and categorized as ended by a means of “potential lethal attack”.
158	Chasing	A tadpole swimming after its counterpart with intent; “intent” was perceived by the observer as rapid changes in swimming speed and direction of swimming being towards the other tadpole

Table 1. Categorization of behaviour observed during aggression trials between tadpoles.



158

159 *Image 1. Illustration of aggressive exchange between *D. tinctorius* tadpoles. Note the tail bite on one*
160 *of the tadpoles, which is a common occurrence both in wild and laboratory encounters. Published*
161 *with artist permission.*

162

163 *Statistical analysis*

164 All models and statistics were performed in the program R (v. 3.6.1, R Development Core Team,
165 2019) with additional packages “glmmTMB” (Magnusson et al., 2020), “coxme” (Therneau, 2020),
166 “dplyr” (Wickham et al., 2018), “tidyr” (Wickham et al., 2019). Activity and aggression analyses (see
167 below) took into account pair identity (Pair_ID) and family (Breeding pair) level random effects
168 (Supp. tables 1 and 2 for AIC comparisons). Differences in duration of trials during experiments (n =
169 3/45 trials ended early due to potential lethal attacks) were taken into account by offsetting models
170 with a trial duration. Aggression and activity models were chosen based on the Akaike Information
171 Criterion (AIC, Akaike, 1973), which compares fit based on log-likelihood and the number of model
172 parameters (see supplementary materials for model AIC comparisons). We considered both negative
173 binomial (with linear and quadratic parameterization) and poisson distributions as model families in
174 AIC comparisons. Residual diagnostics and overdispersion were checked and corrected using the
175 “DHARMa” (Hartig, 2020) package.

176 *Activity levels*

177 Tadpole activity was categorized as “resting” and “swimming” (see Table 1 for details). Tadpole
178 activity was observed during post-acclimation (10 minutes) and experimental (max. 60 minutes)
179 periods. Activity was coded as counts and were modeled in a generalized linear mixed model
180 framework (GLMM). The best fitting model used a negative binomial linear parameterization where
181 activity was predicted by relative tadpole size (a categorical variable (i.e. small, large), where size is
182 relative within pairs) and relatedness (a categorical variable with three levels: full sib, half sib, non
183 sib) (see Supplementary Table 1).

184 *Overall aggression*

185 Aggression between tadpoles was observed as chasing or biting (see Table 1), which were recorded as
186 counts. These two behaviors were combined to represent “total aggression”. The best fitting model for
187 aggression was a generalized linear mixed effect model (GLMM) with a negative binomial linear
188 parameterization. Total aggression was predicted by relatedness (a categorical variable with three
189 levels: full sib, half sib, non sib) and size (a categorical variable (i.e. small, large), where size is relative
190 within pairs)(see Supplementary Table 2), pairs were taken into account as a random variable. We had
191 modeled mass difference (a continuous predictor) between tadpoles as potential predictors which
192 resulted in less parsimonious models (see Supplementary Table 2).

193
194 *Latency to first bite*

195 We modeled latency to first biting behavior using a mixed effect Cox proportional hazards model.
196 Survival object was parameterized with respect to latency to first bite event and absolute biting (0/1,
197 where 0 represents no biting occurred during the trial) in response to the interaction of relatedness and
198 mass difference between tadpole dyads. Latency data was built by selecting the “first biter” within a
199 pair which involved subsetting the original data set. As a result of this, we used mass difference
200 between large and small tadpoles (instead of categorical size) to incorporate trials without aggressive
201 behavior. Mass difference was calculated as the difference between the large and small tadpoles within
202 pairs: this value was always positive since large tadpoles were always more massive. Using subsetted
203 data, each pair identity was independent, so only “Family” was used as a random variable.

204
205 *Game theory model*

206 We modeled pairwise interactions between tadpoles arbitrarily labelled as 1 and 2. We assumed that
207 only one tadpole per pair survives ('wins'), and that the probability of winning depends on each
208 individual's competitive strength. Competitive strength θ_i of tadpole i was calculated based on its **size**,

