

1 **Amazon poison frogs (*Ranitomeya amazonica*) use different phytotelm**  
2 **characteristics to determine their suitability for egg and tadpole deposition.**

3

4 **Erik H. Poelman<sup>1,\*</sup>, René P.A. van Wijngaarden<sup>2</sup>, Ciska E. Raaijmakers<sup>3</sup>**

5

6 <sup>1</sup> Laboratory of Entomology, Wageningen University, PO Box 8031, 6700 EH

7 Wageningen, The Netherlands.

8 <sup>2</sup> ALTERRA, Centre for Water and Climate, PO Box 47, 6700 AA Wageningen, The

9 Netherlands.

10 <sup>3</sup> Netherlands Institute of Ecology, Department of Terrestrial Ecology,

11 Droevendaalsesteeg 10, 6708 PB Wageningen, The Netherlands

12 \* Author for correspondence: Erik H. Poelman, Tel: + 31 317 4 85433, Email:

13 Erik.Poelman@wur.nl

14

15 Running title: Egg and tadpole deposition preference in Amazon poison frogs

16

17 Key-words: phytotelm quality, poison frog, parental care, oviposition, tadpole

18 deposition

19

20 Total word count: 4911

21 Abstract: 286

22 Introduction: 1050

23 Material and Methods: 1528

24 Results: 631

25 Discussion: 1378

- 26 Nr. of references: 47
- 27 Nr. of tables and figures: 1 table, 6 figures
- 28

29 **Abstract**

30 Parents have to assess the multivariate characteristics of their reproductive sites to  
31 maximize their reproductive success through offspring performance. In addition, they  
32 may provide care to ensure optimal performance of their offspring. In poison frogs it  
33 has been identified that ecological characteristics of reproductive sites may underlie  
34 transitions in the involvement of parental sexes in care for offspring. To elucidate the  
35 ecological factors that may drive these transitions, it is important to understand which  
36 characteristics poison frogs use to assess the quality of their reproductive site. We  
37 studied the use of small water bodies in leaf axils of bromeliads, phytotelmata, for egg  
38 and tadpole deposition by Amazon poison frogs (*Ranitomeya amazonica*). We  
39 compared phytotelm quality characteristics for preferred egg and tadpole deposition  
40 sites and used two choice tests with plastic cups to study the causal relationship with  
41 tadpole deposition for the identified characteristics. The differences among quality  
42 characteristics of deposition sites were largest among bromeliad species, and for egg  
43 or tadpole deposition different bromeliad species were preferred. However, males  
44 were also selective in the leaf axils within a bromeliad species that they used for egg  
45 or tadpole deposition. Eggs were deposited in small, resource limited water bodies  
46 that were close to the forest floor. Tadpoles were deposited in leaf axils holding  
47 resource-rich phytotelmata with larger water volumes. Preference of detritus  
48 containing water over clear water in choice tests confirmed that Amazon poison frogs  
49 assess quality of their tadpole deposition sites on food availability. We conclude that  
50 preference for large water volume and resource rich phytotelmata plays an important  
51 role in determining male involvement in parental care and speculate that distribution  
52 of preferred resources may bring about selection on female involvement in parental  
53 care.

54

55 **Introduction**

56 Divergence in reproductive strategies of organisms has intrigued biologists for  
57 centuries. The most elaborate reproductive strategies are found in vertebrates which  
58 parents invest in the quality of few offspring by providing nourishment, protection or  
59 other types of parental care (Trivers 1972; Clutton-Brock 1991). What determines  
60 whether a parent is involved in providing care and what brings about transitions in the  
61 mode of parental care of a species are central questions in evolutionary ecology. The  
62 primary driving forces behind the involvement of one of the sexes in parental care are  
63 their initial investment in gamete size, the certainty of paternity, the chances of  
64 leaving offspring first (for example by the order of gamete release), and the balance  
65 between deserting and caring in terms of fitness gain by additional mating versus loss  
66 of offspring when leaving the current brood (Trivers 1972; Dawkins and Carlisle  
67 1976; Clutton-Brock 1991). Although most of these characteristics and thus mode of  
68 parental care are strongly defined by the ancestral state of a species, ecological factors  
69 may drive transitions in modes of parental care by for example shaping the costs of  
70 deserting offspring. Although ecological factors are now considered capable of  
71 bringing about fast transitions of complex species traits (West-Eberhard 2003), few  
72 studies have provided empirical evidence of a causal relationship between an  
73 ecological parameter and a transition in a mode of parental care (but see Johnson et al  
74 2007 for an example).

75         How ecological parameters may drive reproductive decisions is well studied in  
76 insects that use distinct resources for the development of their offspring (Mayhew  
77 1997). Many insect species desert their offspring upon laying eggs and confine their  
78 offspring to a food source such as a single fruit in fruit flies (Mock and Parker 1997),  
79 a caterpillar host in parasitic wasps (Godfray 1994; Harvey et al 2013) or a small body

80 of water in a tree hole for damselflies (Fincke 1994). The success of development of  
81 offspring in such distinct resources depends on many ecological factors such as the  
82 quantity, quality and stability of the food source as well as the risk of predation and  
83 competition (Fincke 1994; Lehtinen 2004; Refsnider and Janzen 2010; Harvey et al  
84 2013). This implies that mothers need to assess the multivariate quality characteristics  
85 of a distinct resource and adapt their oviposition strategy to this information. Factors  
86 shaping oviposition decisions may be hierarchical in their effect size, for example  
87 predator avoidance may be prioritized above avoidance of competition or preference  
88 for pool size. The hierarchy may depend on the variation in the quality characteristic a  
89 female can choose from and the associated variation in reproductive success for a  
90 female making her choice based on the quality characteristic (Crump 1991). Each of  
91 these ecological factors could potentially drive a shift in reproductive decisions that  
92 may include decisions on parental investment in single offspring (Ekbom and Popov  
93 2004).

94         Interestingly, Neotropical poison frogs (Dendrobatoidea) have both the use of  
95 distinct resources and parental care combined in their reproductive biology, offering a  
96 system in which ecological parameters can be studied for their effect on transition of  
97 modes of parental care. Some clades of poison frogs use small water bodies in leaf  
98 axils or tree holes, i.e. phytotelmata (Kitching 2000), as rearing sites for their tadpoles  
99 (Summers and McKeon 2004). These phytotelmata are distinct resources that may  
100 vary in water volume, nutrient composition, stability and risk of competition or  
101 predation (Lehtinen 2004). Phytotelm volumes may typically be in the range of a few  
102 milliliters to less than 100mL (Kitching 2000). A derived state of parental care in  
103 poison frogs is that males care for the egg clutch and transport tadpoles on their back  
104 to deposit them individually in phytotelmata (Summers et al 1999). Similar to larvae

105 of solitary parasitic wasps that develop individually inside caterpillars and kill other  
106 parasitoid larvae that share the caterpillar (Godfray 1994; Harvey et al 2013), the  
107 tadpoles are cannibalistic and a single phytotelm can yield only a single offspring at a  
108 time (Summers and Amos 1997; Summers and Symula 2001; Poelman and Dicke  
109 2007). Males have been shown to be discriminative for different characteristics of the  
110 phytotelm such as preference for a certain volume, height of the phytotelm above the  
111 forest floor or avoidance of competition and predation (Downie et al 2001; Brown et  
112 al 2008a,b; von May et al 2009a; Ryan and Barry 2011). Interestingly, female  
113 involvement in parental care has evolved in parallel to the use of smaller more  
114 resource limited phytotelmata (Brown et al 2010). In several lineages this has resulted  
115 in species that exhibit bi-parental care or uni-parental female care in which females  
116 provision their offspring with nutritive eggs (Weygoldt 1980; Caldwell 1997;  
117 Caldwell and de Oliveira 1999; Summers et al 1999; Pramuk and Hiler 1999).  
118 Although water volume may correlate with the mode of parental care, little is known  
119 about the characteristics of phytotelmata that poison frogs use to assess the quality of  
120 the pool and that determines their type of care. Some studies have reported on the  
121 water volume preferences and avoidance of cannibalistic conspecific tadpoles in  
122 separate tests (Schulte et al 2011), few studies have evaluated which of these  
123 characteristics most likely explains reproductive site choice by poison frogs.  
124 Moreover, most comparisons have been made across bromeliad species, not looking at  
125 preference for phytotelmata within single bromeliad species and thereby cannot rule  
126 out that poison frogs choose for reproduction in certain bromeliad species rather than  
127 choosing for a certain qualitative trait.

