A comparative study of predator-induced social aggregation of tadpoles in two anuran species from western Madagascar

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In the dry forest of western Madagascar, mixed-species social aggregations of tadpoles are frequent. Two species are often found in one aggregate. We explored the proximate mechanisms leading to the formation of tadpole aggregations that include the two species *Aglyptodactylus securifer* (Mantellidae) and *Dyscophus insularis* (Microhylidae). We show that aggregations are induced by the direct presence of predators, or by indirect chemical cues indicating a predation risk. However, the specific cues that initiated the formation of aggregations differed between the two species. *Aglyptodactylus securifer* reacted to con- and heterospecific tadpole homogenate ("Schreckstoff"). *Dyscophus insularis'* reaction was predator-specific to fish, i.e. directly to fish and indirectly to chemical cues released by fish. Although the ultimate benefit of this behaviour is thought to be to reduce predation, it also has costs. Tadpoles of *A. securifer* in the presence of predatory cues showed reduced growth and retarded development compared to tadpoles in control treatments.

Key words: Aglyptodactylus securifer, Anura, chemical cues, *Dyscophus insularis*, Mantellidae, Microhylidae, predation

INTRODUCTION

Predation is a crucial factor influencing individual fitness, and animals will often react with defensive behaviour to predation threats. In many cases, the predation risk to individuals is thought to be reduced by congregating with conspecific or heterospecific individuals in groups (Siegfried & Underhill, 1975; Diamond, 1981). These groups are termed social when "individuals actively seek the proximity of each other instead of co-occurring in the same spot because of an attraction to the same environmental condition" (the latter is termed non-social aggregation) (Krause and Ruxton, 2002). Studies on different animal species under natural conditions have shown that the formation of aggregations is often positively correlated with the presence of predators (e.g. in fish: Johannes, 1993; hemiptera: Foster & Treherne, 1981; birds: Cresswell, 1994). Aggregative behaviour, however, also involves costs for the socializing individuals. Animals in groups may have reduced food intake and consequently reduced growth and/or slowed development compared to solitarily foraging animals (Krause & Ruxton, 2002). Additionally, they might have increased parasite burdens and are at increased risk of other communicable disease (e.g. Brown & Brown, 1986).

Alarm cues that are used to detect predators and subsequently initiate predator avoidance behaviour include visual detection, alarm calls by con- and heterospecifics (e.g. in birds: Elgar et al., 1984; in primates: Wolters & Zuberbühler, 2003), substrate vibrations caused by the predator (e.g. in spiders: Hodge & Uetz, 1993) and chemicals released by predator or prey (reviewed by Kats & Dill, 1998).

In tadpoles, the proximate mechanisms leading to aggregative behaviour are not well studied. The visual detection of predators (e.g. Rödel & Linsenmair, 1997), mechanical stimuli (e.g. Spieler & Linsenmair, 1999) and alarm substances originating from the body fluid of injured conspecific or heterospecific tadpoles (e.g. Hokit & Blaustein, 1995; Rödel & Linsenmair, 1997), or a combination of two or more such stimuli, appear to account for this reaction (Spieler & Linsenmair, 1999).

In the dry forest of western Madagascar, temporary associations of tadpoles are frequent (Glos et al., 2007). The two most abundant species in these aggregations are *Aglyptodactylus securifer* (Mantellidae) and *Dyscophus insularis* (Microhylidae). The tadpoles of these species are very different with respect to feeding ecology and microhabitat choice (Glaw & Vences, 2007; Glos & Linsenmair, 2004). Nevertheless they form mixed-species aggregations. Here we examined the effects of predation risk on aggregative behaviour, activity and microhabitat choice in tadpoles of these two species. We experimentally assessed the proximate cues used by tadpoles to detect and react to predators, and we analysed the costs for growth and development of behavioural reactions to non-lethal predator presence in *A. securifer*.

MATERIALS AND METHODS

Study site

The Kirindy/CFPF Forest, a deciduous dry forest, is situated near the west coast of Madagascar, 60 km north of Morondava and about 20 km inland (44°39'E, 20°03'S; 18–40 m a.s.l.; Sorg & Rohner, 1996). The climate is characterized by a marked seasonality. Almost all rain falls in the austral summer from November to March, followed by seven months of virtually no precipitation (annual mean rainfall: 800 mm; Sorg & Rohner, 1996).