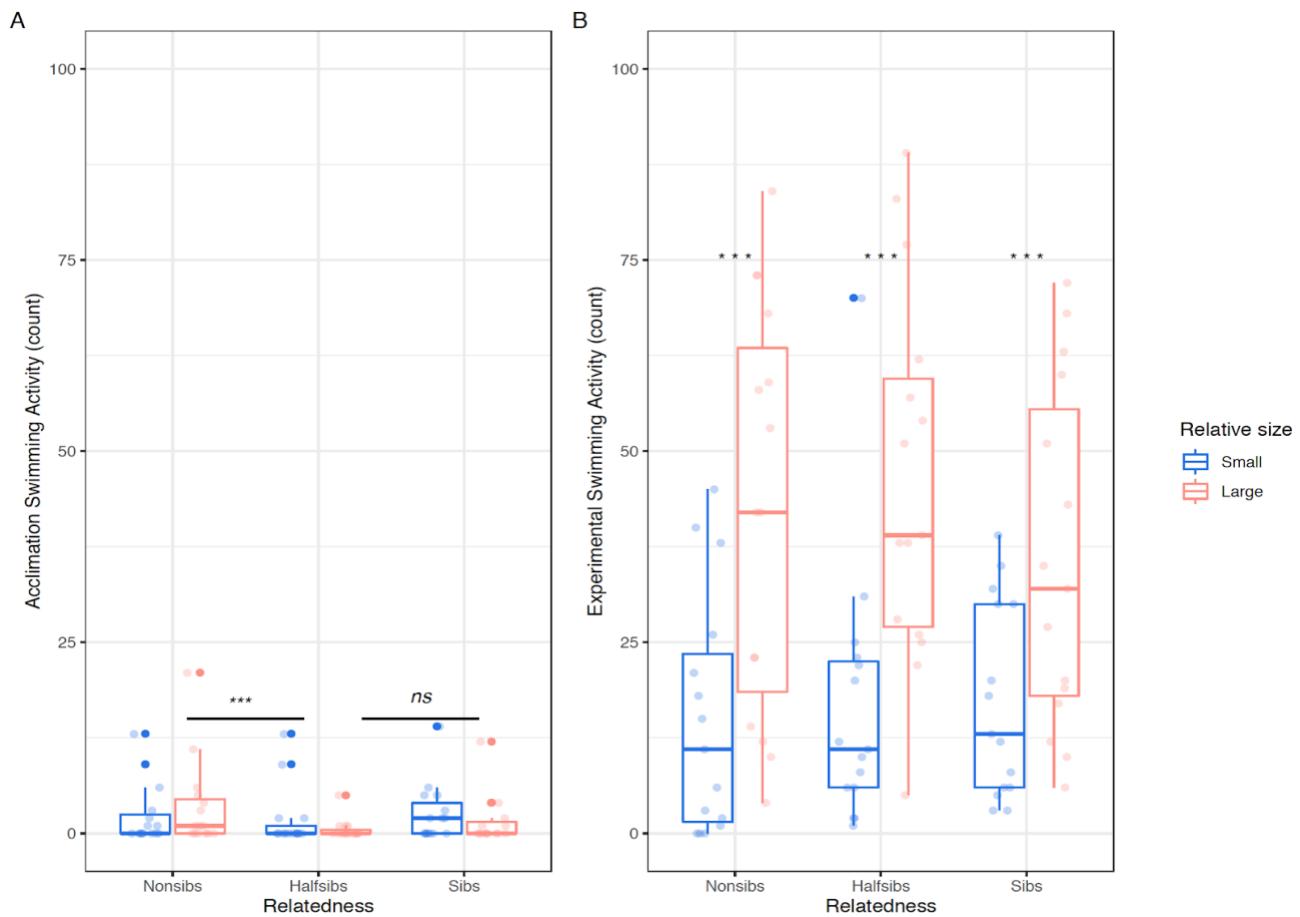
209 s_i and its **aggressiveness**, a_i as $\theta_i = s_i \cdot a_i$. This multiplicative formulation reflects the biological idea
210 that a given increment in aggressiveness should have a greater effect on a large than a small tadpole's
211 competitive strength. Individual 1's probability of winning is given by its **relative competitive**
212 **strength**, as $\omega_1 = \frac{\theta_1}{(\theta_1 + \theta_2)}$. The reproductive success ("**direct fitness**", v_i) of the winning tadpole was
213 modeled under three alternative assumptions: (1a) v_i is size-independent, as $v_i = 1 - a_i^2$; (1b) v_i is
214 proportional to size (for a given level of aggressiveness), as $v_i = s_i - a_i^2$; and (1c) v_i is size-
215 dependent due to aggressiveness being costlier for smaller tadpoles, as $v_i = 1 - \left(\frac{a_i}{s_i}\right)^2$ (see Fig 3 for
216 visualization). In all three formulations costs increased at an accelerating rate, such that low levels of
217 aggression had low costs whereas high levels of aggression could be extremely costly; this was done
218 to account for the increasing danger and energy expense associated with more violent behaviors.

219 Finally, the inclusive fitness of the surviving tadpole was calculated as $v_1 - r v_2$, where r is the
220 relatedness between the pair. This formulation reflects the idea that winning involves the killing of a
221 relative who would have had reproductive success v_2 had it survived. The inclusive fitness of the losing
222 tadpole is zero, because the losing tadpole neither reproduces nor affects the other tadpole's
223 reproduction. We calculated the expected (i.e., probability-weighted mean) inclusive fitness of tadpole
224 1 as $F_1 = \omega_1(v_1 - r v_2)$. For given values of s_1 , s_2 , and a_2 we numerically determined individual 1's
225 optimal aggression level as the value of a_1 that maximises its expected inclusive fitness. By computing
226 individual 1's 'best response' aggression level for any given a_2 which its opponent might exhibit, we
227 then identified pairwise optimal aggression levels that are best responses to each other.