128         We studied phytotelm use for oviposition and tadpole deposition by Amazon  
129 poison frogs (*Ranitomeya amazonica*) under rainy season conditions. We compared

130 phytotelm quality characteristics for preferred egg and tadpole deposition sites. We  
131 specifically addressed whether preference for phytotelmata by *R. amazonica* differs  
132 for egg and tadpole deposition, and what are the underlying characteristics of the  
133 preferred phytotelmata in each context. For deposition of eggs, we hypothesized no  
134 preference for nutrients and water volume since egg development takes on average  
135 only 14 days and needs a moist environment. Since tadpoles of *R. amazonica* require  
136 about three months of development before the metamorphs can leave the phytotelm  
137 they were deposited in (Poelman and Dicke 2007), we hypothesized that tadpoles  
138 would be deposited in phytotelmata that have relatively large water volumes that are  
139 less likely to desiccate. Furthermore, these pools should offer tadpoles a stable food  
140 source for that length of time and therefore should be rich in detritus and have a high  
141 algal growth. The characteristics that were found to correlate with deposition  
142 preferences were studied in two-choice tests for their causal relationship. We discuss  
143 the implications of differences in egg and tadpole deposition sites for the reproductive  
144 ecology and evolution of parental care in poison frogs.

145

## 146 **Material and Methods**

147

### 148 *Study sites*

149 The field study was conducted during two field seasons (10 April – 27 July 2002 and  
150 2 April – 28 April 2008) at three sites in French Guiana. The weather conditions at  
151 these sites are characterized by seasonal rainfall with a dry period from the end of July  
152 until mid-November (Grimaldi and Riéra 2001). Two of the study sites are located in  
153 the Nouragues Nature Reserve (4°05 N, 52°41 W). The first and main study site,  
154 “Inselberg”, is situated on top of a granite outcrop 411 meters above sea level. The

155 patches of forest, of up to a few hundred square meters in size are dominated by trees  
156 of the genus *Clusia* and are separated by bare granite rock. The bromeliad community  
157 at this site is dominated by the epiphytic *Catopsis berteroniana* and the two terrestrial  
158 bromeliads, *Pitcairnia geykessi* and *Aechmea aquilega* (see Sarthou 2001 for a  
159 detailed description of the study area). The second study site “Campsite” was situated  
160 near the campsite of Nouragues. Here a semi-natural habitat is formed by patches of  
161 diverse bromeliad species that have been collected for research purpose and attached  
162 to trees around the campsite (see Poelman and Dicke (2008) for the genera of  
163 bromeliads found here). The third study site “Emerald Jungle Village” was located in  
164 the primary forest behind the lodge at Emerald Jungle Village (4°48N, 52°22 W).  
165 Here a semi-natural habitat is formed by a large private collection of bromeliads. The  
166 two semi-natural sites were only used for experiments on tadpole deposition  
167 preference of *R. amazonica* based on two-choice tests with plastic cups.

168

#### 169 *Study species*

170 Amazon poison frogs, *Ranitomeya amazonica* (Brown et al 2011) (*Ranitomeya*  
171 *ventrimaculata sensu* Grant et al 2006 and *Dendrobates ventrimaculatus sensu* Shreve  
172 1935, the latter name we used in former reports on the populations studied here  
173 (Poelman and Dicke 2007, 2008)) (Fig. 1), belong to a complex of closely related  
174 species that can be found from the Guyana Shield through the Amazon basin of Brazil  
175 into the East Andean lowlands of Colombia, Ecuador, and Peru (Brown et al 2011).  
176 Males are territorial and both males and females can be found around bromeliads or  
177 other plants that hold phytotelmata in their leaf axils (*Heliconia* and aroids) (Poelman  
178 and Dicke 2008). The clutch of 1 up to 6 eggs is laid on the edge of the phytotelm  
179 partly submerged below the water line. Here we refer to egg laying in a phytotelm as



180 oviposition. Tadpoles that hatch are transported one or two at the time on the back of  
181 the male and are deposited in other phytotelmata (Bechter and Lescure 1982;  
182 Summers and Amos 1997; Poelman and Dicke 2007). Here the tadpoles feed on  
183 detritus, algae and cannibalize on conspecific eggs or tadpoles. Metamorphosis takes  
184 place after three months (Poelman and Dicke 2007). None of the six other species of  
185 Dendrobatoidea present in French Guiana, *Dendrobates tinctorius*, *Ameerega hahneli*,  
186 *Allobates femoralis*, *Allobates granti*, *Anomaloglossus baobatrachus*, and  
187 *Anomaloglossus degranvillei* (Born and Gaucher 2001; Kok et al 2006), use similar  
188 phytotelmata as *R. amazonica*.

189

#### 190 *Assessing phytotelm quality*

191 To determine whether *R. amazonica* prefers certain bromeliad species for  
192 reproduction, we counted the number of bromeliads and their number of phytotelmata  
193 for the most common bromeliad species (*Catopsis berteroniana*, *Aechmea aquilega*,  
194 *Pitcairnia geykessi*) on nine forest patches located on the natural study site  
195 “Inselberg”. Over the study period in 2002, the nine patches of a 100 m<sup>2</sup> each were  
196 visited in series of three consecutive days followed by a day without observations. We  
197 monitored all leaf axils for the presence of eggs or tadpoles and noted the bromeliad  
198 species for each encounter.

199 In 2008 at Inselberg, we studied which qualitative characteristics make  
200 phytotelmata or the leaf axils that hold them preferred for oviposition or tadpole  
201 deposition. To determine the characteristics of an average leaf axil and its phytotelm  
202 of *C. berteroniana* and *A. aquilega*, we non-selectively sampled a single leaf axil, in  
203 which no eggs or tadpoles were found, of forty bromeliads for each of the two  
204 bromeliad species. We also searched for leaf axils of *C. berteroniana* and *A. aquilega*

205 that contained eggs or tadpoles by visually inspecting phytotelmata. For each of the  
206 leaf axils that had received oviposition, tadpole deposition and those that were non-  
207 selectively sampled and not in use for reproduction we measured 17 characteristics  
208 (Fig. 2): the bromeliad species (1), the height of the bromeliad above the forest floor  
209 (2), the geographic orientation of the leaf axil (3), the number of leaf rosettes of the  
210 bromeliad (4), the leaf rosette the axil is part of (5), the width of the leaf axil (6), the  
211 depth of the leaf axil (7), the water volume of the phytotelm (8). The water quality of  
212 the phytotelm was measured for its temperature (9), pH (10),  $K^+$  in  $mol/m^3$  (11),  $NO_3^-$   
213 -  $NO_2^-$  in  $mg/l$  (12) and phosphate  $PO_4^{3-}$  in  $mg/l$  (13). The phytotelm was examined  
214 for the presence and amount of detritus (14) that we scored on a scale of 0 to 5 and the  
215 presence of algal growth (15) that we scored on a scale of 0 to 3. On both scales a 0  
216 means absence of the characteristic (detritus or algal growth). For detritus we defined  
217 1-5 by rating how murky the water of the phytotelm was after the water was extracted  
218 from the leaf axil and gently shaken in a plastic vial. We rated each water sample for  
219 transparency (1 = 20%, 2 = 40%, 3 = 60%, 4 = 80% with a maximum of 5 (100%  
220 murky, non-transparent)). Algal growth was determined by visually inspecting the  
221 leaf axil for presence of algae. Leaf axils that were covered for up to 1/3 with algae  
222 were scored as 1, 2/3 as 2 and fully covered scored as 3. Furthermore, we counted the  
223 number of insect larvae (16) and tadpoles of other species (17) present in the  
224 phytotelm. Height of bromeliads above the forest floor was measured to the nearest  
225 centimeter using a tape measure and all other size measurements were taken with dial  
226 calipers to the nearest millimeter. The leaf rosettes were counted as estimate of  
227 bromeliad size by counting from the center of the plant, increasing number to the  
228 outer rosettes (Fig. 2). The position of the measured leaf axil within the bromeliad  
229 was calculated as the rosette number it was part of divided by the total number of

