

1 **From Habitat Use to Social Behavior: Natural History of a**
2 **Voiceless Poison Frog, *Dendrobates tinctorius***

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15

16 Abstract

17

18 Descriptive studies of natural history have always been a source of knowledge on which experimental
19 work and scientific progress rely. Poison frogs are a well-studied group of small Neotropical frogs with
20 diverse parental behaviors, distinct calls, and bright colors that warn predators about their toxicity; and a
21 showcase of advances in fundamental biology through natural history observations. The dyeing poison
22 frog, *Dendrobates tinctorius*, is emblematic of the Guianas region, widespread in the pet-trade, and
23 increasingly popular in research. This species shows several unusual behaviors, such as the lack of
24 advertisement calls and the aggregation around tree-fall gaps, which remain poorly described and
25 understood. Here, we summarize our observations from a natural population of *D. tinctorius* in French
26 Guiana collected over various field trips between 2009 and 2017; our aim is to provide groundwork for
27 future fundamental and applied research spanning parental care, animal dispersal, disease spread, habitat
28 use in relation to color patterns, and intra specific communication, to name a few. We report sex
29 differences in habitat use and the striking invasion of tree-fall gaps; describe their courtship and
30 aggressive behaviors; document egg development and tadpole transport; and discuss how the knowledge
31 generated by this study could set the grounds for further research on the behavior, ecology, and
32 conservation of this species.

33

34

35 Introduction

36

37 Natural history has been long acknowledged as the foundation of new hypotheses in behavioral and
38 evolutionary ecology (Endler 2015). Thus, scientific progress relies greatly on knowing what different
39 organisms are, where they live, what they feed on, how they respond to different stimuli, and what kind of
40 other peculiar behaviors they exhibit (Tewksbury et al. 2014). This, more often than not, is achieved
41 through field observations.

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42 Neotropical poison frogs (Dendrobatidae) and their close relatives are a showcase example of how
43 detailed knowledge of natural history can lead to groundbreaking hypothesis-driven studies (e.g. Santos et
44 al. 2003; Brown et al. 2010; Amézquita et al. 2011; Pašukonis et al. 2014; Tarvin et al. 2017). Exhaustive
45 field observations have revealed the diversity of poison frog parental care behavior (e.g. Crump 1972;
46 Silverstone 1973; Donnelly 1989; Summers 1989; Caldwell 1997), warning coloration (e.g. Silverstone
47 1975; Myers and Daly 1983), and skin alkaloids (e.g. Myers and Daly 1976, 1980; Brodie and
48 Tumbarello 1978; Myers et al. 1978; Summers 1989), aspects that have become a trademark in the group
49 both for research and for the pet-trade. However, there is still a surprising lack of information on the
50 natural history of some species that have become increasingly well studied otherwise, such as the dyeing
51 poison frog, *Dendrobates tinctorius*.

52 Although bred in captivity by hobbyists for decades (Schmidt and Henkel 1995; Lötters et al. 2007), and
53 despite its growing status as a model species for studies on the evolution and function of coloration (e.g.,
54 Wollenberg et al. 2008; Noonan and Comeault 2009; Rojas et al. 2014a, 2014b; Barnett et al. 2018), there
55 are only a handful of studies on *D. tinctorius* in its natural environment; most of these have been carried
56 out and published only after 2010 (Born et al. 2010; Courtois et al. 2012; Rojas and Endler 2013; Rojas
57 2014, 2015; Rojas et al. 2014a). Four other studies in the wild have attempted to understand evolutionary
58 aspects of their variable coloration, using clay or wax models instead of the actual frogs (Noonan and
59 Comeault 2009; Comeault and Noonan 2011; Rojas et al. 2014b; Barnett et al. 2018).

60 Many poison frog field studies over the last five decades have relied on prominent male calls either
61 directly, by studying aspects related to vocal behavior (e.g., Fandiño et al. 1997; Lötters et al. 2003;
62 Forsman and Hagman 2006; Erdtmann and Amézquita 2009; Vargas-Salinas and Amézquita 2013), or
63 indirectly, by using the calls to locate territorial males in the field (e.g., Bee 2003; Hödl et al. 2004;
64 Amézquita et al. 2005; Rojas et al. 2006). Meanwhile, *D. tinctorius* remained almost unstudied, at least in
65 part, due to their lack of a regular calling behavior. Therefore, much of the behavioral and evolutionary
66 ecology of dyeing poison frogs remains unknown.

67 As stated by the IUCN Red List for Threatened Species, *D. tinctorius* is in the category ‘Least Concern’
68 (Gaucher and MacCulloch 2010). According to this report, its major threat is illegal trading, as it is for
69 various other dendrobatid species (Gorzula 1996; Nijman and Shepherd 2010; Brown et al. 2011;
70 Hoogmoed et al. 2012). However, a recent study provided evidence that, despite having seemingly large
71 and stable populations throughout its range, *D. tinctorius* is not safe from the chytrid fungus (*Bd*)
72 infection (which, incidentally, was discovered in a captive individual of *D. tinctorius*; Longcore et al.
73 1999) in its natural habitat (Courtois et al. 2012). Moreover, a recent study by Courtois et al. (2015)
74 raised even greater concern as, of all the species tested for *Bd* in French Guiana, the highest prevalence
75 was found in dendrobatid frogs, including *D. tinctorius*.

76 Alarming declines make it even more urgent to study the natural history of amphibian species and
77 communities, especially of ‘sentinel’ species such as *D. tinctorius* (Courtois et al. 2015), whose declines
78 provide anticipated warning of risks to human or ecosystem health (Beeby 2001). Only by understanding
79 organisms in their own habitat can we produce sensible and timely conservation policies, and sustainable
80 management (Tewksbury et al. 2014). In the particular case of *D. tinctorius*, knowing their habitat use,
81 breeding biology, social behavior, and movement ecology could be of utmost importance for modeling
82 disease spread and the impacts of deforestation, among other current environmental threats. Here, we
83 document the habitat use, and the reproductive, social, and vocal behaviors of *D. tinctorius* in the wild;
84 and provide information about various other aspects of its natural history that will be a valuable
85 groundwork for future fundamental and applied research in behavior, ecology, evolution, and
86 conservation.