228 **Results**

229 *Activity levels*

230 We observed tadpole activity during both post-acclimation and experimental phases. While tadpoles
231 were separated by an opaque barrier during the post-acclimation phase (but water still freely moved
232 throughout the arena) we found that half-siblings demonstrated significantly less swimming behavior
233 than non-siblings (negative binomial GLMM, CI: 0.06- 1.82, $z = -2.08$, $p = 0.037$) but not siblings
234 (negative binomial GLMM, CI: -0.15- 1.65, $z = 1.64$, $p = 0.102$). During the experiment, however,
235 we found that large tadpoles across all relatedness treatments were significantly more active than small
236 tadpoles (negative binomial GLMM, CI: 1.79 - 3.58, $z = 5.23$, $p < 0.001$; see Fig 1, Table 1).



237

238 *Figure 1. Tadpole activity levels before and during experimental trials. Panel (A) shows the post-*
239 *acclimation activity of tadpoles. We found no difference in swimming activity between large and small*
240 *tadpoles during this phase, but found that half-siblings were significantly less active during this period.*
241 *Panel (B) shows experimental activity throughout behavioral trials. Large tadpoles were significantly*

242 more active than small tadpoles during assays. $N_{Trial} = 15$ for each relatedness level. Large tadpoles
 243 are in pink and small tadpoles in blue. Boxplot medians are depicted by thicker lines, whiskers span
 244 $\pm 1.5 * \text{interquartile range}$.

A.

Post-acclimation Activity					Experimental Activity				
<i>Predictors</i>	<i>Estimate</i>	<i>CI</i>	<i>z</i>	<i>p</i>	<i>Predictors</i>	<i>Estimate</i>	<i>CI</i>	<i>z</i>	<i>p</i>
(Intercept)	0.99	0.30 – 1.67	2.82	0.005	(Intercept)	0.00	0.00 – 0.01	-29.09	<0.001
Halfsiblings	-0.94	-1.82 – -0.06	-2.08	0.037	Halfsiblings	1.08	0.76 – 1.53	0.43	0.665
Siblings	-0.19	-0.94 – 0.55	-0.50	0.616	Siblings	0.97	0.68 – 1.39	-0.16	0.874
Size (Large)	-0.07	-0.69 – 0.55	-0.22	0.824	Size (Large)	2.53	1.79 – 3.58	5.23	<0.001

Random Effects		Random Effects	
σ^2	1.71	σ^2	
τ_{00} Pair_ID	0.14	τ_{00} Pair_ID	8.43×10^{-9}
τ_{00} Family	1.82×10^{-8}	τ_{00} Family	5.76×10^{-3}

245

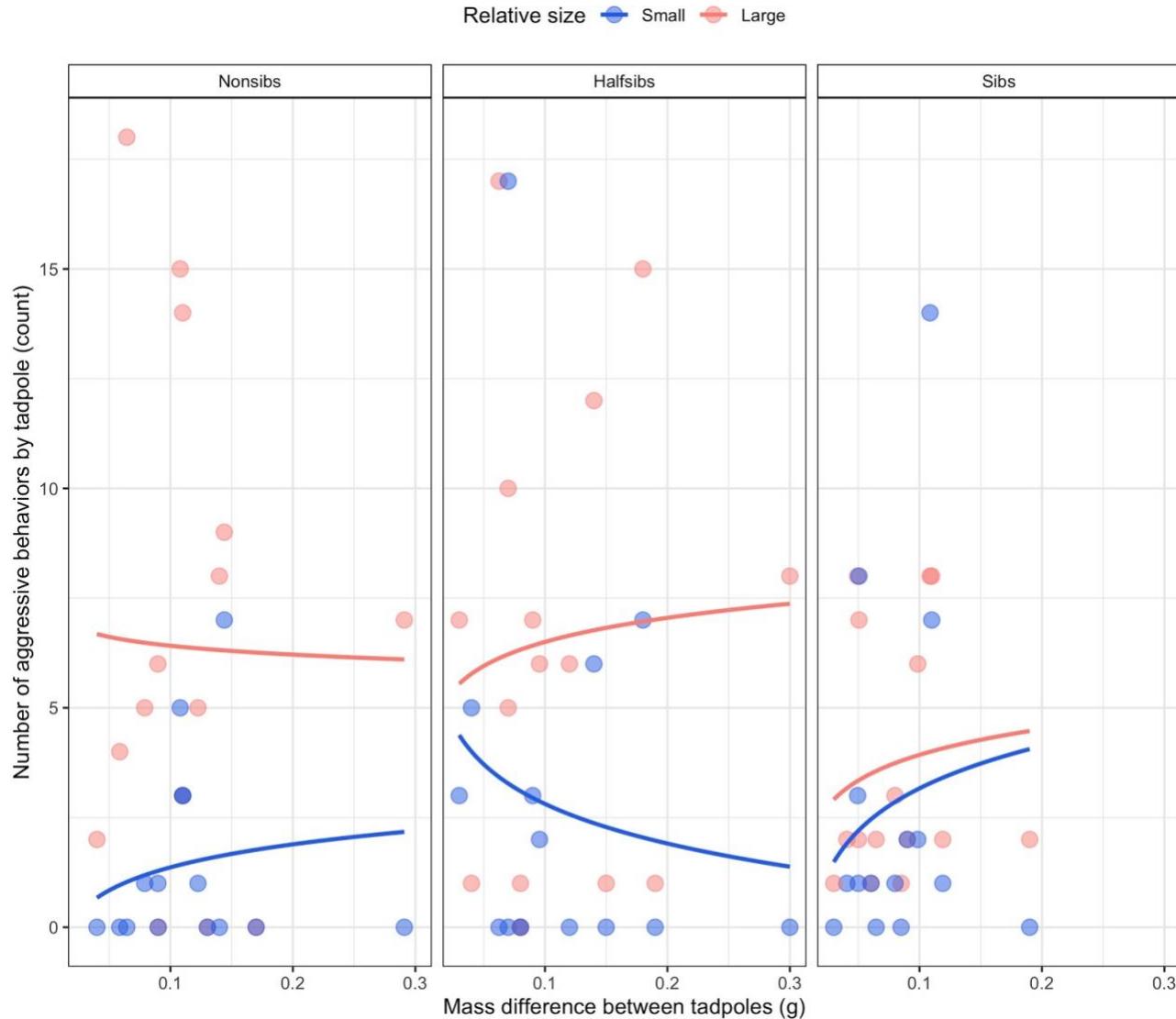
246 Table 1. Summary of negative binomial GLMM with linear parameterization of tadpole activity chosen
 247 from AIC model comparison. Where (A) half-siblings demonstrated significantly less activity
 248 compared to siblings and non-siblings while tadpoles were separated during post-acclimation, which
 249 changes during the experiment (B) where large tadpoles are overall more active. Models for (A) and
 250 (B) were predicted by additive effects of size and relatedness. Tadpole dyads (Pair_ID) and family
 251 were accounted for as random effects, CI represents 95% confidence interval. Differences in trial time
 252 during the experiment ($n = 3/45$) were accounted for by using duration as offset in the model.
 253 Experimental activity model was overdispersed and size was corrected for, hence missing residual
 254 variance (σ^2) in Panel B; τ_{00} represents random intercept variance.