230 rosettes of that bromeliad. The geographic orientation of the leaf axil was assessed  
231 using the degrees derived from the north as pointed by a compass that was held above  
232 the bromeliad. The geographic orientation was computed into eight segments of the  
233 360 degree circle (Fig. 2). Position and geographic orientation of the leaf axils were  
234 assessed because we hypothesized that bromeliads would be heterogeneous in their  
235 axil quality, and that across individual bromeliads the distribution of leaf axil quality  
236 may be similar when caused by the amount of sun light or exposure to wind. Water  
237 volume was assessed by extracting the water from the leaf axil with a 50-ml syringe.  
238 Water temperature was measured using a digital thermometer (Checktemp 1 Pocket  
239 Thermometer, Hanna Instruments BV). We used indicator tests for pH (Duotest pH  
240 3.5-6.8 and 7.0-10.0, Macherey-Nagel), K<sup>+</sup> (Aquadur 0-4.5 mol/m<sup>3</sup>, Macherey-Nagel),  
241 NO<sub>3</sub><sup>-</sup> - NO<sub>2</sub><sup>-</sup> (Quantofix Nitrate 10-500 mg/l Nitrite 1-80 mg/l, Macherey-Nagel) and  
242 PO<sub>4</sub><sup>3-</sup> (Quantofix Phosphat(e) 3-100 mg/l, Macherey-Nagel).

243

#### 244 *Two choice experiments*

245 We experimentally tested whether water volume or presence of detritus affected  
246 tadpole deposition preference of *R. amazonica*. At the start of our field season in 2008,  
247 we attached two-choice tests consisting of a pair of plastic coffee cups to bromeliads  
248 at the three study sites. The plastic cups were exposed to natural tadpole deposition by  
249 Amazon poison frogs for three weeks and were monitored weekly for the presence of  
250 tadpoles. We tested whether water volume affected tadpole deposition by offering a  
251 cup with 10 ml paired to a cup containing 50 ml. Whether detritus of leaf litter  
252 affected tadpole deposition, was tested with paired cups of similar water volume (50  
253 ml). One cup contained collected rain water only, the other cup contained water with  
254 small pieces of leaf litter with a summed size of 4 cm<sup>2</sup>. Water levels were kept at their

255 volume by puncturing a hole in the plastic cup that functioned as drain for excessive  
256 water and the daily rainfall kept water levels at the maximum holding capacity of the  
257 drained cups. At each study site 20 paired cups of each of the two tests were offered  
258 with a minimum distance of ten meters between pairs of cups.

259

#### 260 *Statistical analyses*

261 To test whether certain bromeliad species were preferred by *R. amazonica* for egg or  
262 tadpole deposition, we counted the number of egg and tadpole depositions found in  
263 the three bromeliad species. We used chi-square tests to test whether the observed  
264 distribution differed from an expected distribution of the availability of phytotelmata  
265 of the three bromeliads in the study site as assessed by counting. Differences in the  
266 multivariate characteristics of the two bromeliad species, and differences among non-  
267 selectively sampled leaf axils and those used by *R. amazonica* for egg or tadpole  
268 deposition were analyzed using redundancy analysis (RDA). We used this constrained  
269 multivariate ordination analysis to seek the leaf axil characteristics that best predicted  
270 the difference among bromeliads, or use for reproduction by *R. amazonica*.

271 Characteristics were evaluated for their significance using forward selection and  
272 Monte Carlo Permutation tests (999 tests). To test whether eggs were deposited more  
273 frequently in a specific geographic orientation of leaf axils we used a Mann-Whitney  
274 U test, because most of the eight segments received fewer than 5 ovipositions so that  
275 our data did not meet the test requirements of chi-square. We compared the scores on  
276 the geographic eight segments of the bromeliad among leaf axils that had received  
277 oviposition with those that were randomly selected and were not in use as offspring  
278 rearing site by *R. amazonica*. We used a binomial test to analyze the two-choice tests

279 with paired plastic cups. RDA analysis was performed with CANOCO for Windows  
280 4.5, all other tests were performed in IBM SPSS Statistics 19.

281

## 282 **Results**

283 In the nine study plots on Inselberg, the bromeliad *Pitcairnia geykessi* was the most  
284 abundant over *Catopsis berteroniana* and the uncommon *Aechmea aquilega*. The leaf  
285 axils of *P. geykessi* often contained very small to no phytotelmata and we never found  
286 eggs or tadpoles deposited in *P. geykessi* leaf axils (Figure 3). During the four months  
287 of study in 2002, we found 137 clutches of eggs that were almost exclusively  
288 deposited in leaf axils of *C. berteroniana* that received more oviposition events than  
289 expected by the relative abundance of these leaf axils on the study plots ( $X^2 = 10425.9$ ,  
290  $P < 0.001$ ) (Fig. 3). Of the 106 tadpoles found, half of them were deposited in  
291 phytotelmata of *C. berteroinana* and the other half in *A. aquilega*. Even though *A.*  
292 *aquilega* was absent from many of the study plots and most male territories (Poelman  
293 and Dicke 2008), tadpoles were more frequently deposited in phytotelmata of *A.*  
294 *aquilega* than expected from the low availability of these tadpole deposition sites ( $X^2$   
295  $= 10490.4$ ,  $P < 0.001$ ) (Fig. 3).

296 The two bromeliad species differed widely in their characteristics as identified  
297 with RDA (first PC explained 78%, Fig. 4a). *Aechmea aquilega* is a terrestrial species  
298 that has on average 7 leaf rosettes with over 30 leaf axils per bromeliad. These leaf  
299 axils are deeper, wider and contain eight times as much water compared to the smaller  
300 *C. berteroniana* (80 and 10 ml respectively) (Table 1, Fig. 4a). *Catopsis berteroniana*  
301 is an epiphytic bromeliad that can be found close to the forest floor up to two meters  
302 high in the *Clusia* trees. It has 4-5 leaf rosettes holding about 18 small phytotelmata.  
303 The water temperature and composition of  $K^+$ ,  $NO_3^-$  -  $NO_2^-$  and  $PO_4^{3-}$  was similar for

304 both species. Phytotelmata of *A. aquilega* contained more detritus, but fewer mosquito  
305 or other insect larvae than phytotelmata of *C. berteroniana*.

306 In 2008, we found 24 leaf axils of *C. berteroniana* that contained eggs of *R.*  
307 *amazonica* and 7 that contained a freshly hatched tadpole. As identified from  
308 significant correlation of the bromeliad characteristic in RDA comparison among leaf  
309 axils that received eggs and non-selectively sampled leaf axils, leaf axils that had  
310 received oviposition were smaller and had lower water volume than the average leaf  
311 axil of a *C. berteroniana* plant (first PC explained 92%, Fig. 4b). These leaf axils  
312 were generally in the outer rosettes of bromeliads that were growing close to the  
313 forest floor. Moreover, egg clutches were found to be deposited more frequently in  
314 leaf axils that were directed to the geographic south than our random set of leaf axils  
315 of *C. berteroniana* (Mann Whitney U-test,  $Z = -2.546$ ,  $P = 0.011$ ) (Fig. 5). In RDA of  
316 a comparison among random leaf axils and those of *A. aquilega* that had received  
317 tadpole deposition none of the leaf axil characteristics correlated significantly with the  
318 Principle Components (PC).