There are two types of breeding sites in the Kirindy Forest, pools in the riverbed and pools in the forest. The first waters that are used by amphibians for spawning

usually arise in the rocky parts of the Kirindy riverbed. As a rule, the density of invertebrate predators in these pools is low at this early stage. During the course of the rainy season, these pools eventually become connected with each other and fish immigrate from the few permanent pools in the riverbed, resulting in a very high predation risk for amphibian larvae. The first breeding ponds in the closed forest usually fill in December. There are no fish in the forest ponds throughout the rainy season. In contrast to the riverbed ponds, the density of invertebrate predators (Dytiscidae, Belostomatidae, Anisoptera, Zygoptera) in larger forest ponds (>200 m^2) can be very high (mean 37 m^{-2}; range 7–160 m^{-2}; unpubl. data).

Study species

Aglyptodactylus securifer Glaw, Vences & Böhme 1998 (Mantellidae: Laliostominae).

This frog is limited to a few localities in Madagascar (Glaw & Vences, 2007). As an explosive breeding species it reproduces primarily after the first heavy rains at the beginning of the rainy season. Breeding takes place mainly in rock pools, before the river is running. Tadpoles are benthic and feed primarily on plant detritus, but also on carcasses of con- and heterospecific tadpoles (Glos & Linsenmair, 2004). Their mode of locomotion is characterized by long periods of low activity, followed by short bursts of swimming movement. Together with their brown dorsal coloration this leads to a rather cryptic appearance. Predation pressure on *A. securifer* tadpoles is potentially very high. Waters that contain fish are avoided for breeding. However, strong rainfall may raise the water level in the riverbed, interconnecting ponds already spawned in, and thus enable the immigration of fish. Birds (kingfisher *Alcedo vintsioides*, paradise flycatcher *Terpsiphone mutata*) and turtles (*Pelomedusa subrufa*) are further major predators of tadpoles. Aquatic insects occur only in low densities in these ponds and have a relatively small size at the time of spawning and tadpole development. Invertebrate predators therefore constitute only a relatively low predation risk for *A. securifer* tadpoles.

Dyscophus insularis Grandidier 1872 (Microhylidae: Dyscophinae).

This frog occurs in dry habitats all over western Madagascar (Glaw & Vences, 2007). It is an explosive breeder, reproducing only after heavy rains. However, unlike *A. securifer*, reproduction occurs throughout the whole rainy season, although it peaks at its start. It uses a wide variety of habitats and pond types for reproduction (Glos, 2003). Hence, predation pressure is presumably very variable. The filter feeding *D. insularis* tadpoles mainly swim in mid-water and move almost constantly.

Proximate factors causing aggregations

Experimental setup. Experiments were conducted in December and January of 2000–2001 and 2001–2002. To avoid genetic effects of related siblings, tadpoles of *A. securifer* were taken from eight different clutches that were deposited by eight amplectant couples under controlled conditions. Tadpoles of *D. insularis* in young developmental stages (25–28; Gosner, 1960) were dip-net-

ted from different parts of a fish-free pond to increase the probability of collecting members of different sibships. Tadpoles of both species were raised separately in the field camp in a predator-free environment under natural temperature conditions. They were kept in mixed-sibling groups in stock tanks (55 cm in diameter, 25 cm in depth) and fed *ad libitum* with commercial fish food (TetraMinTabs®).

The aggregation experiments were conducted in green polyethylene arenas (45 × 26 cm), the bottoms of which were subdivided into 18 equal sections (7.5 × 8.7 cm each). The arenas were filled with rain water to a depth of 12 cm. To control for light regime, and to exclude possible effects of rain dropping into the arenas or of ground vibrations emitted by the observer, all arenas were positioned on elevated roofed frames. These frames were 30 cm above the ground, and the roof was at a height of about 3 m. The arenas were covered with wire mesh to prevent disturbance by other animals. All tadpoles were used only once in the experiment. Tadpoles within one species were of similar size and developmental stage. A haphazardly selected sample of *A. securifer* tadpoles at the time of the experiments had a body size of 7.0±0.6 mm (mean±SD; *n*=44) and a developmental stage of 31.0±1.0 (*n*=20); *D. insularis* tadpoles had a body size of 8.4±1.1 mm and a developmental stage of 29.8±3.5 (*n*=90). Tadpoles of these sizes and stages were found aggregating under natural conditions.