87 88 **Materials & Methods**

89 90 **Study species**

91 *D. tinctorius* is a diurnal, relatively large (Snout-Vent Length 37—53mm at the study site; Rojas and
92 Endler 2013) poison frog of the Neotropical family Dendrobatidae (more specifically of the *tinctorius*
93 group; Grant et al. 2006), which occurs around canopy gaps in primary forests in the Eastern Guiana

94 Shield, at elevations between 0 and 600 m (Noonan and Gaucher 2006; Wollenberg et al. 2006). It has
95 skin alkaloids (Daly et al. 1987), and is characterized by a great color pattern variation both within (Fig.
96 1a; Rojas and Endler 2013) and among populations (Fig. 1b; Noonan and Gaucher 2006; Wollenberg et
97 al. 2008).

98 In our study area, color patterns can be used reliably for individual identification (Born et al. 2010;
99 Courtois et al. 2012; Rojas and Endler 2013; Fig. 1a), and sex can be determined by the size of males' toe
100 discs, which are wider than females' in relation to their body size (Rojas and Endler 2013). In contrast to
101 most frogs (including closely related poison frogs), male *D. tinctorius* do not produce advertisement calls,
102 and when they do vocalize, they do it very softly (Lescure and Marty 2000). Newly hatched tadpoles are
103 carried by males to pools formed in tree-holes or palm bracts at variable heights (Fig.2; Supplementary
104 video 1; Rojas 2014, 2015), where they remain unattended until metamorphosis, which occurs after
105 approximately two months (BR, pers. obs. in the field). As in some other species of *Dendrobates*
106 (Caldwell and De Araujo 1998, Gray et al. 2009, Summers 1990, Summers and McKeon 2004), larvae
107 feed on detritus and on larvae of insects and frogs (BR, pers. obs.), including conspecifics (Rojas 2014,
108 2015; Supplementary movie 2). In captivity, individuals reach maturity after approximately 18 months
109 (Lötters et al. 2007), but their age at sexual maturity in the field is unknown to date.

110

111 **Reproductive and social behavior**

112 BR did systematic data collection during three field seasons between 9 January and 20 February 2009, 17
113 January and 19 March 2010, and 17 January and June 6 2011, at Camp Pararé, Nouragues Ecological
114 Research Station, French Guiana (4°02'N, 52°41'W), in primary lowland terra-firme forest, where *D.*
115 *tinctorius* is one of the most common leaf-litter frogs (Courtois, et al. 2013). In addition, AP made
116 opportunistic observations on social and reproductive behavior at the same study site between January
117 and March 2016 and 2017. The study periods correspond to the early rainy season and high reproductive
118 activity of *D. tinctorius* in the study area.

119 During each study period between 2009 and 2011, BR surveyed a 1.5 km transect on a near-daily basis,
120 between 8:00 and 17:30. Each frog found was captured, when possible, and photographed for future
121 individual identification on the basis of its color patterns. When two individuals seemed to be interacting,
122 they were followed for as long as it was necessary to determine the nature of the interaction (i.e. courtship
123 or agonistic encounter). Two individuals were considered to be in courtship when they were less than 1
124 m apart (as in Pröhl 2002) and one was clearly following and touching the other (pers. obs.) for at least 15
125 min. A 15 min waiting time was chosen on the basis of previous studies of mate choice and assortative
126 mating in captive dendrobatids (Maan and Cummings 2008, 2009). When possible, we followed pairs in
127 courtship until they were no longer visible or until oviposition occurred, which proved difficult most of
128 the time due to lack of accessibility and poor visibility under forest structures. Agonistic encounters were
129 more difficult to follow because of their usually short duration and the high movement speed of the frogs,
130 but we observed them for as long as both individuals were visible. Fragments of the two types of
131 interactions were filmed for documentation purposes. Observations were done at irregular time intervals
132 during the day.

133 Males carrying tadpoles were found during daily surveys along a 1.5 km transect. BR recorded the
134 number of tadpoles on the back of each tadpole-carrying male and captured it when possible. Upon
135 capture, each male was photographed (with the tadpole(s) still attached) against graph paper. Later these
136 photos were used to measure the size of both the frog and the tadpoles with the software ImageJ
137 (Schneider et al. 2012). Tadpole size was measured dorsally, from the tip of the snout to the base of the
138 tail. The sizes of tadpoles carried by one male at the same time were compared (always the small vs. the
139 large of each pair) with a paired *t*-test.

140

141 **Vocal behavior**

142 *Dendrobates tinctorius* vocalizes rarely and at very low intensities, making it difficult to obtain audio
143 recordings. We were able to obtain a high-quality audio recording of one male. In addition, to measure
144 the acoustic properties of the call, we extracted lower quality audio from video recordings of social

145 interactions. In total, we obtained sufficient quality recordings of eight calls produced by three males
146 (four, three, and one call per individual). We manually measured the duration, pulse rate and dominant
147 frequency of each call using Praat (v. 5.3.85; Boersma and Weenink 2014) acoustic analysis software.
148 We averaged the measures between calls within each male and then between the three males. We used
149 one call of the highest quality to visually illustrate call structure.

150

151 **Treefall-gap invasion**

152 In a previous study, Born et al. (2009) reported frequent sightings of adult *D. tinctorius* in recently
153 formed tree-fall gaps. BR witnessed the formation of nine tree-fall gaps over the study periods (one in
154 2009, eight in 2011); these were discovered rapidly because they occurred in the 1.5 km transect surveyed
155 daily. BR inspected each gap within the first 24 hours of its formation and caught as many frogs as
156 possible, moving fallen branches until no frogs were seen (after 2-3 hours). During the next two
157 consecutive days BR carefully looked for new frogs over a similar period of time (2-3 h); most of the
158 individuals seen then had been found on the first day. When frogs were seen but not caught, BR
159 photographed them from a distance to record their color pattern for further identification upon capture.
160 The presence of individuals in these tree-fall gaps continued to be recorded during daily surveys for up to
161 two months after their occurrence.