319 Because *A. aquilega* was preferentially used for deposition of tadpoles and  
320 phytotelmata of this bromeliad are typically holding larger phytotelmata that are  
321 richer of detritus than leaf axils of *C. berteroniana*, we studied these two  
322 characteristics in tadpole deposition preference tests. The paired cups consisting of  
323 one holding a small water volume (10ml) and one with a large water volume (50ml)  
324 received only five tadpole depositions that were divided with 2 and 3 over the  
325 treatments. These observations were too few for statistical analysis, and give no  
326 indication on a preference for water volume of phytotelmata by *R. amazonica*. The  
327 pairs of cups that had similar water volume but to which detritus was added in one  
328 cup received 14 tadpoles, summed for the three study sites. *Ranitomeya amazonica*

329 males preferentially deposited their tadpoles in cups that had received detritus  
330 (Binomial test,  $P = 0.013$ ) (Fig. 6).

331

### 332 **Discussion**

333 Amazon poison frogs use different characteristics of leaf axils to assess their  
334 suitability as deposition site for eggs or tadpoles. The differences among quality  
335 characteristics of deposition sites were strongest among bromeliad species largely  
336 partitioning egg and tadpole deposition over bromeliad species. However, males were  
337 also selective about the leaf axils within a bromeliad species that they used for egg or  
338 tadpole deposition. Eggs were deposited in small, resource limited water bodies that  
339 were close to the forest floor. Tadpoles were deposited in leaf axils holding  
340 phytotelmata that are resource rich and have larger water volume.

341       Only few poison frog species use phytotelmata for both egg and tadpole  
342 deposition. Most of the other poison frogs lay their eggs in leaf litter on the forest  
343 floor and eggs are kept moist by one of the parents (Summers et al 1999). Terrestrial  
344 egg deposition has been hypothesized to be an escape to the many aquatic predators as  
345 compared to a lower predation risk of eggs on land (Wells 1981). The use of the  
346 smallest phytotelmata for egg deposition by *R. amazonica* in our study and avoidance  
347 of insect larvae, may suggest that also this species selects egg deposition sites that  
348 have low predation risk. Since eggs are partly submerged below the water line in  
349 phytotelmata, significant mortality may be caused by aquatic predators that include  
350 conspecific tadpoles (Summers and Amos 1997; Poelman and Dicke 2007). The  
351 presence of conspecific tadpoles in many of the phytotelmata of the terrestrial  
352 bromeliad *A. aquilega* may for the same reason explain why these leaf axils were not  
353 used for oviposition. In contrast to a preference for egg deposition in bromeliads on

354 the forest floor by *R. amazonica*, the sister species *Ranitomeya variabilis* was found to  
355 select phytotelmata that were located two meters above the forest floor. Schulte et al  
356 (2010) hypothesized that this may be a strategy to avoid the many predators on the  
357 forest floor. Since eggs of *R. variabilis* are deposited above the water line of the  
358 phytotelm (Brown et al 2008a,b), terrestrial predation may be selecting stronger on  
359 oviposition site selection than aquatic predators in this species. Interestingly, we  
360 found eggs to be deposited primarily in leaf axils that were directed towards the  
361 geographic south. Although these leaf axils were also containing a smaller volume of  
362 the phytotelm than the average leaf axil in *C. berteroniana*, we noted that this is  
363 confounded with a more horizontal position of the leaf axil. Most of the *C.*  
364 *berteroniana* bromeliads were tilted so that their southern half was more horizontal  
365 and leaves on the northern half were standing more vertically. We speculate that the  
366 choice for phytotelmata on the southern half of the bromeliad is due to the preference  
367 of horizontal surfaces for oviposition and courtship. Space requirements for egg  
368 deposition were found to explain oviposition preference in *R. sirensis* (*R. biolat* in von  
369 May et al 2009b) that uses bamboo internodes as egg deposition site. Here eggs were  
370 deposited in internodes that provided sufficient space above the water line of the  
371 phytotelm to attach eggs to the bamboo so that they would not run the risk of sliding  
372 into the phytotelm (von May et al 2009b).

373 In contrast to eggs, tadpoles were found in larger water bodies that were rich  
374 in detritus on which the tadpoles feed. Phytotelmata of *A. aquilega* were offering  
375 larger water volume and contained higher abundance of detritus than phytotelmata in  
376 *C. berteroniana* and therefore *A. aquilega* may have been preferred for tadpole  
377 deposition. Although phytotelm volume and detritus abundance were confounding  
378 factors explaining the tadpole deposition preference in *A. aquilega*, our choice tests



379 revealed that *R. amazonica* males select phytotelmata on the abundance of detritus, i.e.  
380 select on phytotelmata that offer food to their offspring (compare von May 2009b).  
381 Nevertheless, water volume may still co-account for preference of tadpole deposition  
382 in *A. aquilega*, since our choice test for different water volumes received too few  
383 depositions to rule out this parameter. Before releasing the tadpole off their back,  
384 males were observed to repeatedly dive into the phytotelm. Potentially, males may  
385 visually explore the phytotelm for presence of predators or food, but may also use  
386 taste or smell to assess the quality of the phytotelm (Schulte et al 2011). Water  
387 exposed to conspecific tadpoles was found to be avoided for egg and tadpole  
388 deposition by *R. variabilis*, confirming that chemical cues in water may be used to  
389 assess phytotelm quality (Schulte et al 2011, Schulte and Lötters 2013). The use of  
390 chemical cues to assess the quality of reproductive sites is well known for insects. For  
391 example, several aquatic insects use both odours and contact with water to assess  
392 presence of conspecifics, predators or nutrients (Fincke 1994; Silberbush and  
393 Blaustein 2008). Also terrestrial insects rely on both odour profiles and taste of their  
394 food plant to assess the content of for example defensive chemistry of the plant and  
395 therefore suitability as food plant for their offspring (Mayhew 1997; Schoonhoven et  
396 al 2005).

397         As in insects there is only a single phase in which females decide to which  
398 reproductive site her offspring will be confined by oviposition, parental care in poison  
399 frogs includes relocation of tadpoles after they hatch from their eggs and offers  
400 parents an opportunity to partition eggs and tadpoles over reproductive sites. Here we  
401 show that parents have distinct preferences for egg and tadpole deposition sites and  
402 can make this distinction because of tadpole transport on the back of the parent.  
403 However, preferred tadpole sites were absent from many of the male territories in our

404 study. Males were observed to visit these preferred tadpole deposition sites in  
405 territories of other males (Summers and Amos 1997; Poelman and Dicke 2008). This  
406 behaviour has resulted in an array of reproductive strategies that infer costs to the  
407 reproductive success of other males and may result in sexual conflicts among parents  
408 of the tadpole. Tadpole carrying males that intrude other territories, may have the  
409 opportunity to deposit their tadpoles in phytotelmata containing eggs sired by an  
410 unrelated male (Brown et al 2009). Deposition of tadpoles in egg containing  
411 phytotelmata may be beneficial for the male siring the tadpole, as the eggs provide the  
412 tadpoles with a high quality food source. Males that pirate on reproductive sites of  
413 other males, reduce the reproductive success of other breeding pairs (Summers and  
414 Amos 1997; Poelman and Dicke 2007; Brown et al 2009). Such deposition strategies  
415 may however also result in sexual conflict when males deposit tadpoles in  
416 phytotelmata that contain eggs or tadpoles they sire, but are offspring of another  
417 female (Poelman and Dicke 2007; Schulte and Lötters 2013). These food provisioning  
418 strategies may be beneficial to females when some of her eggs or tadpoles are  
419 provided to her own offspring and enhance survival chances of the provisioned  
420 tadpole (Poelman and Dicke 2007; Schulte and Lötters 2013). To ensure provisioning  
421 only to her own offspring, females may be selected for involvement in parental care.