The following protocol was used for all experimental trials. Tadpoles were haphazardly selected from the stock tanks to ensure mixing of sibling groups among treatments. Thirty tadpoles were transferred into each test arena. Tadpoles were allowed to acclimatize overnight (>15 hours) to the arenas. Food (one TetraMinTabs® per arena) was added at night and evenly distributed in the arena. The experimental arenas were arranged in a row and randomly assigned to either experimental or control treatment. In ten daily trials, including all treatments and controls, 78 arenas were stocked with *A. securifer* tadpoles and 78 with *D. insularis* tadpoles.

A glass of rain water (200 ml) containing either a live predator or a predator's chemical cue (see below) was added to the experimental arenas at 1100. In the simultaneous control treatments, the same mechanical manipulations were performed by adding a glass of rain water. Measurements were taken by an observer from above, every 20 min over the course of two hours (= six measurements per arena). The mean of these six measurements per arena represents one datum point. The effect of the experimental treatment was analysed by one-way ANOVA and Dunnet *t*-test post hoc comparisons. To acquire information on the persistence time of the aggregations, we continued the measurements at 20 min intervals for two more hours. After each experiment, all used tadpoles were released into their natural habitats.

Response variables

Five parameters were measured during the experiments: first, we recorded the *presence of aggregations* in the arena (0 = absence, 1 = presence). Aggregations were

Table 1. Effects of predatory treatments on response variables in tadpoles of a) *Aglyptodactylus securifer* and b) *Dyscophus insularis* (mean±SD). Aggregation index = number of tadpoles in that square of the arena with the highest number of tadpoles; aggregation presence = % of control checks with aggregations; aggregation size = number of tadpoles per aggregation; swimming activity = crossing of the lateral axis in the arena / h * animal; microhabitat choice = vertical position in the water column (b = bottom, c = centre, s = surface); survival = number of tadpoles present at the end of the experiment. ANOVA with Dunnet *t*-test *post hoc* comparisons (shown are *P* values); microhabitat choice was tested with χ^2 goodness of fit tests; significant results are highlighted in italics. *n*=number of tested arenas including controls. "–" = not measured.

a) *A. securifer*

	ANOVA	Control	Fish	Fish water	A. securifer-homogenate	D. insularis-homogenate	Diving beetle	Turtle	Kingfisher
Aggregation index	*P<0.001* *F=9.67, n=77*	7.59±1.81	9.42±5.40 / 0.91	7.89±1.81 / 1.00	*16.53±5.87* / *<0.001*	10.79±4.89 / 0.14	6.32±1.65 / 0.92	7.43±1.26 / 1.00	*13.16±4.47* / *<0.01*
Aggregation presence	*P<0.001* *F=13.49, n=77*	10.6±19.5	12.5±15.8 / 1.00	4.6±7.9 / 1.00	*79.6±37.1* / *<0.001*	*38.9±40.8* / *<0.05*	10.0±14.0 / 1.00	18.4±19.3 / 0.93	*81.7±36.6* / *<0.001*
Aggregation size	*P=0.126* *F=2.32, n=39*	6.16±3.65 ANOVA n.s.	5.25±1.77	4.15±0.25	8.09±4.09	5.68±2.15	3.96±0.13	5.27±0.84	10.30±4.18
Swimming activity	*P=0.02* *F=2.54, n=77*	27.48±20.60	21.43±7.74 / 1.00	24.36±13.88 / 1.00	14.23±15.55 / 0.21	18.51±7.25 / 0.63	18.45±13.06 / 0.59	20.36±18.16 / 0.86	*0.79±1.42* / *0.001*
Microhabitat choice (%)		b 56.80 c 34.40 s 8.80	b 75.43 c 22.63 s 1.93	b 45.87 c 43.73 s 10.40	b 67.70 c 30.23 s 2.10	b 55.07 c 38.97 s 6.00	b 72.17 c 20.23 s 7.60	b 79.83 c 19.07 s 1.10	*b 96.77* *c 2.60* *s 0.67*
			>0.05 χ^2=2.84, df=2	>0.05 χ^2=0.72, df=2	>0.05 χ^2=1.65, df=2	>0.05 χ^2=0.28, df=2	>0.05 χ^2=1.70, df=2	>0.05 χ^2=0.94, df=2	*<0.01* χ^2=13.44, df=2
Survival		29.61±0.77	29.00±1.41	28.89±2.09	29.86±0.38	29.89±0.33	29.70±0.82	23.25±5.60	23.00±4.00