162

163 **Habitat use**

164 During the field season of 2010, BR captured 109 frogs (55 females and 54 males), each of which was
165 assigned to one of two microhabitats according to where they were first seen: leaf litter (when frogs were
166 on a relatively open patch of leaf litter without any obvious structure in a 1 m radius), or associated to the
167 following structures: fallen logs (when frogs were visibly exposed on top of the log), fallen branches
168 (when individuals were in fallen tree crowns) and tree/palm roots (when the frogs were within the
169 exposed roots or next to them, or inside hollow trunks). Frogs were only included in the analyses once
170 (recaptures of the same individual were excluded in order to avoid pseudoreplication, and only the site at

171 first sighting was taken into account). We tested for differences between the sexes in the microhabitat
172 where they were found (open vs. associated with the aforementioned structures) using a Generalized
173 Linear Model with binomial distribution. All statistical analyses were done with the software R v. 3.3.3
174 (RCoreTeam 2014) using the RStudio interface (RStudio Team 2015).

175

176 **Ethics statement**

177 Our research was approved and authorized by the scientific committee of the Nouragues Ecological
178 Research Station. We strictly adhered to the current French and European Union law, and followed the
179 Association for the Study of Animal Behaviour's (ASAB) Guidelines for the use of live animals in
180 teaching and research.

181

182 **Results**

183

184 During three field seasons between 2009 and 2011, we identified 629 individuals unequivocally, 597 of
185 which were captured. We photographed the remaining 32 frogs from a distance that allowed the record of
186 their unique color patterns and, thus, their individual identification. There was no statistically significant
187 difference between the number of females (N=276) and the number of males (n=321) found, although
188 there was a non-significant trend towards a larger number of males ($\chi^2 = 3.392$, $df = 1$, $P = 0.066$).

189

190 **Habitat use**

191 We found clear differences between the sexes in terms of the microhabitat where they were found.
192 Females were predominantly found in open areas of leaf litter (60% of females vs. 31.5% of males),
193 whereas males were mostly found associated to structures (68,5% of males vs. 40% of females; estimate \pm
194 SE = 1.183 ± 0.402 , $Z = 2.943$, $P < 0.001$, $n=109$; Fig. 3), specially fallen logs and branches (31.5% and
195 22.2%, respectively).

196

197 **Invasion of treefall gaps**

198 A total of 140 individuals (65 females and 75 males) arrived in the nine fresh gaps studied, mostly on the
199 same day of their occurrence or within the first three days. Males were as likely as females to arrive
200 within this timespan (males: mean = $1.24 \pm$ (SE) 0.08 d; females: mean = $1.08 \pm$ (SE) 0.06 d; $\chi^2 = 0.714$ df
201 = 1, $P = 0.398$; Fig. 4A). In the long-term (i.e., up to 60 days after the occurrence of the treefall),
202 however, more males than females were found in treefall gaps ($\chi^2 = 11.137$, df = 1, $P = 0.001$; Fig. 4B).

203 204 **Vocal behavior**

205 *Dendrobates tinctorius* produces a call that can be described as a very low intensity ‘buzz’
206 (Supplementary sound file). The call is audible to humans only from within a few meters; at times males
207 inflate the vocal sac without anything audible to us from a distance of up to 1 m. Males call rarely and
208 only when in courtship or during agonistic interactions with other males. We never observed a male
209 calling alone. We were able to record and measure calls from two males in courtship and one in an
210 agonistic interaction. Calls produced in courtship and agonistic contexts sounded similar to us and had
211 similar acoustic parameters, although more recordings would be needed for a detailed comparison. All
212 measured calls shared the same general structure: a short broadband burst of pulses produced at a high
213 rate (Fig. 5B, C). The measured call duration was 0.55–0.98 s (mean = 0.76 s), the within-call pulse rate
214 was 143–175 Hz (mean = 160 Hz), and the dominant frequency band centered around 2700–3270 Hz
215 (mean = 3109 Hz).

216 217 **Courtship and egg laying**

218 We found 47 pairs engaged in courtship (10 in 2009, 14 in 2010 and 23 in 2011), involving 40 males and
219 39 females. Courtship was observed throughout the day and lasted several hours. In one case a courting
220 pair was followed for nearly 7 hours before oviposition took place. Courtship consists of several bouts of
221 moving together and stationary tactile interactions (Fig. 5A, Supplementary video 3) that are interrupted,
222 for example, when one of the individuals starts to feed. In general, each bout is initiated by tactile
223 interactions in which the female repeatedly places one of her forelimbs on the male’s limbs, back or head.

224 The male then usually faces her before moving away, followed by the female, in search of an egg-laying
225 site. When a female stops following for several minutes, for example because she starts to forage, the
226 male usually turns back and calls. Males also produce the same soft calls and vocal sac movements
227 during some tactile interactions and following bouts. If the female does not approach the male, he
228 occasionally approaches her and touches her head or back. Altogether, the courtship sequence in *D.*
229 *tinctorius* appears to be very similar to that in *D. auratus* (Wells 1978), with females taking the most
230 active role. Both males and females vibrate the second digit of the hind-legs at high frequency ('toe
231 trembling', *sensu* Hödl and Amézquita 2001; see Supplementary video 3) during courtship. Toe-
232 trembling behavior can also be observed during foraging and agonistic interactions.

233 The courting pair does not seem to move over great linear distances (mean = 4.5 m; range, 0–8 m; n = 6),
234 but moves in circles within an area of a few square meters instead. Every now and then the pair stops at
235 certain places under the leaves or inside a hollow trunk, and the female starts to move in circles on the
236 same spot, with alternating movements of her hind limbs in what appears as wiping of the leaves (See
237 Supplementary video 4). The pair sometimes rests on the same spot for several minutes and the tactile
238 interactions increase considerably during these breaks. More often than not, the pair does this a few
239 times, at different places, before they choose the place where egg laying occurs.

240 In addition to the clutches laid by pairs we followed during courtship (n= 3), we found 18 clutches (for a
241 total of 21) with 2–5 embryos (mean = 3.6) at different developmental stages. The eggs were laid under
242 or within fallen logs and other wooden structures, leaf-litter, palm bracts and leaves, and animal burrows,
243 usually completely sheltered from the rain (Fig 6A, B). Egg diameter is ~ 4.2 mm and hatching occurs
244 after approximately two weeks (BR, pers. obs.; Fig. 6A). Eleven clutches were followed during
245 development and only 14 out of 46 embryos (30,4 %) from eight out of 11 clutches survived until
246 hatching. Other embryos did not develop, were destroyed by fungus, or disappeared possibly due to
247 predation, although no predation was observed directly. Twelve clutches in total were observed with 1–4
248 (mean = 2.2) tadpoles ready for male transport (Fig. 6C). Males were found occasionally sitting near or
249 on top of egg clutches, most likely inspecting and moistening them.