422 In addition to the ecological parameter of reproductive sites such as water  
423 volume or food availability that may directly drive transitions in parental care (Brown  
424 et al 2010), the distribution of reproductive sites with preferred quality characteristics  
425 may indirectly contribute to selection on parental care by sexual conflicts involved in  
426 reproductive strategies to exploit reproductive sites. Such transitions may be enhanced  
427 by co-existence of several species of poison frogs that use phytotelmata for  
428 reproduction and in which at least one of the species has predatory tadpoles. To avoid

429 predation by other poison frog tadpoles, poison frog species with less competitive  
430 larvae may colonize (smaller) phytotelmata that are not used for tadpole deposition by  
431 species with predaceous tadpoles, i.e. niche partitioning (Brown et al 2008a,b; Ryan  
432 and Barry 2011). Selection of smaller phytotelmata to avoid predation, coincides with  
433 reduced resource availability and potentially increased risk of desiccation (Summers  
434 and McKeon 2004). Through plastic reproductive strategies that include provisioning  
435 of offspring with either eggs (Poelman and Dicke 2007) or young tadpoles (Schulte  
436 and Lötters 2013), for example driven by seasonal changes is phytotelm stability,  
437 females may have been selected for involvement in parental care. This dynamic  
438 interplay between ecology and behavioral plasticity may have driven transitions in the  
439 mode of parental care by poison frogs and explain the association of species with  
440 female involvement in parental care with small phytotelmata as reproductive sites.  
441 Simple ecological parameters may therefore drive evolution of parental care (Brown  
442 et al 2010) and these insights from studies on poison frogs should encourage to  
443 explore potential ecological parameters that underlie evolution of parental care in  
444 other organisms.

445

#### 446 **Acknowledgements**

447 We thank CNRS for permission to conduct our study at Nouragues Nature Reserve;  
448 Joep Moonen for research permission at Emerald Jungle Village. Schure-Beijerinck-  
449 Popping Fonds for financial support to EHP.

450

#### 451 **References**

452

453 Bechter R, Lescure J (1982) *Dendrobates quinquevittatus*, Fortpflanzungsverhalten  
454 im Terrarium und Vielgestaltigkeit der Art (Teil 1). Herpetofauna 4:26–30  
455

456 Born MG, Gaucher P (2001) Amphibian and reptile species at the Nouragues Nature  
457 Reserve. In: Bongers F, Charles-Dominique P, Forget PM, Théry M (eds) Nouragues  
458 Dynamics and Plant-Animal Interactions in a Neotropical Rainforest. Kluwer  
459 Academic Publisher, Dordrecht, The Netherlands, pp. 371–379  
460

461 Brown JL, Morales V, Summers K (2008a) Divergence in parental care, habitat  
462 selection and larval life history between two species of Peruvian poison frogs: An  
463 experimental analysis. J Evol Biol 21:1534-1543  
464

465 Brown JL, Twomey EM, Morales V, Summers K (2008b) Phytotelm size in relation  
466 to parental care and mating strategies in two species of Peruvian poison frogs.  
467 Behaviour 145: 1139-1165  
468

469 Brown JL, Morales V, Summers K (2009) Tactical reproductive parasitism via larval  
470 cannibalism in Peruvian poison frogs. Biol Letters 5:148-151  
471

472 Brown JL, Morales V, Summers K (2010) A key ecological trait drove the evolution  
473 of biparental care and monogamy in an amphibian. Am Nat 175:436-446  
474

475 Brown JL, Twomey E, Amézquita A, Barbosa de Souza M, Caldwell JP, Lötters S,  
476 von May R, Melo-Sampaio PR, Mejía-Vargas D, Perez-Peña P, Pepper M, Poelman  
477 EH, Sanchez-Rodriguez M, Summers K (2011) A taxonomic revision of the

478 neotropical poison frog genus *Ranitomeya* (Amphibia: Dendrobatidae). Zootaxa  
479 3083:1–120  
480  
481 Caldwell JP (1997) Pair bonding in spotted poison frogs. Nature 385:211  
482  
483 Caldwell JP, de Oliveira VRL (1999) Determinants of biparental care in the spotted  
484 poison frog, *Dendrobates vanzolinii* (Anura: Dendrobatidae). Copeia 1999:565-575  
485  
486 Clutton-Brock TH (1991) The evolution of parental care. Princeton University Press,  
487 Princeton  
488  
489 Crump ML (1991) Choice of oviposition site and egg load assessment by a treefrog.  
490 Herpetologica 47:308-315  
491  
492 Dawkins R, Carlisle TR (1976) Parental investment, mate desertion and a fallacy.  
493 Nature 262:131-133  
494  
495 Downie JR, Livingstone SR, Cormack JR (2001) Selection of tadpole deposition sites  
496 by male Trinidadian stream frogs, *Mannophryne trinitatis* (Dendrobatidae): an  
497 example of anti predator behavior. Herpetol J 11:91–100  
498  
499 Ekbom B, Popov SYA (2004) Host plant affects pollen beetle (*Meligethes aeneus*)  
500 egg size. Physiol Entomol 29:118–122  
501

502 Fincke OM (1994) Population regulation of a tropical damselfly in the larval stage by  
503 food limitation, cannibalism, intraguild predation and habitat drying. *Oecologia*  
504 100:118–127  
505  
506 Godfray HCJ (1994) *Parasitoids: behavior and evolutionary ecology*. Princeton  
507 University Press, Princeton  
508  
509 Grant T, Frost DR, Caldwell JP, Gagliardo R, Haddad CFB, Kok PJR,  
510 Means BD, Noonan BP, Schargel W, Wheeler WC (2006) Phylogenetic systematics  
511 of dart-poison frogs and their relatives (Anura: Athesphatanura: Dendrobatidae). *B*  
512 *Am Mus Nat Hist* 299:1-262  
513  
514 Grimaldi M, Riéra B (2001) Geography and climate. In: Bongers F, Charles-  
515 Dominique P, Forget PM, Théry M (eds) *Nouragues Dynamics*  
516 *and Plant-Animal Interactions in a Neotropical Rainforest*. Kluwer Academic  
517 Publisher, Dordrecht, The Netherlands, pp. 9–18  
518  
519 Harvey JA, Poelman EH, Tanaka T (2013) Intrinsic inter- and intra-specific  
520 competition in parasitoid wasps. *Annu Rev Entomol* 58:333–351  
521  
522 Johnson LS, Brubaker JL, Ostlind E, Balenger SL (2007) Effect of altitude on male  
523 parental expenditure in mountain bluebirds (*Sialia currucoides*): are higher altitude  
524 males more attentive fathers? *J Ornitol* 148:9-16  
525

526 Kitching RL (2000) Foodwebs and container habitats: the natural history and ecology  
527 of phytotelmata. Cambridge University Press, Cambridge  
528

529 Kok P JR, Macculloch RD, Gaucher P, Poelman EH, Bourne GR, Lathrop A, Lenglet  
530 GL (2006) A new species of *Colostethus* (Anura, Dendrobatidae) from French Guiana  
531 with redescription of *Colostethus beebei* (Noble, 1932) from its type locality.  
532 *Phyllomedusa* 5:43–66  
533

534 Lehtinen RM (2004) Tests for competition, cannibalism, and priority effects in two  
535 phytotelm dwelling tadpoles from Madagascar. *Herpetologica* 60:1–13  
536

537 Mayhew PJ (1997) Adaptive patterns of host-plant selection by phytophagous insects.  
538 *Oikos* 79:417–428  
539

540 Mock DW, Parker GA (1997) The evolution of sibling rivalry. Oxford University  
541 Press, Oxford  
542

543 Poelman EH, Dicke M (2007) Offering offspring as food to cannibals:  
544 oviposition strategies of Amazonian poison frogs (*Dendrobates ventrimaculatus*).  
545 *Evol Ecol* 21:215-227  
546