b) *D. insularis*

	ANOVA	Control	Fish	Fish water	A. securifer-homogenate	D. insularis-homogenate	Diving beetle	Water bug	Turtle
Aggregation index	*P<0.001* *F=15.17, n=78*	6.01±1.09	*10.60±1.98* / *<0.001*	6.24±0.87 / 0.99	6.87±0.56 / 0.41	5.56±1.36 / 0.92	6.51±0.76 / 0.88	5.75±0.59 / 0.78	6.16±1.24 / 0.99
Aggregation presence	*P<0.001* *F=10.60, n=64*	32.0±31.1	*97.2±6.9* / *<0.001*	*68.9±37.8* / *0.01*	0.0±0.0 / 0.06	42.9±34.3 / 0.91	–	19.3±16.2 / 0.86	6.9±12.4 / 0.17
Aggregation size	*P<0.001* *F=9.66, n=33*	5.84±1.51	*11.06±1.98* / *<0.000*	*8.57±2.74* / *0.02*	–	6.49±0.99 / 0.91	–	–	6.25±1.77 / 0.99
Swimming activity	*P=0.07* *F=2.38, n=40*	19.57±9.20 ANOVA n.s.	–	11.86±15.30	28.50±15.39	14.34±11.84	–	–	11.86±7.30
Microhabitat choice (%)		b 30.33 c 35.20 s 34.47	–	b 56.67 c 11.67 s 31.67	b 43.77 c 32.77 s 23.47	b 40.67 c 52.67 s 6.67	–	–	*b 72.03* *c 21.63* *s 6.33*
			–	>0.05 χ^2=5.98, df=2	>0.05 χ^2=1.41, df=2	*<0.05* χ^2=7.13, df=2	–	–	*<0.01* χ^2=11.88, df=2
Survival		28.75±2.40	26.67±3.08	30.00±0.00	30.00±0.00	30.00±0.00	29.29±1.11	24.33±6.71	22.25±4.69

defined as at least four individuals being not more than 2 cm apart from each other. This distance between individuals reflects the natural situation in the field, and was based on field studies on natural aggregations in these species (Glos et al., 2007) and earlier studies on tadpole aggregations (O'Hara & Blaustein, 1985). If there was an aggregation present, the number of tadpoles per aggregation was counted (*aggregation size*). The third parameter, the *aggregation index*, is a quantitative measure of aggregative behaviour, defined as the number of tadpoles in that section of the arena (of 18 sections) with the highest number of tadpoles. Fourth, we measured *swimming activity* of the tadpoles. For that, we recorded the number of tadpoles crossing the centre line of the arena within a 10 sec period. Finally, we recorded microhabitat choice of all tadpoles as the vertical position in the water column. We distinguished bottom (0–3 cm), middle (3–9 cm) and surface (9–12 cm) microhabitat. For practical reasons, not all parameters were measured for all predators or predatory cues ("–" = not measured in Table 1). At the end of the experiment, we counted the number of tadpoles in each arena. This number was used to determine *survival*. As the predators were free to move within the arenas and to prey on the tadpoles this number regularly dropped below 30 in the experimental treatments. We corrected the variables *aggregation index*, *aggregation size*, *swimming activity* and *microhabitat choice* according to the number of tadpoles that were present in the arena during the actual observation.

Predatory cues. For both tadpole species, we tested predators that represent different taxa and predation modes: 1) juvenile fish (*Oreochromis* sp., Cichlidae; mean body length 36.2 mm ± 4.1 SD; $n=6$); 2) young turtles (*Pelomedusa subrufa*; carapax length 68.7±17.3 mm; $n=9$); 3) adult diving beetles (Dytiscidae; body length 27.8±1.4 mm, $n=10$). For *A. securifer* tadpoles only, 4) Malagasy kingfishers (*Alcedo vintsioides*, $n=7$) were tested. This was done by allowing these naturally occurring birds to prey in the arenas by removing the wire mesh cover that kept them from doing so otherwise. Generally, the birds sat on the edge of the arena about 20 cm above water level, and eventually flew up and foraged on tadpoles in the arena. For *D. insularis* only, 5) giant water bugs (*Lethocerus* sp., Belostomatidae, body length 72.0±10.5 mm; $n=6$) were tested. All these predators are present in the tadpoles' natural habitats. All predators were allowed to move freely in the arenas and prey on the tadpoles. Individual predators were used only once. Only kingfisher predators might have been the same individuals in two or more replicates.