250

251 Larval development and patterns of tadpole transport

252 Hatching occurs after approximately 14 days (Fig. 6), but the tadpoles may remain viable in the clutch for
253 several days before being transported (AP, pers. obs.). The male eventually returns and sits on the clutch,
254 allowing the tadpoles to wriggle on his back, and takes them to suitable bodies of water where they will
255 remain unattended until metamorphosis, feeding on detritus and the larvae of some insects (e.g., Diptera
256 and Odonata) and other frogs (pers. obs.), even conspecifics (Fig. 7A; Rojas 2014, 2015). Tadpole
257 mouthparts are well suited for their carnivorous diet, with hardened serrated jaw sheath (Silverstone 1975;
258 Fig. 7B). Size at metamorphosis ranges 10.94–15.62 mm (mean = $13.15 \pm (\text{SE}) 0.24$ mm, $n=24$), and at
259 that point the color patterns are already completely visible (Fig. 2D).

260 We found 102 males (7 in 2009, 17 in 2010 and 78 in 2011) carrying one (Fig. 8A 79.4%), two (18.6%) or
261 three (2.0%) tadpoles (mean = $1.23 \pm (\text{SD}) 0.465$; Fig. 8c) ranging 4.78–6.87 mm long (from the tip of the
262 snout to the base of the tail; mean = $5.52 \pm (\text{SD}) 0.50$ mm). On one exceptional occasion BR also found
263 one female carrying two tadpoles with a visible difference in size (Fig. 8B). Pairs of tadpoles transported
264 by a male simultaneously differed significantly in size (paired *t*-test: $t= 4.719$, $df=13$, $n=14$, $P < 0.01$; Fig.
265 8D). The tadpoles of the two males carrying three at a time ($n=2$) were excluded from this analysis.
266 Some males carrying more than one tadpole were seen depositing one of them in a pool and leaving with
267 the second tadpole still attached to their back, whereas other males were seen depositing their two
268 tadpoles in the same pool, at the same time. Some males were also seen visiting more than one pool
269 before the tadpole(s) detached from their back. The visits consisted of jumping into the pool and
270 sometimes repeatedly diving inside for several minutes while the tadpole remained attached (see
271 Supplementary video 1).

272

273 Aggressive behavior

274 We observed 23 agonistic encounters involving both male - male ($n = 10$) and female - female ($n = 13$)
275 pairs. On one occasion, a male shortly attacked a female while attacking another male but resumed

276 courting the same female shortly after. The agonistic interactions ranged from short instances of chasing
277 without any physical contact to prolonged continuous physical combat lasting at least 20 min.
278 In both sexes, the physical fights involved kicking, jumping on each other's back, and pressing either the
279 head or the dorsum against the substrate (Fig. 9; Supplementary video 5). In most cases, we were unable
280 to identify the origin of the conflict, but it seemed to occur both in the presence and absence of an
281 individual of the opposite sex. While this was not always the case, both male and female aggressive
282 interactions were observed while one of the contestants was involved in courtship. For example, on one
283 occasion, while observing a courting pair in which the female was following the male closely, a second
284 female who had been under a log suddenly appeared and immediately assaulted the courting female. The
285 intruding female jumped on top of the courting female, trying to press the body of the latter against the
286 substrate. The courting female recovered, and tried to go on top of the intruder, and these alternating
287 attacks rapidly became a seemingly intense physical combat, in which movements and attacks occurred at
288 a high speed. The male turned away from the females and started to call at a high repetition rate. The
289 combat lasted for about ten minutes at the end of which the intruder female moved away, presumably
290 defeated by the courting female. The courting pair continued to be together for a couple more hours until
291 egg-laying occurred. On other occasions, we noticed the presence of a female in the vicinity of two males
292 engaged in a physical combat after which one of the males courted the female while the other moved
293 away. Some agonistic interactions both between males and between females occurred with no visible
294 involvement of the opposite sex. Interestingly, on two occasions males carrying tadpoles were also seen
295 engaged in physical combats with other males.

296

297 **Discussion**

298

299 The purpose of this study was to provide basic information about various aspects of the natural history of
300 *D. tinctorius* in the wild that could be used as background knowledge for future research on the
301 behavioral ecology, evolution and conservation of the species. We describe their reproductive and social

302 behaviors, habitat use, and their remarkable colonization of tree-fall gaps as soon as they occur. The
303 implications of these findings, as well as some hypotheses derived from our observations, are discussed
304 below.

305

306 *Dendrobates tinctorius* males were most often found climbing, foraging, and hiding around forest
307 structures, such as dead logs, fallen branches, roots, tree buttresses, and palm leaves. These structures are
308 used as oviposition sites (this study), and are also the types of structures that accumulate rainwater,
309 forming pools where newly hatched tadpoles are deposited (Rojas 2014). Females, in contrast, were more
310 often found foraging on the ground in open areas. Sex differences in microhabitat use might thus be
311 related to differences in parental duties, as males periodically attend developing clutches and are in charge
312 of tadpole transport and deposition. Forest structures can also be used by both sexes as communal retreats
313 during dry periods (Born et al. 2010), as has also been reported for *D. truncatus*, a closely related species
314 (Gualdrón et al. 2016). Such microhabitat likely provides higher humidity and shelter from potential
315 predators.

316

317 Males' association with structured habitats may also be the key reason why they are more likely to be
318 found in tree-fall gaps long after their formation. A recent study reported higher tadpole deposition rates
319 in pools at recent tree-fall gaps in comparison to pools in the closed forest (Rojas 2015), suggesting that
320 the availability of new places for tadpole deposition is one of the drivers of tree-fall gap invasion in this
321 species. However, the immediate arrival in tree-fall gaps is not exclusive of males. Females are as likely
322 to get to new tree-fall gaps within the first three days of their formation (Fig. 4B), possibly attracted by
323 the sudden abundance and diversity of food (pers. obs.); in fact, frogs captured in recently-formed tree-
324 fall gaps have shown a tendency to have more prey items in their stomach than frogs caught in the closed
325 forest (Born et al. 2010). Moreover, as suggested by Born et al. (2010), the simultaneous presence of
326 many individuals (Fig. 4A) can make tree-fall gaps a perfect mating arena.