255 When comparing models we found that random effects of pair ID had higher between subject variance
 256 ($\tau_{00} = 0.14$) than tadpole family ($\tau_{00} = 1.82 \times 10^{-8}$) during post-acclimation activity (Table 1, Panel A),
 257 indicating that when separated, there was less variation in behavior on a family level. Yet, while
 258 interacting during the experiment this difference disappears (Table 1, Panel B). In both cases,

259 between-subject variance is low, indicating that across families and pairs of tadpoles, activity levels
260 are similar.

261 *Overall aggression*

262 We found that large siblings were significantly less aggressive than the large non-sibling treatment,
263 exhibiting almost half the amount of aggressive behaviors as large non-siblings (Fig. 2, negative
264 binomial GLMM, $z = -2.07$, $p = 0.039$, Table 2). Half-siblings were not significantly different from
265 either treatment. Following our expectations of creating unique pair interactions, the random effect of
266 pair identity had a high between group variation ($\tau_{00\text{Pair_ID}} = 0.55$, Table 2), but families were
267 unexpectedly consistent ($\tau_{00\text{Family}} = 0.06$, Table 2).



268

269 *Figure 2. Differences in aggression across relatedness treatments with respect to weight difference*
270 *between dyads. Pink dots represent large tadpoles and blue dots represent small tadpoles (sizes*
271 *relative to dyads); $N_{Trial} = 15$ for each relatedness level. There was a higher level of aggression by*
272 *large tadpoles overall, but significantly less aggression by large siblings when compared to non-*
273 *siblings. Lines fit with GLM smoother ($y \sim \log(x)$) for relative sizes.*

274

Total aggression					
<i>Predictors</i>	<i>Estimate</i>	<i>CI</i>	<i>z</i>	<i>p</i>	
(Intercept)	-7.95	-8.75 – -7.16	-19.63	<0.001	
Halfsiblings	0.41	-0.62 – 1.44	0.77	0.440	
Siblings	0.58	-0.40 – 1.56	1.15	0.249	
Size (Large)	1.44	0.68 – 2.20	3.71	<0.001	
Halfsiblings: Size (Large)	-0.44	-1.47 – 0.58	-0.84	0.399	
Siblings: Size (Large)	-1.02	-1.99 – -0.05	-2.07	0.039	
Random Effects					
σ^2	0.58				
τ_{00} Pair_ID	0.55				
τ_{00} Family	0.06				