Additionally, (6, 7) we tested in both species the reaction to homogenate of both conspecific and heterospecific tadpoles (*A. securifer* and *D. insularis*, respectively). To this end, one tadpole per arena was homogenized using a surgical blade and suspended in 200 ml of rain water. To test for the reaction to the chemical stimulus of fish, 8) "fish water" was added. Fish water was standardized: for each replicate, we kept one different juvenile fish (*Oreochromis* sp.; total length about 40 mm each) for 24 h in 5 l of fresh rain water. Subsequently, one glass (200 ml) of the water was taken for the experiments.
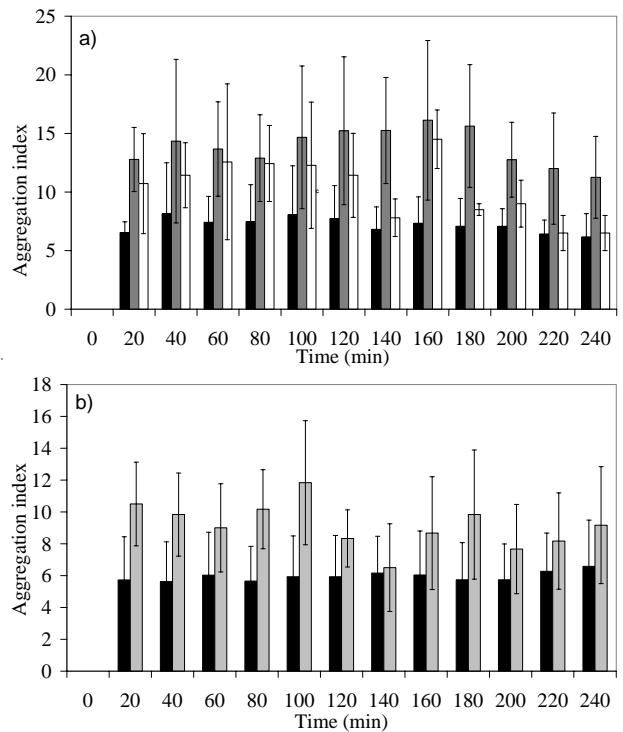

Fig. 1. Reaction of a) *A. securifer* and b) *D. insularis* tadpoles to the experimental treatment over time. The aggregation index (mean ±SD) for the control treatment (black bars) is significantly lower than for the predatory treatments a) *A. securifer*-homogenate (grey bars) and kingfisher (white bars) for *A. securifer* tadpoles, and b) fish (hatched bars) for *D. insularis* tadpoles. For definitions of variables, see Table 1. Predator or predator stimulus was introduced at 0 min.

Costs of behavioural reactions

Experimental setup. Experiments were conducted on *A. securifer* during the rainy season 2001–2002. Tadpoles from eight different clutches were pooled and raised in stock tanks (55 cm in diameter, 25 cm in depth) filled with rain water and fed *ad libitum* with commercial fish food (TetraMinTabs®). Tadpoles of similar size and developmental stage were haphazardly collected from these tanks. The experiments were conducted in circular arenas (diameter 40 cm, volume 15 l, water depth 12 cm). A line on the bottom divided each arena into halves. Environmental conditions were similar to those described above. Twenty tadpoles were kept in each arena for 12 days (density 1.3 tadpoles/l). One arena represents one replicate (treatment: $n=8$, control: $n=8$).

The following protocol was used. To induce aggregations, *A. securifer*-homogenate (one tadpole per basin; for extraction see previous experiment) suspended in a glass of rain water was added daily at 0900 to the experimental treatments. A glass of rain water was added to the control treatments. Tadpoles were fed with one TetraMinTab® per day, which was evenly distributed at 0700 in each arena. To reduce handling effects, we did not take measurements on these tadpoles before the experiment.

Independently of this, we haphazardly selected a representative sample of 30 tadpoles from the stock tanks at the beginning of the experiment and measured body length and developmental stage to determine average size and developmental stage at the beginning of the experiment. These tadpoles were subsequently released into their natural habitat.

Response variables

To detect whether behavioural reactions were induced by the addition of homogenate over longer time periods, *aggregation presence*, *swimming activity* and *microhabitat choice* (as defined above) were recorded four times per day. Times of data acquisition were at least 90 min apart. After 12 days, *size* (as body length) and *developmental stage* were measured in all tadpoles in the experimental arenas. The mean of all 20 tadpoles in one arena represents one replicate.