327 The mechanism by which these frogs detect and locate treefalls remains unknown. However, the sound
328 and seismic cues produced during a treefall might be sufficient, as some frogs are known to detect
329 vibrational signals from conspecifics (Lewis and Narins 1985; Caldwell et al. 2010), heterospecifics
330 (Warkentin et al. 2007) and abiotic factors like rain (Caldwell et al. 2010); these three kinds of signals are
331 presumably much weaker than those produced by a treefall. Low-frequency seismic cues could be
332 detected at long distances but are short in duration; thus, it is possible that strong olfactory cues and light
333 gradients produced by a fresh treefall provide the additional information needed for orientation.

334

335 One of the most unusual aspects of *D. tinctorius*' reproductive behavior, and likely one of the
336 reasons why their behavior has been rarely studied in the wild (but see Born et al. 2010; Rojas 2014,
337 2015; Rojas et al. 2014a for examples of field studies on the species), is the lack of advertisement calls.
338 Most male frogs, including other dendrobatids, use calls to attract females and to repel rival males
339 (Gerhardt and Huber 2002; Erdtmann and Amézquita 2009; Santos et al. 2014), making them also easier
340 to locate by researchers. The structure of these calls shows great variation across the poison frog family
341 (Erdtmann and Amézquita 2009), and a recent large-scale comparative study (Santos et al. 2014) argued
342 that a reduced predation pressure has facilitated this diversification in acoustic signals in aposematic
343 species. Paradoxically, and in contrast to the vast majority of frogs, aposematic *D. tinctorius* appears to
344 have lost the advertisement function of its call altogether. Two closely related species, *D. auratus* and *D.*
345 *truncatus*, also vocalize less frequently and at lower intensities than most other poison frogs, but still use
346 calling both for territorial advertisement and courtship (Wells 1978; Summers 1989; Erdtmann and
347 Amézquita 2009; Gualdrón-Duarte et al. 2016). What factors drove or facilitated the loss of typical
348 calling behavior in *D. tinctorius* remains an intriguing evolutionary puzzle. Despite their toxicity, recent
349 studies indicate that predation risk by naïve predators may still be an important selective pressure
350 (Noonan and Comeault 2009; Comeault and Noonan 2011; Rojas et al. 2014b), suggesting that the
351 increased exposure associated with prominent calling behavior should be selected against. However, this
352 situation is not exclusive to *D. tinctorius*, and poison frogs in the genus *Oophaga*, for instance, have kept

353 their advertisement calls and an active vocal behavior despite their conspicuous coloration (Pröhl 2003;
354 Vargas-Salinas and Amézquita 2013; Willink et al. 2013). On the other hand, male and female *D.*
355 *tinctorius* tend to segregate in and around tree-fall gaps and other forest structures, potentially facilitating
356 mating pair formation by direct encounter without the need of acoustic signals. We speculate that such
357 microhabitat segregation and the availability of putative visual signals for communication in a diurnal
358 colorful frog (discussed below) has promoted the loss of the advertisement call in *D. tinctorius*.

359 Male *D. tinctorius* use calls, however, in courtship and agonistic interactions. The courtship calls
360 resembles a lower intensity version of calls produced by closely related species, such as *D. auratus* and *D.*
361 *truncatus* (Wells 1978, Gualdrón et al. 2016; BR, pers. obs.). In addition to advertisement calls, many
362 other dendrobatid frogs use soft courtship calls (e.g., Roithmair 1994), which are thought to facilitate the
363 contact with the female during the prolonged courtship while reducing the potential detection and conflict
364 with competitor males (Wells 2007). Courtship calls may also stimulate the ovulation in females, signal
365 territory ownership or function as visual signals because of the slow and prominent vocal sac inflation. In
366 *D. tinctorius*, males often take a distinct elevated posture when calling both during courtship and
367 agonistic encounters, and this posture is retained at times in the absence of vocalizations. This so-called
368 ‘upright posture’ is thought to function as a visual signal in both contexts (Hödl and Amézquita 2001).
369 Visual signals (Summers et al. 1999, Santos et al. 2014, Narins et al. 2003, de Luna et al. 2010) and
370 tactile interactions (Bourne et al. 2001, Pröhl & Hödl 1999, Summers 1992) have been long thought to
371 play an important role in poison frog communication. Aspects of dorsal coloration, for example, are
372 known to influence mating decisions (Summers et al. 1999; Maan and Cummings 2008) and agonistic
373 encounters (Crothers et al. 2011) in at least one species of poison frog, *O. pumilio*. However, in *O.*
374 *pumilio* and other species, acoustic signals still mediate the initial mate attraction (Lötters et al. 2003;
375 Pröhl 2003; Dreher and Pröhl 2014) and male-male competition (Crump 1972; Bee 2003; Amézquita et
376 al. 2006; Rojas et al. 2006; Tumulty et al. 2018). In the absence of advertisement calls, the use of tactile
377 stimuli and both static (such as dorsal colour patterns) and dynamic visual signals most likely plays a
378 predominant role in *D. tinctorius* communication. Dorsal color patterns might mediate mate choice

379 (Rojas 2017), given that individuals follow each other for a considerable amount of time while searching
380 for a suitable place for oviposition. Males have been found to have a higher proportion of yellow in their
381 dorsal area than females in our study population (Rojas and Endler 2013). This has been suggested to be
382 particularly beneficial during tadpole transport (Rojas and Endler 2013), a task that requires long
383 displacements and prolonged exposure, especially when climbing trees (A. Pasukonis, M. Loretto and B.
384 Rojas, unpubl. data). Male coloration might thus indicate parental male quality and be subject to sexual
385 selection (Rojas 2017). We suggest, however, that the variable coloration patterns on these frogs' front,
386 forelimbs, and flanks, also have the potential to be used as signals, as a lot of the time the frogs are either
387 facing or next to each other during courtship (Rojas 2012). These color patterns may also be used for
388 species, sex, or even individual recognition from the distance. Individual recognition has not been shown
389 in any amphibian, but the relatively complex social behavior, the lack of acoustic communication, and the
390 repeated encounters in their shared micro-habitat might have promoted such ability in *D. tinctorius*.
391 Both male and female *D. tinctorius* engage in intra-sex aggression that may escalate to intense physical
392 combats, which involve chasing, wrestling and prolonged pressure over the opponent's head or dorsum.
393 These types of behaviors have been also reported for the closely related *D. auratus* (Wells 1978;
394 Summers 1989) and *D. leucomelas* (Summers 1992). Aggression in male poison frogs is usually a result
395 of male competition for mates and territorial defense mediated by acoustic interactions (reviewed in Pröhl
396 2005). To the best of our knowledge, males of all dendrobatid species studied to date show some degree
397 of territoriality (Pröhl 2005). *Dendrobates tinctorius* seems also unusual in this respect, as they do not
398 appear to defend exclusive areas. Similar to Born et al. (2010), we have observed males foraging in close
399 proximity without aggressive escalations in large aggregations around fresh tree-fall gaps, as well as
400 around structures where a few males might take refuge. We observed that the presence of individuals of
401 the opposite sex, especially during courtship, was the cause of some of the agonistic encounters both
402 between males and between females. Inter-female aggression has been also reported for *Mannophryne*
403 *trinitatis* (Wells 1980), *D. auratus* (Wells 1978; Summers 1989), *D. leucomelas* (Summers 1992), and *O.*
404 *pumilio* (Meuche et al. 2011). Just like in *D. tinctorius*, in the closely related *D. auratus*, tadpoles are