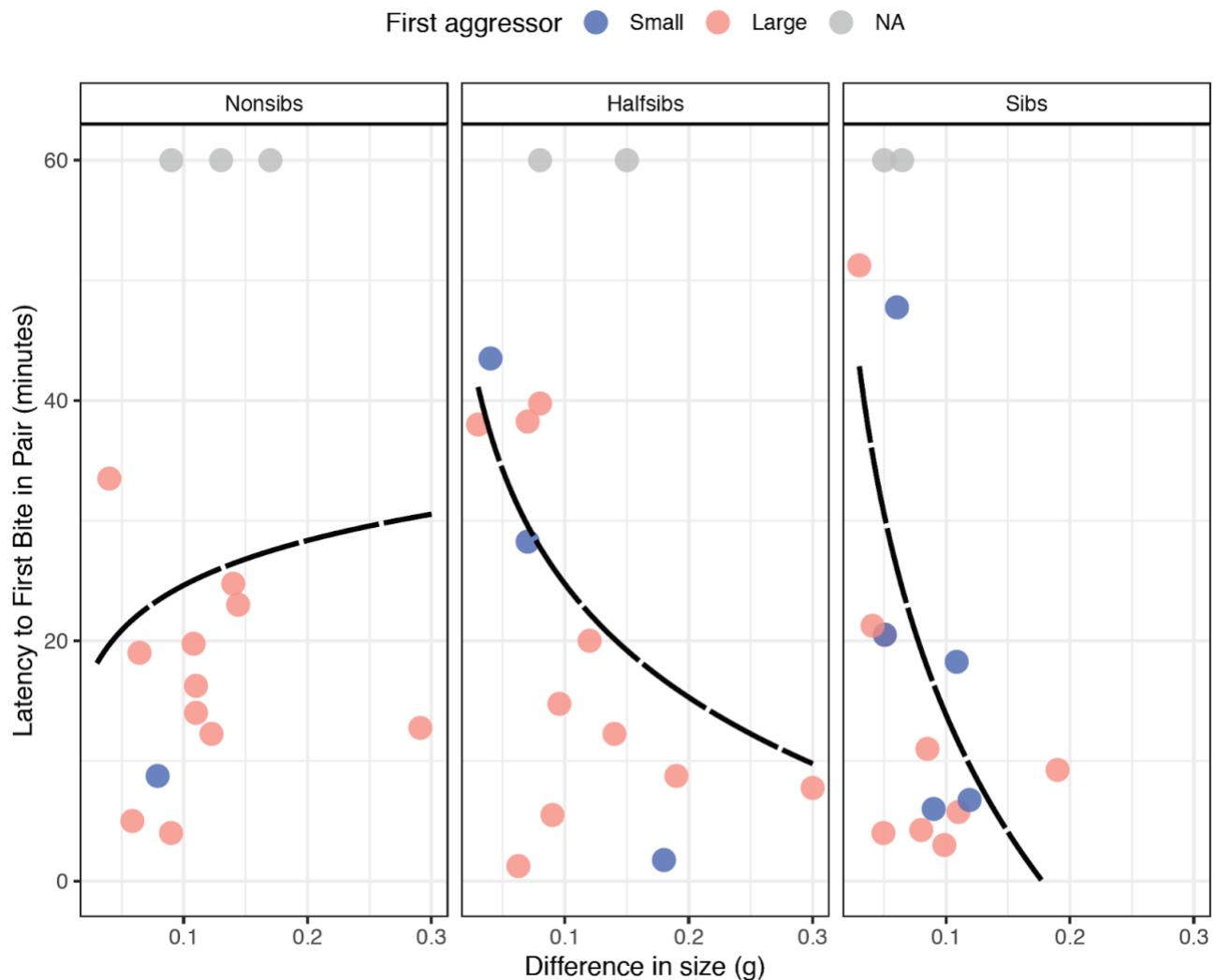
275

276 *Table 2. Summary of negative binomial GLMM with linear parameterization of tadpole aggression.*
277 *Where total aggression (total count of biting and chasing) was predicted by the interaction between*
278 *size (two level categorical variable) and relatedness. Tadpole dyads (Pair_ID) and family were*
279 *accounted for as random effects, CI represents 95% confidence interval. Differences in trial time*
280 *during the experiment (n = 3/45) were accounted for by using duration as offset in the model. σ^2*
281 *represents residual variance and τ_{00} represents random intercept variance.*

282 *Latency to first bite*

283 We observed that initial aggression between tadpoles can change based on physical and genetic
284 attributes. We used biting behavior as a measurement of first aggression because it consistently
285 represented the first aggressive contact in tadpole dyads. Based on a mixed effect Cox proportional
286 hazards model, we assessed the risk of first attack when considering relatedness and size difference
287 between pairs. We found a significant interaction between relatedness and size, where a higher degree
288 of relatedness led to a shorter latency to aggression when the size difference within dyads was larger.

289 In other words, siblings demonstrated more immediate aggressive behavior towards each other when
290 their size differences were greater (Cox mixed effects, $z = 2.209, p = 0.022$, see Table 3). For example,
291 at a large mass difference (> 0.15 g between tadpoles) siblings were more than 40 percent more likely
292 to bite than non-siblings within the first five minutes of a trial. Interestingly, non-siblings demonstrated
293 a seemingly inverted behavioral trend, where dyads with large size differences had delayed aggressive
294 behaviors. Half-siblings did not behave significantly differently from either treatment. In trials where
295 biting was exhibited, large tadpoles were most often the first aggressor ($n = 8/13$ for siblings; $n = 10/13$
296 for half siblings; $n = 11/12$ for non siblings).