RESULTS

Proximate factors causing aggregations

Effects of predatory cues. Tadpoles of *A. securifer* reacted significantly to the presence of some of the potential predators or predatory cues, but not to all (Table 1a). Adding both conspecific and heterospecific homogenate caused tadpoles to aggregate more often (*aggregation presence*), and their overall aggregative tendency (*aggregation index*) was increased by conspecific homogenate. The same was true with birds as predators. In addition, the presence of the birds greatly reduced the *swimming activity* of the tadpoles and altered their *microhabitat choice*, with the effect that a greater proportion of tadpoles were found on the bottom of the experimental arenas. The *aggregation size* was not changed by predators or predatory cues.

Dyscophus insularis tadpoles formed significantly larger and significantly more frequent aggregations when fish were present or fish water was added (*aggregation presence*, *aggregation size*) compared to the control, and the *aggregation index* was higher in the presence of fish (Table 1b). The *microhabitat choice* was altered by the addition of conspecific homogenate and the presence of turtles; significantly fewer tadpoles were recorded close to the water surface than in the control treatment. There was no change in the *swimming activity*.

Aggregative behaviour did not decline greatly in either species for up to four hours after the initiation of the ag-

gregation (Fig. 1). In general, aggregations persisted for several hours, and dissolved with diminishing day-light.

Costs of aggregations in *A. securifer*

Formation of aggregations over longer periods of time. We first tested whether the variables *aggregation presence*, *aggregation size*, *swimming activity* and *microhabitat choice* differed between the experimental and the control treatment over a period of 12 days. We found significant differences in *aggregation presence* and *swimming activity* (Table 2). Accordingly, we found aggregations more often and for longer periods of time in the experimental treatments. The aggregations, initiated by the daily addition of conspecific homogenate, normally persisted until nightfall. Whenever aggregations were found in the control treatment, there was no difference in the *aggregation size* as compared to the experimental treatment. Therefore, the results of these experiments (when tadpoles were exposed to homogenate in the long-term) are consistent with the results of the experiment when they were exposed only for a short time as shown above, with the exception that *swimming activity* was additionally reduced when predator cues were presented over a longer period of time.

Costs for growth and development. Initial body length of the *A. securifer* tadpoles before the experiment was 6.63±0.31 mm (mean±SD; $n=30$) and the developmental stage was 30.93±1.05. After the 12 days of the experiment, the *size* of the tadpoles in the homogenate treatment (7.98±0.45 mm) was significantly smaller than in the control treatment (8.48±0.26 mm; t-test: $P=0.018$, $t=2.69$, $n=8$; Fig. 2). Furthermore, the presence of homogenate had a significant negative effect on *development* (control: stage 35.88±0.53, homogenate: stage 34.90±1.09; t-test: $P=0.039$, $t=2.28$, $n=8$; Fig. 3). Mortality during the experiment was very low (control: 19.62±0.70, homogenate: 18.87±0.33 surviving tadpoles per arena at the end of the experiment) and did not differ between treatment and control (Mann–Whitney U-test: $P=0.65$, $Z = –0.69$, $n= 8$).

DISCUSSION

Most anurans in the dry forest of western Madagascar use ephemeral pools as breeding sites. Next to pond drying, predation is thought to contribute most to tadpole mortality (e.g. Hero et al., 1998; Rödel, 1998). Selection pressure for the development of predation avoidance by morphological, chemical and behavioural adaptations

Table 2. Effects of addition of conspecific homogenate on response variables in tadpoles of *A. securifer* (mean±SD) in the growth experiment. For definition of variables see Table 1. Mann Whitney U-tests and χ^2 test; significant results (α=0.05) are highlighted in italics. *n* refers to the number of tested arenas.