405 cannibalistic and males may deposit tadpoles from multiple clutches in the same pool (Summers 1989,
406 1990). As suggested for *D. auratus*, female aggression thus might be the result of attempts to monopolize
407 males and reduce the potential competition and risk of cannibalism by unrelated tadpoles in shared pools.
408 Interestingly, we also observed aggressive interactions that seemingly did not involve a third individual,
409 suggesting aggression triggers other than access to mates. These observations should, however, be
410 interpreted with caution, as we cannot be certain that a third individual was not hiding in the area. In
411 addition to mating context, aggression in some dendrobatid frogs has been linked to defense of shelter and
412 feeding areas (Wells 1980; Meuche et al. 2011), but *D. tinctorius* does not appear to defend exclusive
413 territories (Born et al. 2010). Some of the aggressive interactions resulted in the defeated individual being
414 chased away, as if in a territorial displacement, but others terminated with both individuals continuing to
415 forage nearby. This hints at an establishment of dominance hierarchies between opponents, which we
416 suggest could be the result of repeated encounters of individuals in their shared microhabitat. Dominance
417 hierarchies are well documented in all other vertebrates, where dominant individuals get preferential
418 access to food, mates, and shelter (reviewed in Huntingford 2013). However, the formation of such
419 potential hierarchies has not been described for any anuran species in the wild, despite being suggested to
420 arise among poison frogs in captivity (Zimmermann and Zimmermann 1988) and to mediate conflict
421 resolution in *O. lehmanni* (Rojas 2002). This is, therefore, a subject that merits further investigation.
422 Aggressive behavior and territoriality in *D. tinctorius* might be context-dependent and related to
423 population density, variation in food abundance and other resources, such as structures for shelter or
424 oviposition. In the absence of vocalizations, *D. tinctorius* may be using visual signals to get information
425 about the fighting abilities of their opponents, as it has been reported for male *O. pumilio* (Crothers et al.
426 2011; Crothers and Cummings 2015), and settle their conflicts before escalating to physical combats
427 (Rojas 2017). Social behavior in *D. tinctorius* is a promising avenue of research, which could provide
428 insights into the evolution of visual communication and factors influencing anuran aggressive and
429 territorial behavior in the absence of acoustic communication.

430 Egg clutches at our study site have high mortality and are much smaller than those reported in captivity,
431 which may have up to 14 eggs (Lötters et al. 2007). This does not seem to be an exception, as levels of
432 hatching failure of up to 80% have been previously reported for *O. pumilio* (Pröhl and Hödl 1999). Loss
433 of most eggs or embryos is likely due to predation (e.g., Juncá and Rodrigues 2006), or to fungal
434 infections (Fig 6B). On one occasion, we observed a female unrelated to the clutch on top of the missing
435 eggs, indicating possible cannibalism. This behavior has been previously reported in *D. auratus* as a
436 mechanism of intra-female competition (Summers 1989; 1990).

437 Upon hatching, males take tadpoles, either all of them or one at a time, to bodies of water. The latter is
438 thought to be the case of most *Dendrobates*, although the evidence supporting this pattern comes mostly
439 from observations in captivity (Lötters et al. 2007). Transport of single tadpoles, one by one, implies
440 several trips between the place where clutch was laid and the pools, a task that has been shown to require
441 remarkable spatial abilities (Pašukonis et al. 2016) and probably a high energetic cost (Beck et al. 2017).
442 In *D. tinctorius*, males carry one or two (sometimes three) tadpoles at a time. In combination with the
443 high clutch mortality rates observed, this suggests that males often take all the larvae that survive within a
444 given clutch at once. However, we have observed at least a few instances in which males take one
445 tadpole to a pool and then return to get the rest (A. Pašukonis, M. Loretto and B. Rojas, unpubl. data).
446 Furthermore, males have been seen depositing one tadpole in the same pool where they had deposited
447 another tadpole (Rojas 2014), which could also mean that males carrying two tadpoles at once are
448 transporting tadpoles from different clutches. This is supported by the fact that tadpoles transported at the
449 same time on a male's back differ significantly in size. In *D. auratus* for example, males have been seen
450 moistening a fresh clutch and a hatching clutch within the same hour in captivity (Wells 1978), and
451 attending multiple clutches of different stages in the field (Wells 1978; Summers 1990). However, these
452 differences in size could also be inherent to within-clutch variation in hatching size. Size difference
453 between tadpoles transported simultaneously was particularly noticeable in the tadpoles on the back of the
454 only female found performing these duties. While rare (1 in >100 tadpole transport events reported here),

455 tadpole transport might be taken over by females if males go missing. This type of flexible compensation
456 is known to occur in at least one other species of poison frog, *Allobates femoralis* (Ringler et al. 2015).
457 As reported before, we observed tadpole deposition in different water-holding structures in the forest,
458 from palm bracts on the ground to tree holes high up. However, the specific characteristics that influence
459 pool choice by a male and favor successful tadpole development are currently unknown. It has been
460 previously suggested that, despite the high levels of tadpole cannibalism, parents might use the presence
461 of larger tadpoles as a cue of pool quality. Presence of large tadpoles may indicate that basic
462 requirements, such as sufficient nutrients and water stability, have been met to allow tadpole development
463 (Rojas 2014). Even less is understood about the role that *D. tinctorius* plays in the ecology of other
464 phytotelm-breeding anurans, especially considering that most species are restricted to terrestrial or
465 arboreal habitat; meanwhile, *D. tinctorius* and their carnivorous tadpoles are capable of exploiting pools
466 at all heights (Gaucher 2002). How *D. tinctorius* finds canopy pools is unknown, but it has been
467 speculated that they may eavesdrop on the calls of treefrog species such as *Trachycephalus resinifictrix*
468 and *T. hadroceps*, which breed in arboreal water bodies (Gaucher 2002). We further hypothesize that
469 enlarged male toe-pads (apt for climbing) and aposematic coloration gave *D. tinctorius* access to a wider
470 variety of aquatic habitats despite being exposed to would-be predators for prolonged periods of time
471 during tadpole transport.