297

298 *Figure 3. Latency to first bite between tadpole dyads. Points are colored by relative tadpole size within*
299 *dyads. Lines are fit with a GLM smoother with a $y \sim \log(x)$ formula. There is an inversion in behavior*
300 *as size difference increases, where sibling pairs with large size differences attacked significantly faster*
301 *than non-siblings. Dyads where there were no aggressive behaviors were accounted for by assigning*
302 *them the maximum time limit (60 minutes). $N_{Trial} = 15$ for each relatedness level.*

303

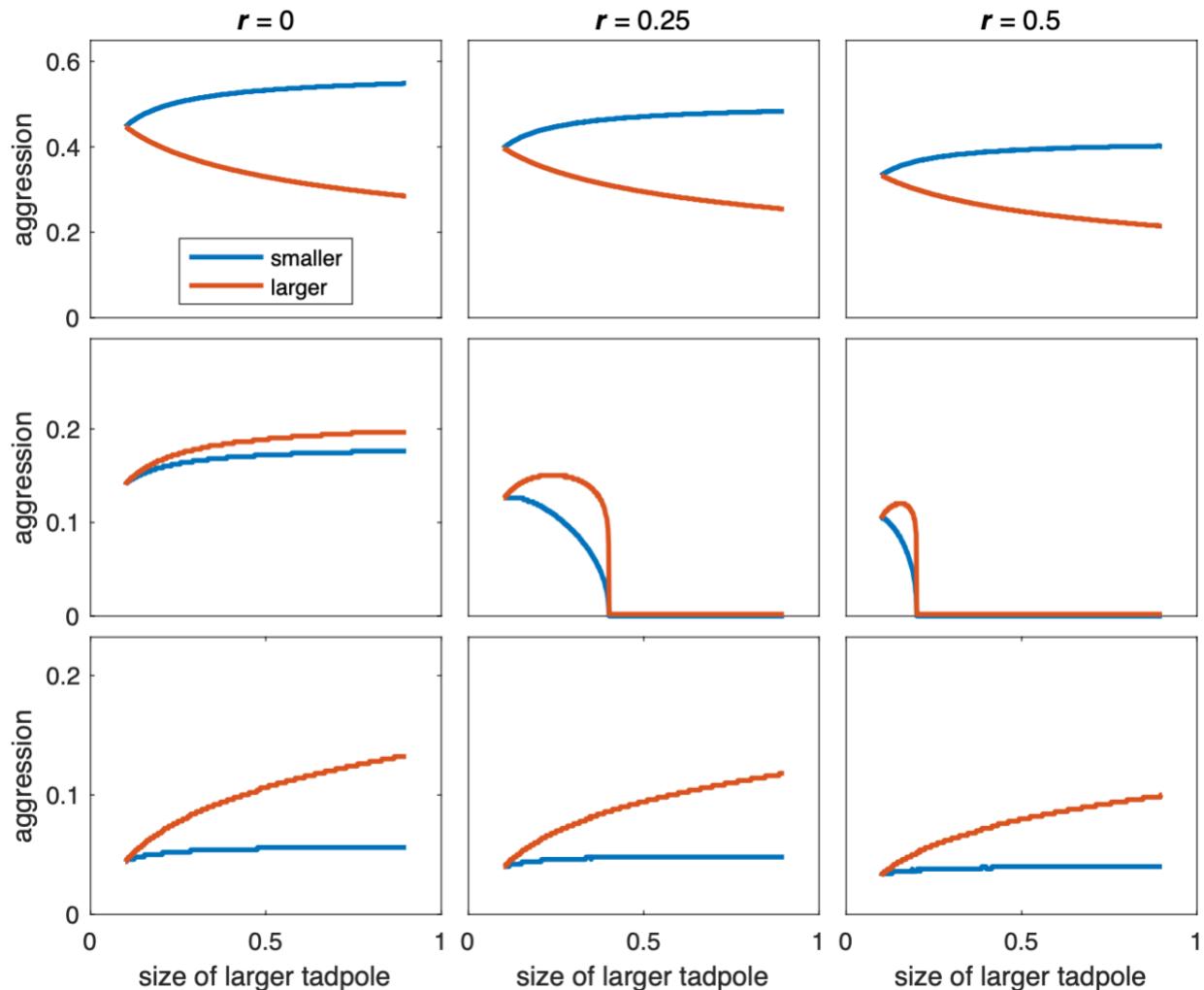
Latency to first bite					
<i>Predictors</i>	<i>Estimate</i>	<i>CI</i>	<i>z</i>	<i>p</i>	
Halfsiblings	-1.27	-2.83 – 0.30	-1.59	0.113	
Siblings	-1.44	-3.12 – 0.24	-1.68	0.093	
Mass_Diff	0.89	-8.65 – 10.44	0.18	0.854	
Halfsiblings: Mass_Diff	9.62	-2.14 – 21.38	1.60	0.109	
304 Siblings: Mass_Diff	16.32	1.80 – 30.84	2.20	0.028	

305 *Table 3. Mixed effects Cox proportional hazards model. Time to first aggressive behavior was*
306 *predicted by the interaction of the mass difference between tadpoles and their relatedness; family is*
307 *taken into account as a random effect. There is a significant interaction between relatedness and size,*
308 *where more similarly sized siblings have a shorter latency to aggression than non-siblings. Mass_Diff*
309 *is the difference in weight between large and small tadpoles.*

310 *Game theory model*

311 Based on our three formulations (1a-c) we varied the impact of size to model aggression levels of
312 tadpoles with different degrees of relatedness. The version where aggression was both size-dependent
313 and costlier for the smaller tadpoles (Fig 4, third row) appeared most consistent with our empirical
314 data (Fig 2), in that larger tadpoles were consistently predicted to be more aggressive than their smaller
315 counterparts, and overall aggression decreased with relatedness.