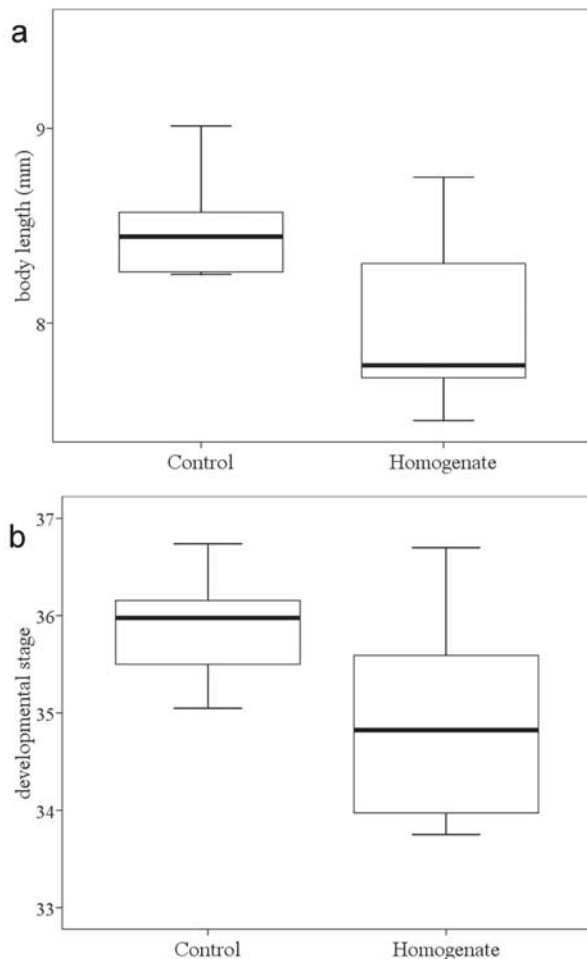| Response variable	Control ($n=8$)	Homogenate ($n=8$)	Z value	P
Aggregation presence (%)	39±9	70±25	2.53	*0.01*
Aggregation size	5.39±0.47	6.40±1.17	1.68	0.11
Swimming activity	12.02±3.15	3.92±3.62	2.89	*<0.01*
Microhabitat choice (%)	b 94.40±3.90	b 99.05±0.55	χ^2=0.71	>0.05
	c 4.90±3.20	c 0.90±0.50		
	s 0.70±0.80	s 0.05±0.05		

Fig. 2. Costs of behavioural reactions to predators (i.e. aggregation formation, reduced swimming activity, change of microhabitat) shown as a) body length and b) developmental stage of *A. securifer* tadpoles. Tadpoles in the control treatment (*n*=8) are significantly larger than those in the homogenate treatment (*n*=8), and development is faster. Shown are median, 25% and 75% percentiles, minima and maxima.

should therefore be strong. This study shows that a high predation risk induces tadpoles of *A. securifer* as well as of *D. insularis* to form aggregations, and that this behaviour is often associated with a decrease in swimming activity and a shift in their microhabitat. We found reactions not only to the immediate presence of predators (fish, bird, turtle), and therefore possibly to a combination of visual, mechanical and olfactory cues, but also to purely chemical stimuli. However, the specific proximate cues that initiated the formation of aggregations, activity decrease or microhabitat change were different between the two species.

Aglyptodactylus securifer responded strongly to the homogenate of conspecifics and still significantly, but less intensively, to that of heterospecific tadpoles. The presence of chemical cues deriving from injured tadpoles (called "Schreckstoff" by Pfeiffer, 1966) presents an indirect but reliable signal of immediate predation risk and is a generalized response, i.e. is not predator specific. *Dyscophus insularis*, on the other hand, also uses indi-

rect signals to assess predation risk and release aggregation behaviour. In contrast to *A. securifer*, its reaction is specifically to fish or to chemicals released by fish, respectively. Prey defences to chemical cues emitted by predators have been demonstrated across a wide range of taxa, and the ability to recognize potential predators through chemical cues clearly has adaptive value (see Kats & Dill, 1998 for review).

In general, the formation of aggregations was triggered by the presence of vertebrate predators, but not invertebrate predators (diving beetle, giant water bug). It is known that the formation of aggregations as a predator avoidance behaviour is a reaction to active, mostly visually hunting predators (Krause & Ruxton, 2002). Aquatic invertebrates, however, are predominantly sit-and-wait predators, and aggregating might not pay off the costs of this behaviour in this case (but see Stav et al., 2007). Furthermore, *A. securifer* and to a lesser extent also *D. insularis* breed at the very beginning of the rainy season, directly after the filling of the breeding ponds (Glos, 2003). Invertebrate size and density and hence predation probability from this group is low at that season, and presumably does not exert any selective pressure. Accordingly, no correlation between invertebrate predator density and the presence of aggregations was found under natural conditions (Glos et al., 2007).

Depending on environmental conditions and predator identity, behavioural defences that are triggered by specific predators – as shown by *D. insularis'* reaction to fish – are advantageous in comparison to those reactions that are triggered by more general cues (e.g. *A. securifer* reaction to tadpole homogenate). In the former cases, the prey's reaction can specifically oppose the predator's sensory abilities and foraging mode, improving the prey's chances of survival. *Dyscophus insularis* is exposed to fish in only some of its natural habitats, while *A. securifer* lives sympatrically with the cichlid *Oreochromis* sp. over much of its range. Although fish-free waters are initially preferred as breeding sites (Glos & Linsenmair, 2004), *Oreochromis* sp. regularly immigrates into the breeding waters of *A. securifer* presenting a potentially high predation threat. Therefore, at first sight it appears paradoxical that it is *D. insularis* and not *A. securifer* that reacts directly to fish. However, *Oreochromis* sp. is exotic to Madagascar (Reinthal & Stiassny, 1991). Hence, predator avoidance behaviour to this fish might simply not have evolved yet in *A. securifer*.