472 Approximately 43% of the amphibian species worldwide are experiencing population declines (Stuart, et
473 al. 2004), largely as a consequence of the spread of a deadly disease caused by the fungus
474 *Batrachochytrium dendrobatidis* (*Bd*) (Lips et al. 2006; Lötters et al. 2009; Lips 2016; Bower et al. 2017).
475 Despite having a low *Bd* prevalence compared to species in other families and regions (e.g. Flechas et al.
476 2012), phytotelm-breeding dendrobatids, including *D. tinctorius*, have been found to have the highest
477 prevalence of *Bd* in recent studies done in French Guiana, indicating that dendrobatid species may be
478 more vulnerable to *Bd* infection than previously suspected (Courtois et al. 2015). Furthermore, the same
479 study reports an increase in *Bd* prevalence in *D. tinctorius* between 2009 and 2012, hinting at a relatively
480 recent *Bd* establishment and its current spread in French Guiana. While *Bd* research has been mostly

481 focused on adult frogs, it is known that tadpoles can also get infected due to their keratinized mouthparts
482 (Berger et al. 1998; Blaustein et al. 2005). However, there is currently no information on *Bd* prevalence
483 in *D. tinctorius* tadpoles, or studies assessing the presence of *Bd* in the pools where tadpoles develop,
484 despite reports of *Bd* occurrence in phytotelmata and phytotelm-breeding frog species in other
485 Neotropical areas such as Panama and Ecuador (Cossel and Lindquist 2009; McCracken et al. 2009).
486 Furthermore, considering that dispersal of *Bd* outside large bodies of water may imply an amphibian
487 vector (Kolby et al. 2015), we urge studies evaluating the role of *D. tinctorius* adults as *Bd* vectors across
488 forest strata (i.e., from the ground to the canopy), and advocate that increasing our knowledge on the
489 behavior and ecology of this species may improve our understanding on the dynamics of *Bd* transmission
490 in their habitat.

491 Another major threat for anurans is habitat destruction (Cushman 2006). While our study population
492 occurs within in a natural reserve, many populations of *D. tinctorius* are in unprotected areas, which are
493 under threat primarily by gold mining-driven deforestation. It is estimated that approximately 41% (~684
494 km²) of the deforestation in the South American tropical rainforest between 2001 and 2013 occurred in
495 the so-called Guianan moist forest ecoregion due to gold mining activities (Alvarez-Berríos and Aide
496 2015). Because *D. tinctorius* is often distributed in small patchy populations (Noonan and Gaucher
497 2006), deforestation even at the small-scales used for gold mining, can have a detrimental, probably
498 irreversible effect on the life histories and survival of this species and other phytotelm-breeders. We thus
499 fully support the long-term monitoring strategies suggested by Courtois et al. (2015) and currently
500 implemented across several nature reserves in French Guiana (e.g., [http://www.reserve-tresor.fr/en/our-](http://www.reserve-tresor.fr/en/our-actions/studies-and-surveys/herpetology)
501 [actions/studies-and-surveys/herpetology](http://www.reserve-tresor.fr/en/our-actions/studies-and-surveys/herpetology)) to allow the timely assessment of changes in population size and
502 sudden declines, especially of ‘sentinel species’ such as *D. tinctorius* (Courtois et al. 2013, 2015). These
503 types of strategies, together with basic research on the natural history of threatened species are key, not
504 only for the formulation of successful conservation policies, but also for the education and future
505 engagement of public essential for the preservation of wildlife at a local scale.

506

507 **Additional Information and Declarations**

508 **Competing Interests**

509 The authors declare no competing interests.

510

511 **Author Contributions**

512 Both authors collected information in the field, analyzed data, and prepared figures for the paper. BR
513 wrote a first draft of the manuscript, which was then edited and approved by AP.

514

515 **Animal Ethics**

516 Our research was approved and authorized by the scientific committee of the Nouragues Ecological
517 Research Station. We strictly adhered to the current French and European Union law, and followed the
518 ‘Guidelines for use of live amphibians and reptiles in the field and laboratory research’ by the
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532 Supplementary videos

533 1. Tadpole transport and pool inspection

534 <https://youtu.be/KE5LB-2IsTU>

535

536 2. Cannibalism/tadpole aggression

537 <https://youtu.be/Utvrnqi-VOK>

538

539 3. Courtship

540 <https://youtu.be/gNIZLBpivMI>

541

542 4. Egg laying

543 <https://youtu.be/zf-4aOXeir0>

544

545 5. Male and female aggressive behavior

546 https://www.youtube.com/watch?v=g5x_K0x7Lcg&feature=youtu.be

547

548

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558

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800

801 **Figure legends**

802 **FIGURE 1.** Colour pattern variation (a) within the studied population and (b-e) between different
803 populations of the dyeing poison frog in French Guiana. (a) Top row males, bottom row females. Lines
804 on the background paper mark 5 mm. Note the enlarged toe discs in males, but overall larger female body
805 size. Photos by: A. Pašukonis and Matthias-Claudio Loretto (a: Nouragues nature reserve, French
806 Guiana), A. Fouquet (c: Bakhuis, Suriname; d: Mt. Galbao, French Guiana), and B. Rojas (b: Mt.
807 Matoury, French Guiana; e: Mt. Bruyere, French Guiana).

808

809 **FIGURE 2.** Examples of phytotelmata used as tadpole deposition sites at the studied population. Photos
810 by: B. Rojas (a, c) and A. Pašukonis (b, d).

811

812 **FIGURE 3.** Habitat use in relation to sex. Numbers in the boxes indicate the total number of individuals in
813 each category (N =109).