547 Poelman EH, Dicke M (2008) Space use of Amazonian poison frogs: testing the  
548 reproductive resource defense hypothesis. *J Herpetol* 42:270-278  
549

550 Pramuk JB, Hiler BI (1999) An investigation of obligate oophagy of *Dendrobates*  
551 *pumilio* tadpoles. Herpetol Rev 30:219–221  
552  
553 Refsnider J, Janzen F (2010) Putting eggs in one basket: ecological and evolutionary  
554 hypotheses for variation in oviposition-site choice. Annu Rev Ecol, Evol Syst 41:39-  
555 57  
556  
557 Ryan MJ, Barry DS (2011) Competitive interactions in phytotelmata - Breeding pools  
558 of two poison-dart frogs (Anura: Dendrobatidae) in Costa Rica. J Herpetol 45:438-  
559 443  
560  
561 Sarthou C (2001) Plant communities on a granitic outcrop. In: Bongers F, Charles-  
562 Dominique P, Forget PM, Théry M (eds) Nouragues Dynamics and Plant-Animal  
563 Interactions in a Neotropical Rainforest. Kluwer Academic Publisher, Dordrecht, The  
564 Netherlands, pp 65-78  
565  
566 Schoonhoven LM, van Loon JJA, Dicke M (2005) Insect–Plant Biology. Oxford,  
567 Oxford University Press  
568  
569 Schulte LM, Rödder D, Schulte R, Lötters S (2010) Preference and competition for  
570 breeding plants in coexisting *Ranitomeya* species (Dendrobatidae): does height play a  
571 role? Salamandra 46:180-184.  
572



573 Schulte LM, Yeager J, Schulte R, Veith M, Werner P, Beck LA, Lötters S (2011) The  
574 smell of success: Choice of larval rearing sites by means of chemical cues in a  
575 Peruvian poison frog. *Anim Behav* 81:1147-1154  
576

577 Schulte LM, Lötters S (2013) The power of the seasons: rainfall triggers parental care  
578 in poison frogs. *Evol Ecol* in press  
579

580 Shreve B (1935) On a new Teiid and Amphibia from Panama, Ecuador, and Paraguay.  
581 *Occ Pap Bost Soc Nat Hist* 8:209–218  
582

583 Silberbush A, Blaustein L (2008) Oviposition habitat selection by a mosquito in  
584 response to a predator: Are predator-released kairomones air-borne cues? *J Vector*  
585 *Ecol* 33: 208-211  
586

587 Summers K, Amos W (1997) Behavioral, ecological and molecular genetic  
588 analysis of reproductive strategies in the Amazonian dart-poison frogs, *Dendrobates*  
589 *ventrimaculatus*. *Behav Ecol* 8:260-267  
590

591 Summers K, Weight L, Boag P, Bermingham E (1999) The evolution of female  
592 parental care in poison frogs of the genus *Dendrobates*: evidence from mitochondrial  
593 DNA sequences. *Herpetologica* 55:254-270  
594

595 Summers K, Symula R (2001) Cannibalism and kin discrimination in tadpoles  
596 of the Amazonian poison frog, *Dendrobates ventrimaculatus*, in the field. *Herpetol J*  
597 11:17-21

598

599 Summers K, McKeon CS (2004) The evolutionary ecology of phytotelmata use  
600 in neotropical poison frogs. In: Lehtinen RM (ed) Ecology and Evolution of  
601 Phytotelm-breeding Anurans. Miscellaneous publications of the Museum of Zoology  
602 University of Michigan 193:55-73

603

604 Trivers RL (1972) Parental investment and sexual selection. In: Campbell B (ed)  
605 Sexual selection and the descent of man. Aldine, Chicago, pp 139-179

606

607 Von May R, Reider KE, Summers K (2009a) Effect of body size on intraguild  
608 predation between tadpoles of bamboo-breeding poison frogs and predaceous  
609 mosquito larvae. J Freshwater Ecol 24:431-435

610

611 Von May R, Medina-Müller M, Donnelly MA, Summers K (2009b) Breeding-site  
612 selection by the poison frog *Ranitomeya biolat* in amazonian bamboo forests: An  
613 experimental approach. Can J Zool 87:453-463

614

615 Wells KD (1981) Parental behavior of male and female frogs. In: Alexander RD,  
616 Tinkle DW (eds) Natural selection and social behavior. Chiron Press, New York, pp  
617 184–198

618

619 West-Eberhard MJ (2003) Developmental plasticity and Evolution. Oxford University  
620 Press, New York

621

622 Weygoldt P (1980) Complex brood care and reproductive behavior in captive poison-  
623 arrow frogs, *Dendrobates pumilio* O. Schmidt. Behav Ecol Sociobiol 7:329–332

624 **Figure legends**

625

626 **Fig. 1:** A male *Ranitomeya amazonica* at Inselberg in the Nouragues nature reserve

627

628 **Fig. 2:** Top view of a *Catopsis berteroniana* bromeliad showing its leaf axils that hold  
629 phytotelmata and two panels showing the methodological numbering of the position  
630 of a leaf axil. The middle panel depicts the rosette numbering to indicate the position  
631 of the leaf axil towards the central rosette of the bromeliad. The right panel depicts the  
632 geographic orientation of the leaf axil computed from degrees into eight segments of  
633 the 360 degree circle.

634

635 **Fig. 3:** Distribution of eggs and tadpoles over phytotelmata of three bromeliad species,  
636 *Pitcairnia geykessi* (dark grey), *Aechmea aquilega* (black), *Catopsis berteroniana*  
637 (light grey), compared to the availability of these phytotelmata in the study area.

638

639 **Fig. 4:** RDA plots of leaf axel characteristics that differ among bromeliads (A) and  
640 empty control versus phytotelmata used for egg deposition in *Catopsis berteroniana*  
641 (B). (A) Individual points represent single leaf axils and their phytotelm measured for  
642 16 parameters of random axils of *Catopsis* (black circles) and *Aechmea* (green  
643 squares). (B) Individual points represent leaf axils of *Catopsis* that were randomly  
644 selected and not used for egg deposition (black circles) or leaf axils in which eggs  
645 were found (orange squares). Blue arrows in both plots indicate the direction of  
646 increase in value of a parameter and parameters that significantly correlated with one  
647 of the PC axis are identified with their name.

648

649 **Fig. 5:** Orientation distribution of *Catopsis* leaf axils that were used for egg  
650 deposition. Each black dot represents a single case of egg deposition and its position  
651 corresponds with the degrees derived from the north on a 360 degrees circle as well as  
652 the eight segments in which the degree values were computed for statistical analysis.  
653

654 **Fig. 6:** Number of tadpoles deposited in plastic cups containing 50ml of clear water or  
655 water with detritus that were offered in pair wise attached to bromeliads in the field.  
656 Cups with detritus received significantly more tadpoles than cups holding water only  
657 (Binomial test  $P = 0.013$ ).

**Table 1:** Measurements of leaf axil and phytotelm characteristics of control axils and those that had received egg or tadpole deposition by *R. amazonica* in two bromeliad species, *Catopsis berteroniana* and *Aechmea aquilega*. Bold face type indicate axil characteristics that explained variation in the RDA analyses between bromeliad species or axils used for reproduction.