The intriguing question remains: how do these two species aggregate in mixed-species associations, as observed in nature at the study site (Glos et al., 2007), with both species reacting to different predatory cues? Several explanations are feasible. If predation risk is high, multiple cues might be present simultaneously in the water. For example, if a fish captures several tadpoles, alarm substances from injured or killed tadpoles might be released and cause tadpoles of *A. securifer* to aggregate and join a *D. insularis* aggregation that had already formed in reaction to the presence of the fish itself. Furthermore, the presence of an already existing aggregation might lower the threshold (e.g. of the concentration of alarm substance) at which further aggregations are initiated or at

which individuals join an aggregation. Alternatively, joining an aggregation might be induced solely by the presence of an already existing aggregation (Wassersug & Hessler, 1971). By doing so, group members may benefit from other species' abilities to detect and react to predators earlier (Diamond, 1981; Peres, 1993). Therefore, under some circumstances members of a mixed-species group may be better protected than those of single-species groups.

The ultimate benefit of predator-induced aggregations with decreased individual activity and microhabitat change in tadpoles is primarily seen as an increase in individual survival chances by reducing predation risk. All group members within aggregations may benefit from mere group-size-related anti-predator benefits, such as the dilution effect (Hamilton, 1971; Riipi et al., 2001; Spieler, 2005), the confusion effect (Heller & Milinski, 1979), the Trafalgar effect (Siegfried & Underhill, 1975) and also the learning effect, if the group includes unpalatable individuals (Brodie & Formanowicz, 1987). Furthermore, reducing the swimming activity may lower the encounter rate of or detectability by predators and shifting the microhabitat may help in predator avoidance. Both behaviours have been shown to increase survival of individual tadpoles (e.g. Watt et al., 1997; Spieler, 2001). *Aglyptodactylus securifer* and *D. insularis* tadpoles reacted flexibly to predation threats. In the experiments, the tendency to aggregate was greatly increased when a predator cue existed, and the swarms usually dissolved with the diminishing daylight, as is the case in *Phrynomantis microps* (Rödel & Linsenmair, 1997; Spieler, 2003). Accordingly, aggregations of these species in natural ponds are temporary (Glos et al., 2007). These inducible reactions may return higher fitness benefits than constitutive defences would provide. Constitutive reactions would not allow for shifts in foraging strategies to increase growth and developmental rates when predation pressure is low (Sih, 1987). Indeed, aggregation probability decreases when water transparency and therefore visibility is low (Glos et al., 2007; see also Rödel & Linsenmair, 1997; Spieler, 2003). In a natural situation, the predation risk is presumably strongly decreased under low light conditions, in particular by vertebrate predators (fish, birds, turtles), as these predominantly hunt visually.

This flexibility in behaviour also indicates that fitness costs are associated with aggregating, reduced swimming activity or change of microhabitat, or any combination of these. In fact, tadpoles of *A. securifer* that were induced to perform these behaviours over a prolonged time had reduced growth and retarded development in comparison to control tadpoles. The timing of and size at metamorphosis are seen as important factors in amphibian life history (Wilbur & Collins, 1973; Rowe & Ludwig, 1991), affecting reproduction (Berven, 1981, 1982), survival during hibernation (Lyapkov, 1998) and/or desiccation and predation risk (Wilbur, 1997). Accordingly, *A. securifer*, whose tadpoles have a prolonged larval development in combination with a reduced metamorphic size, are predicted to suffer from negative fitness consequences. Mechanisms that generate reduced growth and retarded

development in tadpoles that react behaviourally to predators include increased food competition in groups (reviewed by Krause & Ruxton, 2002), decreased foraging activity as a result of decreased swimming activity (Werner, 1986; Skelly & Werner, 1990; Skelly, 1992), staying in less profitable food patches as a result of microhabitat change, and hormonal–physiological effects associated with high density.

Temporary mixed-species social associations of tadpoles are frequent in the dry forest of western Madagascar. They are formed as a reaction to predatory threats, although generating fitness costs for the species involved. This indicates that aggregation behaviour may play a key role in the ability of amphibians to successfully develop in predator-rich breeding sites.

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