814

815 **FIGURE 4.** Dozens of adult *D. tinctorius* can aggregate at once at a newly formed treefall gap. There are
816 no sex differences in arrival within the first three days of gap formation (a), but males are more likely to
817 be found in treefall gaps in the long term (b).

818

819 **FIGURE 5.** (a) Example of tactile interactions observed in *D. tinctorius* during courtship: a female with a
820 limb on a male's head. (b) Waveform and (c) spectrogram of *D. tinctorius* call recorded from close range
821 (approx. 30 cm) during courtship. The normalised waveform reveals the relative amplitude modulation
822 and the pulsating structure of the call (pulse rate = 154 Hz); the spectrogram (FFT window length = 0.01
823 s, Gaussian window, frequency range 0 - 6000 Hz) show the broadband spectral structure of the call with
824 dominant frequency band centered around 3150 Hz. Photo by: B. Rojas.

825

826 **FIGURE 6.** Clutch development in *D. tinctorius* in the field. (a) Freshly laid clutch of 5 eggs; (b) The same
827 clutch five days later. Note that two of the initial eggs have been infected by fungus; (c) 15 days after egg
828 laying, two surviving tadpoles are ready to be picked by the male and taken to a body of water where they
829 will continue to develop until metamorphosis; (d) A tadpole attached to the male's back. Photos by: A.

830 Pašukonis.

831

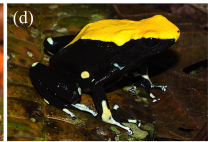
832 **FIGURE 7.** (a) A cannibalistic tadpole with the remainings of its victim; (b) oral apparatus (anterior side
833 up) of a stage 25 (Gosner 1960) *D. tinctorius* tadpole. Photos by: B. Rojas (a) and E. K. Fischer (b).

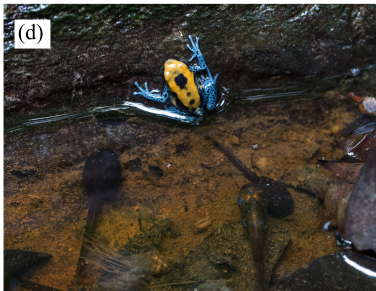
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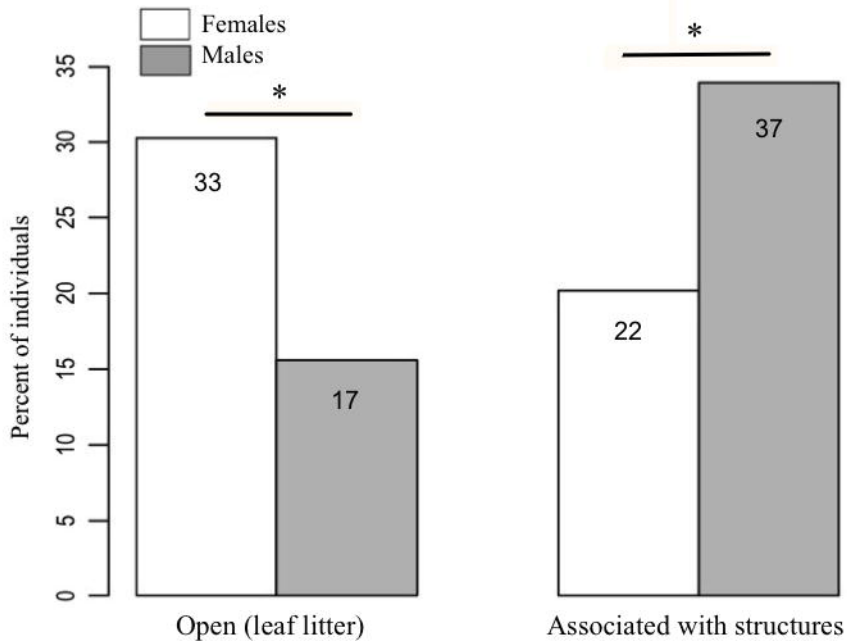
835 **FIGURE 8.** A male (a) and a (exceptional) female (b) with tadpoles on their back (indicated by white
836 arrows). (c) Most individuals were found carrying one tadpole, but two and three tadpoles can also be
837 carried at once. (d) Tadpoles transported simultaneously differ significantly in size. Photos by: B. Rojas.

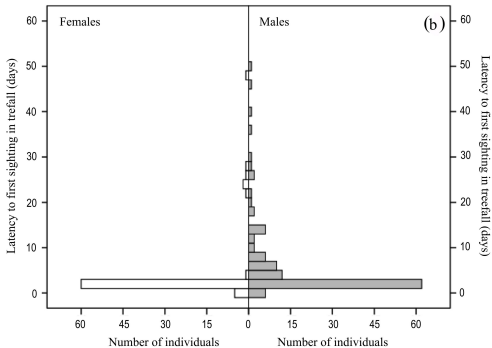
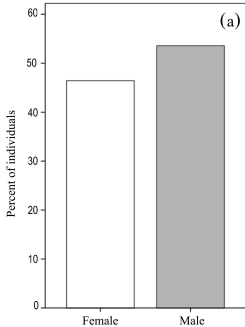
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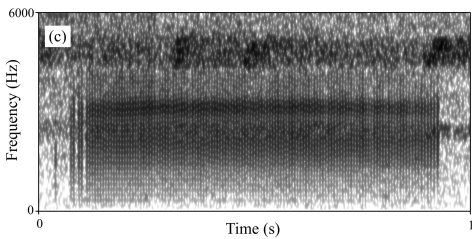
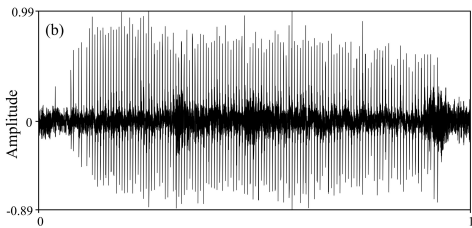
839 **FIGURE 9.** Sequence of an agonistic encounter between two male *D. tinctorius*. Physical combats involve
840 wrestling, pressing the opponent against the substrate (with either the forelimbs or the whole body), and
841 kicking. Occasionally males also vocalize during fighting, as seen by the inflated vocal sac in the middle
842 photograph. Photos by: B. Rojas.













(a)



(b)