Leaf axil characteristic	<i>Catopsis berteroniana</i>			<i>Aechmea aquilega</i> <sup>(e)</sup>	
	control (n = 40)	egg (n = 24)	tadpole (n = 7)	control (n = 40)	tadpole (n = 17)
<b>Height above forest floor (cm)</b>	<b>45.58 (36.09)</b>	<b>16.58 (20.34)</b>	<b>13.00 (23.13)</b>	<b>0 (0.00)</b>	<b>0 (0.00)</b>
<b>Geographic orientation<sup>(a)</sup></b>	<b>4.25 (2.01)</b>	<b>5.71 (2.44)</b>	<b>6.14 (2.10)</b>	<b>4.45 (2.24)</b>	<b>4.41 (2.14)</b>
<b>Nr. of leaf rosettes</b>	<b>4.55 (0.71)</b>	<b>4.38 (0.82)</b>	<b>4.14 (0.69)</b>	<b>6.75 (1.01)</b>	<b>8.00 (1.12)</b>
Leaf rosette number <sup>(b)</sup>	0.66 (0.19)	0.90 (0.13)	0.84 (0.18)	0.65 (0.19)	0.56 (0.24)
<b>Width (cm)</b>	<b>2.83 (0.64)</b>	<b>2.50 (0.46)</b>	<b>2.66 (0.49)</b>	<b>5.29 (1.76)</b>	<b>5.39 (1.21)</b>
<b>Depth (cm)</b>	<b>9.52 (2.88)</b>	<b>5.95 (2.95)</b>	<b>7.19 (3.96)</b>	<b>14.03 (5.72)</b>	<b>16.52 (5.42)</b>
<b>Water volume (ml)</b>	<b>10.63 (6.57)</b>	<b>4.93 (3.50)</b>	<b>6.43 (3.55)</b>	<b>81.38 (35.68)</b>	<b>85.65 (40.35)</b>
Water temperature (°C)	26.60 (0.88)	25.74 (0.90)	25.57 (1.09)	23.99 (0.70)	24.45 (0.58)
Water Ph	5.42 (0.21)	5.48 (0.28)	5.51 (0.29)	5.41 (0.18)	5.37 (0.13)
K <sup>+</sup> (mol/m <sup>3</sup> )	< 0.5 (0.00)	< 0.5 (0.00)	< 0.5 (0.00)	< 0.5 (0.00)	< 0.5 (0.00)
NO <sub>3</sub> <sup>-</sup> - NO <sub>2</sub> <sup>-</sup> (mg/l)	50 – 10 (0.00)	50 – 10 (0.00)	50 – 10 (0.00)	50 – 10 (0.00)	50 – 10 (0.00)
PO <sub>4</sub> <sup>3-</sup> (mg/l)	2.10 (2.71)	0.67 (1.69)	0.43 (1.13)	3.83 (3.46)	3.71 (2.71)
<b>Amount of detritus<sup>(c)</sup></b>	<b>1.20 (0.85)</b>	<b>1.54 (0.83)</b>	<b>1.29 (0.95)</b>	<b>2.13 (1.28)</b>	<b>2.94 (0.90)</b>
Presence of algae <sup>(d)</sup>	0.30 (0.69)	0.17 (0.48)	0.14 (0.38)	0.35 (0.77)	1.00 (0.79)
Nr. Of insect larvae	0.40 (0.55)	0.17 (0.38)	0.14 (0.38)	0.03 (0.16)	0.00 (0.00)
Nr. of other tadpoles	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.12 (0.33)	0.00 (0.00)

(a) Computed compartment number from circular degree of geographic orientation (Figure 1)

(b) Number of rosette of measured leaf axil divided by the total number of rosettes in the bromeliad (Figure 1)

(c) Scored visually on a scale of 0 to 5

(d) Scored visually on a scale of 0 to 3

(e) Only a single egg clutch was found in a waterless leaf axil of *A. aquilega*

Fig 1





Fig 2

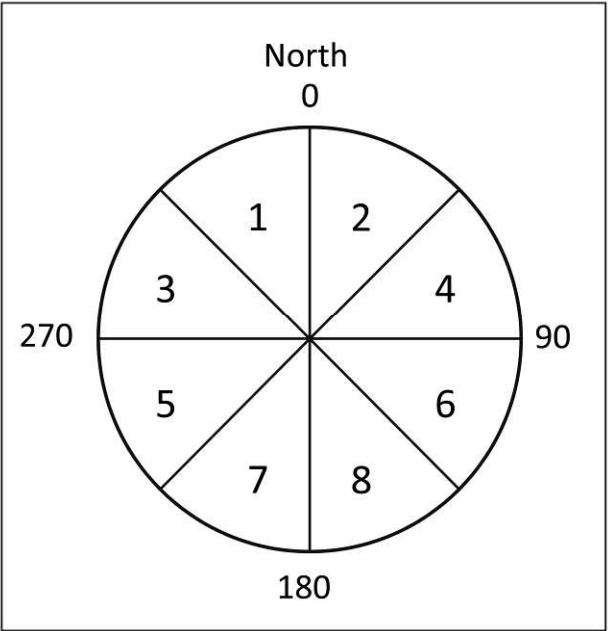
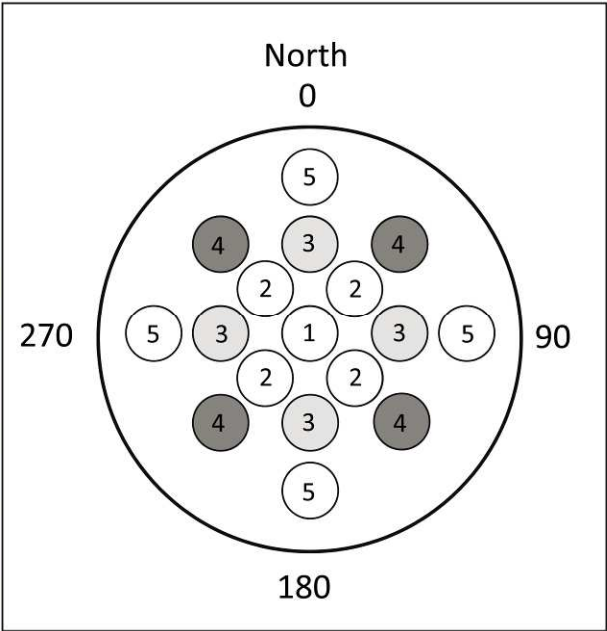