316



317

318 *Figure 4. Optimal aggressiveness of dyads of tadpoles as a function of size difference for three*
319 *different levels of relatedness (represented in panel columns) and three sets of assumptions*
320 *(represented in panel rows). First row: direct fitness was assumed to be size-independent. Second*
321 *row: direct fitness was assumed to be proportional to size. Third row: aggressiveness was assumed*
322 *to be costlier for smaller tadpoles. The smaller tadpole's size was held fixed at $s_i = 0.1$; the larger*
323 *tadpole's size is shown on the x-axis.*

324

Discussion

326 Larval cannibalism or, more broadly, juvenile aggression has most often been studied in the context
327 of resource competition (Baras et al., 2000; Tershy et al., 2000), as juveniles do not usually defend
328 territories (Stamps and Krishnan, 1994), nor compete for sexual partners. Here, we observed
329 aggressive behaviors between *D. tinctorius* tadpoles in resource-abundant, low-density conditions. We
330 found that aggression is common (also see Fischer et al., 2020; Rojas, 2014), and depends on the
331 interaction between relative size and relatedness between tadpoles. Our results show that, together,
332 relatedness and physical attributes shape overall aggression, latency to aggression, and even activity
333 levels between dyads.

334

335 *Cannibalism and the environment*

336 Aggressive attacks between pairs were recorded across all relatedness treatments and sizes. Although
337 less common, small tadpoles were sometimes quicker to exhibit aggressive behaviors than their larger
338 counterparts (Fig 3) and, in some instances, were even more aggressive than large tadpoles (this was
339 observed only in sibling and half-sibling treatments, see Supplementary Fig 1). These intense
340 aggressive responses were elicited under vastly different conditions from which cannibalism is usually
341 reported, such as in response to starvation (spiders: Mayntz and Toft, 2006; cephalopods: Ibáñez and
342 Keyl, 2010; earwigs: Dobler and Kölliker, 2011), pathogens (caterpillars: Wang and Daane, 2014;
343 salamanders: Pfennig et al., 1991), high population densities (crabs: Moksnes, 2004), and in the
344 context of sexual selection (chimpanzees: Takahata, 1985; seals: Bishop et al., 2016). These
345 associations suggest that cannibalism is triggered by immediate stress and competition. However, a
346 handful of studies have been invoked in decoupling stress from aggression and cannibalism (poison
347 frogs: Dugas et al., 2016b; egrets: Mock et al., 1987, vultures: Margalida et al., 2004, snails: Baur,

348 1987), contributing to the mounting evidence that across diverse taxa, immediate physical and
349 environmental stress are not the only triggers for cannibalistic behavior (Fox, 1975).

350
351 We report here that aggression in *D. tinctorius* is not primarily driven by immediate nutritional need.
352 This raises the question of whether there is a long-term fitness advantage to a cannibal that pays off
353 later, during the reproductive stages of the animal. For example, attackers may accrue fitness
354 advantages by having a shorter latency to metamorphosis, larger size as an adult, and higher fecundity
355 as an outcome of their behavior during the larval stage (Crump, 1990; Polis, 1981).

356

357 *Interactive predictors of cannibalism*

358 Aggression between tadpoles was predicted by the interaction between relative size and relatedness
359 within dyads. We found that large non-siblings were the most aggressive, expressing almost twice the
360 amount of aggressive behaviors than large siblings (Fig. 2). When able to physically interact in the
361 arena, the behavior of half-siblings did not differ significantly from the two other relatedness
362 treatments, which demonstrates that even if there does appear to be some kin discrimination in *D.*
363 *tinctorius*, it may not function on as fine of a scale as for other species (e.g., Pfennig et al., 1994).

364
365 With respect to the interaction with size, Rojas (2014) had already established that cannibalism
366 between *D. tinctorius* tadpoles occurs faster with increasingly size-mismatched pairs. In fact, across
367 the animal kingdom the aggressor in a pair/group is most often the larger individual (Ibáñez and Keyl,
368 2010; Mayntz and Toft, 2006; Mock et al., 1987). However, our findings highlight the fact that in this
369 system aggression is not solely mediated by size differences. This suggests that there may be many
370 other cases of context-specific discrimination across taxa that have been overlooked by not considering
371 the interaction between relatedness and physical attributes (such as illness, injury, or phenotype). The

372 context-specific kin discrimination observed in this study could have potentially evolved because the
373 benefit to cannibals only outweighs the cost of harming kin in particular circumstances.

374
375 In the salamander system, the cost of consuming kin is high (i.e. disease rates, Pfennig, 1999; Pfennig
376 et al., 1991), thus the ability to discriminate against them is valuable; even first cousins have been
377 shown to be attacked less than nonrelatives (Pfennig et al., 1994). Costs may be lower for *D. tinctorius*
378 tadpoles if they do not face similar consequences of cannibalism such as acquiring pathogens
379 (although the effects of pathogens on cannibalism in this species remain unexplored). Costs may also
380 be decreased by the impact of parental care, whose protective effects have been shown in other poison
381 frog species (Schulte et al., 2011; but see Rojas, 2014, 2015) for seemingly counterintuitive deposition
382 choices in *D. tinctorius*). Although discrimination is less precise than in salamanders (Pfennig et al.,
383 1994), our results support the presence of kin discrimination in *D. tinctorius*, and warrant further
384 investigation into the possible proximate mechanisms regulating kin recognition in this species.