Fig 3

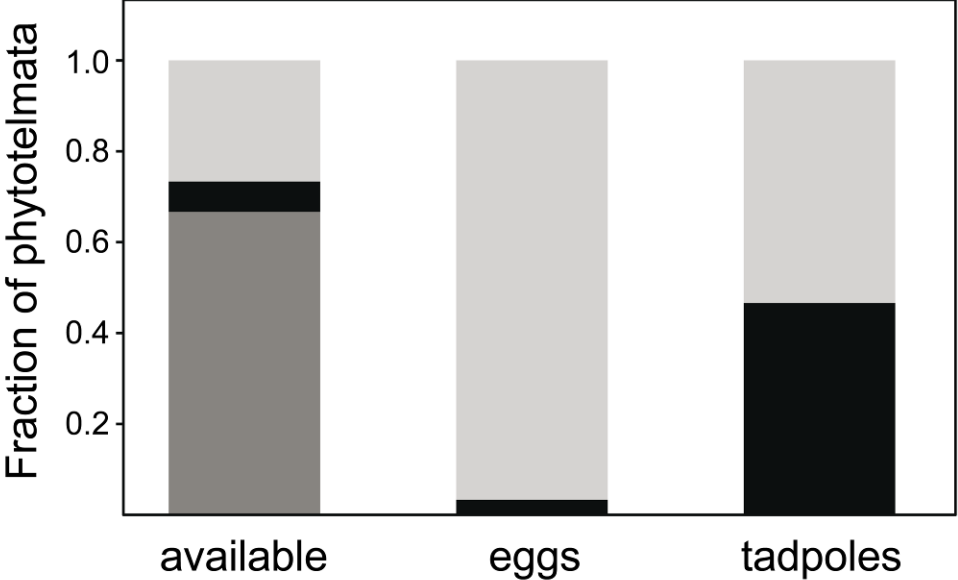


Fig 4

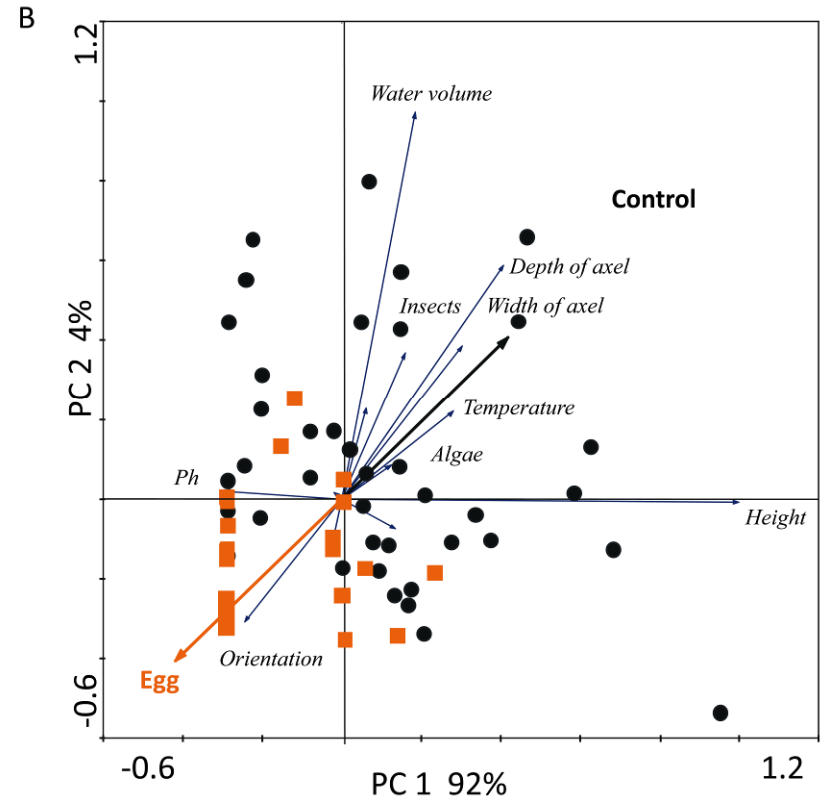
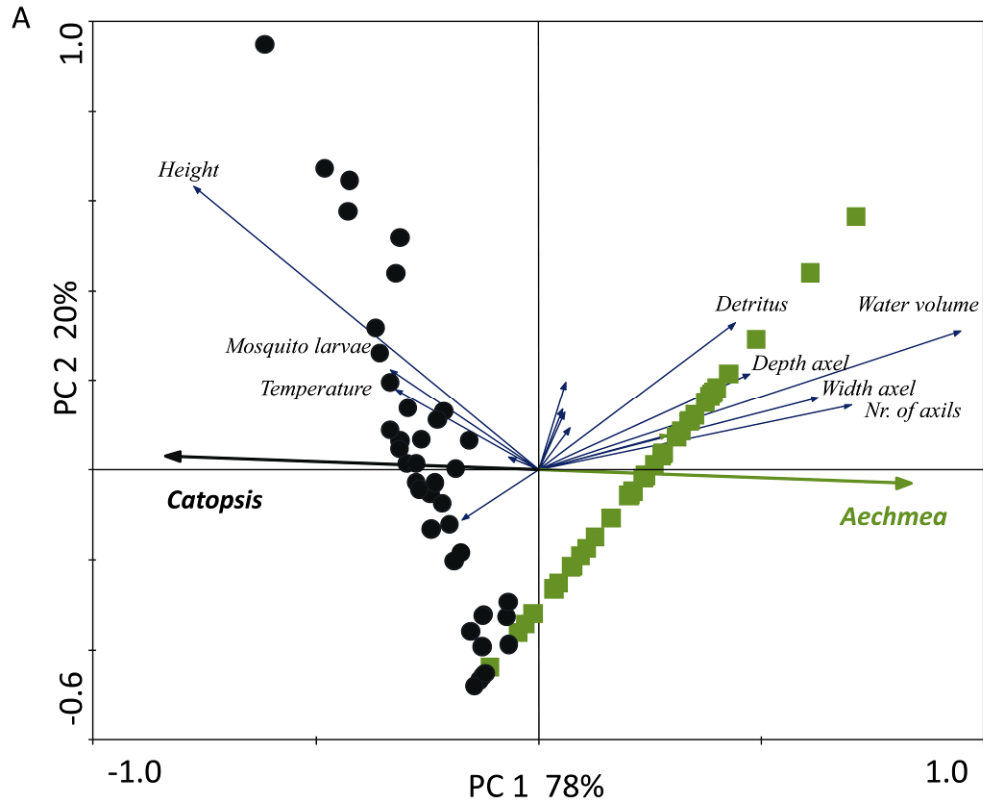


Fig 5

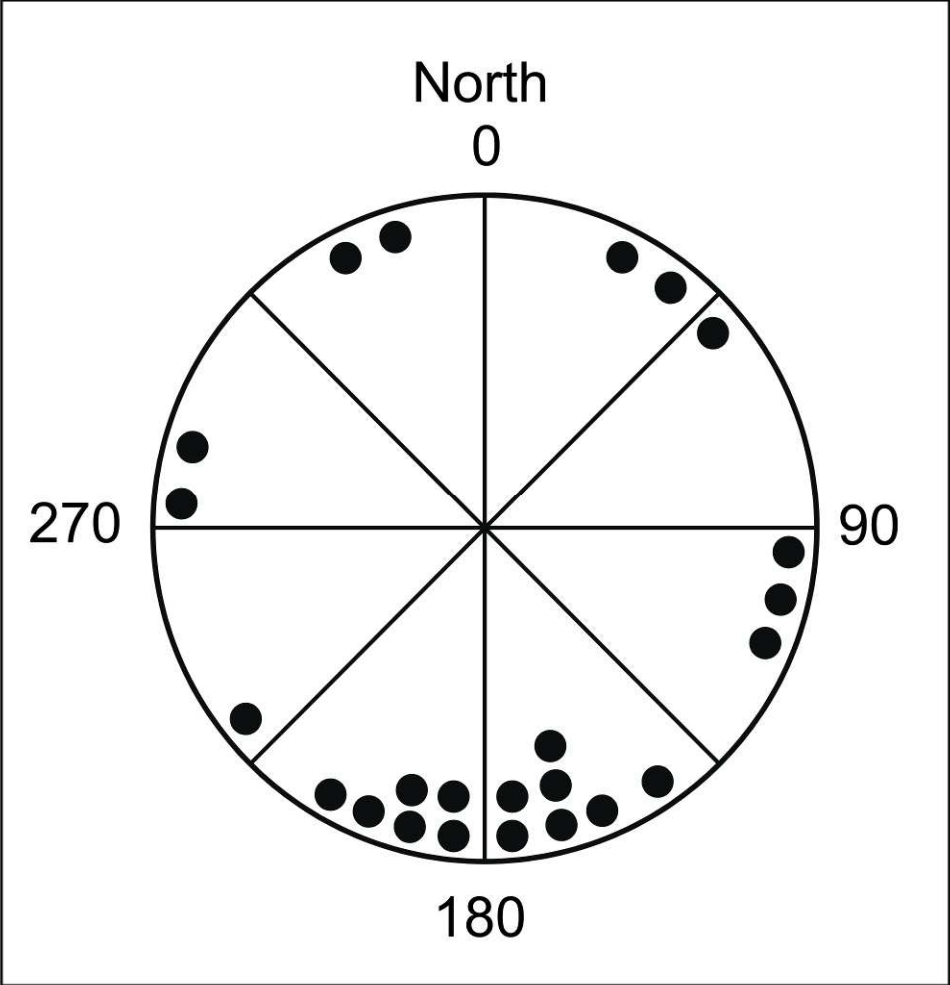


Fig 6