385

386 *Latency to aggressive behavior*

387 Latency to attack changed unexpectedly as a function of both size difference and relatedness between
388 tadpoles. When pairs were more similarly sized, non-siblings attacked faster; in contrast, when
389 mismatched in size, non-siblings delayed aggression (Fig. 3). This trend was inverted for siblings,
390 which were tolerant of a similarly-sized counterpart, but were quickly aggressive in size-mismatched
391 pairings. In other words, although large siblings were less aggressive overall, they had a shorter latency
392 to aggression in dyads with large size differences. We speculate that fast ‘attacking’ may serve
393 different functions in different contexts. For example, when performed between size-mismatched
394 siblings, it may serve not to initiate an escalated fight but to ascertain by taste the first impression of
395 relatedness.

396
397 When considering latency to aggression, it may be important to take into account at which point
398 tadpoles processed that they were not alone in the arena. The earliest occasion where this could have
399 occurred is during the acclimation phase, during which tadpoles were separated by an opaque barrier
400 which allowed water to pass through the entire testing arena (for an hour during acclimation, and
401 actively recorded for 10 minutes before the experiment). It is probable that chemical cues (if any) and
402 vibrations from tadpoles moving in the water circulated throughout the entire arena during this time.
403 Unexpectedly, half siblings were significantly less active than non-siblings and did not differ in
404 activity from siblings while dyads were separated (Fig 1, Panel A); relatedness-level differences in
405 activity disappeared once pairs could physically interact (Fig 1, Panel B). It should be noted that post-
406 acclimation behaviour was observed for only 10 minutes, and that tadpoles could have behaved
407 differently during the unobserved acclimation period (60 minutes). Nevertheless, higher activity levels
408 by non-siblings could indicate the presence of some kind of chemical cue whose meaning then shifted
409 with the presence of visual contact.

410
411 While we are unsure of the mechanisms *D. tinctorius* may be using to recognize each other, we believe
412 that tadpoles could potentially be using both olfactory and taste cues to discriminate kin, as shown in
413 salamanders (Pfennig et al., 1994) and *Xenopus* sp. (Dulcis et al., 2017). Fischer et al., (2020) recently
414 described the neural basis of conspecific aggression in *D. tinctorius* and found differences in brain
415 region activity based on the “winning” or “losing” status of paired tadpoles after fights; their findings
416 lay the groundwork for understanding the proximate mechanisms of aggression and provide the
417 backdrop to understanding its triggers in *D. tinctorius* tadpoles. Following the establishment of kin
418 discrimination in the species (this study), experiments investigating the possible mechanisms
419 underlying recognition are warranted.

420

421 *Game theory model*

422 As intuitively expected, our model robustly predicts decreasing aggression levels with increasing
423 relatedness (Figure 4). Predictions about size-dependent behavior, however, turn out to be sensitive to
424 details. When aggression is assumed to be costlier for small tadpoles (bottom row of panels in Fig. 4),
425 larger tadpoles are always more aggressive, and are increasingly so towards larger size differences and
426 more distant relatedness. This formulation (1c) results in a pattern that is the most consistent with
427 experimental results (Fig 4). The model also reveals interesting theoretical possibilities which can be
428 rejected for *D. tinctorius*. For example, if (adult) reproductive success and aggressiveness costs were
429 independent of tadpole size, smaller tadpoles should compensate for their size disadvantage by being
430 more aggressive (top row of panels in Fig. 4). Moreover, if tadpole size strongly predicted adult
431 reproductive success, then above a certain size difference the smaller tadpole should let its larger
432 relative win without fighting (middle row of panels in Fig. 4).

433

434

Conclusions

435 In this study, we attempted to better understand the roles of physical attributes and relatedness in
436 predicting aggressive behavior under stable conditions. We found that large siblings were significantly
437 less aggressive than large non-siblings towards their smaller counterpart, demonstrating evidence for
438 kin discrimination in *D. tinctorius*. These findings are complicated by latency to aggression, which
439 showed different trends based on dyad relatedness, although the evolutionary explanation for this
440 pattern remains unclear. We contribute to the growing body of literature demonstrating aggressive
441 behavior (that may escalate to cannibalism) is independent of environmental stress, and conclude that
442 (1) size and relatedness interact in predicting aggressive behaviors, and (2) the ability to discriminate
443 kin does not guarantee kin bias. These findings set the stage for studies to consider cannibalistic

444 behavior in more complex ways, and to better understand the value and purpose of kin discrimination
445 in cannibals.

446
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453
454 **Statement of Authorship**

455 BR conceived the study, with input from CF and JV in the design of the assays; CF performed the
456 study; LF developed the mathematical model; CF analyzed the data; and CF wrote the first draft with
457 input from BR. All authors critically commented on previous drafts and approved the final version of
458 the manuscript.

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