

## BEHAVIORAL RESPONSES BY TADPOLES OF SIX AUSTRALIAN SPECIES TO CHEMICAL CUES FROM OTHER TADPOLES

MATTIAS HAGMAN<sup>1</sup>

School of Biological Sciences A08, University of Sydney, NSW 2006, Australia,  
e-mail: mattias.hagman@zoologi.su.se

<sup>1</sup>Present Address: Department of Zoology, Stockholm University, 106 91 Stockholm, Sweden

**Abstract.**—Anuran tadpoles from a variety of phylogenetic lineages display strong responses to chemical cues from injured conspecifics. Some of these responses may simply involve attraction to a potential food source; whereas, others may reflect avoidance responses to potential presence of a predator. Australian anurans have received little attention in this respect. This study revealed significant behavioral responses (e.g., attraction, aggregation, movement) of six species of anuran larvae to chemical cues from injured conspecifics and, in at least two cases, from injured heterospecific tadpoles. None of the species that I tested showed dramatic aversion responses to chemical cues from injured conspecifics, unlike many anuran species from other parts of the world.

**Key Words.**—alarm pheromone; anti-predator behavior; chemical cues; tadpoles

### INTRODUCTION

The larvae of a variety of amphibians react strongly to chemical cues from injured conspecifics. Presumably recognition of these cues communicates to the signal receiver that a predator is in the vicinity and risk of predation is high (Petranka 1989). Kin selection (i.e., acting for the benefit of relatives: Hamilton 1964; Harvey and Greenwood 1978) or selfish manipulation of group members (e.g., inducing protective aggregations: Hamilton 1971; Dawkins 1976; Harvey and Greenwood 1978; Mathis et al. 1995) can be important mechanisms for the evolution of such alarm signals. The exact nature of the behavioral response varies depending upon the stimulus and the study taxon, but may involve changing activity levels, increased shelter use, repulsion, or aggregation (Rajchard 2006). For example, tadpoles of the Common European Toad (*Bufo bufo*) typically flee when exposed to chemical cues from damaged or injured conspecifics ("Schreckstoff": Eibl-Eibesfeldt 1949; Hrbacek 1950; Kulzer 1954; Pfeiffer 1966). Predator-associated cues also may induce longer-term changes in morphology (e.g., body shape, coloration, growth rate: Rajchard 2006) or life-history traits (e.g., age and size at metamorphosis: Rajchard 2006).

Most of the scientific research on this topic has focused on anurans from North America and Europe, with species from other continents relatively neglected. Frogs are diverse and abundant over much of Australia, with up to 45 species occurring sympatrically in some areas (Slatyer et al. 2007). Most Australian anurans have an aquatic larval stage as part of their life cycle, and hence natural waterbodies often contain a phylogenetically diverse array of tadpoles (Lemckert et al. 2006). Although data are limited, it appears that Australian tadpoles are highly vulnerable to predation from a range of taxa, including (in some cases) conspecific and

heterospecific tadpoles as well as both invertebrates (e.g., insect larvae) and vertebrates (e.g., wading birds, snakes: Bridges 2002). Adult anurans often possess potent antipredator toxins in the skin, but such defense mechanisms seem to develop in the metamorphic stage and hence may be less common in the larvae of the same species (Duellman and Trueb 1986). Accordingly, intense selection on antipredator behavior of tadpoles likely exists, especially on detection of predators (Burger 2002).

Such responses to chemical cues from other species may allow the evolution of complex "biological warfare" within ponds (Mokany and Shine 2003a, b, c), with interspecific and intraspecific competition mediated via such chemical signals. For example, tadpoles of *Rana sphenoccephala* can decrease growth rates and survival of *Hyla cinerea* tadpoles via chemical interference (Faragher and Jaeger 1998). Most chemical signals produced by tadpoles elicit responses in conspecifics, although heterospecifics can sometimes detect these messengers. Thus, tadpoles of the sympatric toad species *Bufo bufo* and *B. calamita* respond to each other's alarm pheromones (Pfeiffer 1966); whereas, no intergeneric communication occurs between tadpoles of *B. boreas* and *H. regilla* (Hews and Blaustein 1985). Intergeneric communication is an important phenomenon because it can shed light on potential homologies, and because some species can learn to detect alarm cues from heterospecifics.

Chemoreception can be an effective means of detecting predators in aquatic environments. An ability to perceive and respond to chemical cues may be more important in turbid water than in clear water, where visual detection of predators might be more efficient. To test this prediction, I explored behavioral responses to potential alarm cues in six Australian species of tadpoles that inhabit different aquatic environments, by exposing these animals to chemical cues from injured conspecific tadpoles. I also tested

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**TABLE 1.** Geographic locations of origin for tadpoles used in the present study. Collection locality for *Litoria infrafrenata* was unknown, because these were long-term captives for which original collection data had been lost. Similarly, elevational data are not available for most sites.

Species	Locality	State	GPS	Elevation (a.s.l.)
<i>Limnodynastes peronii</i>	Lane Cove, Sydney	New South Wales	33° 81' 42" S 151° 16' 98" E	
<i>Litoria dentata</i>	McPherson State Forest	New South Wales	33° 14' 24" S 151° 09' 23" E	235m
<i>Litoria freycineti</i>	Heathcote, Sydney	New South Wales	34° 08' 01" S 151° 00' 08" E	
<i>Litoria infrafrenata</i>	Unknown	Queensland		
<i>Litoria verreauxii</i>	Olney State Forest	New South Wales	33° 08' 05" S 151° 12' 18" E	365m
<i>Pseudophryne coriacea</i>	Chaelundi State Forest	New South Wales	30° 02' 26" S 152° 24' 33" E	700m

for cross-reactions between two of my study species to examine possible homology of alarm cues across taxa.

## MATERIALS AND METHODS

*Study species and animal husbandry.*—I obtained four to seven egg clutches from each of six species (*Limnodynastes peronii*, *Litoria dentata*, *L. freycineti*, *L. infrafrenata*, *L. verreauxii*, *Pseudophryne coriacea*). Most of these originated from New South Wales and Queensland (Table 1). Two of these species (*Limnodynastes peronii* and *Pseudophryne coriacea*) frequently occur in murky waters and in muddy environments; whereas, the other four species more often inhabit clear waters (Anstis 2002; Mattias Hagman, pers. obs.). I transported the eggs to the University of Sydney where, after hatching, I separated the tadpoles into groups of 200-300 larvae housed in 30 L of water (pH = 7.5), conditioned with 1 ml of aquaria Water Ager (Pets Paradise, Pty Ltd, Hallam, Victoria, Australia) per 10 L of water. I fed the tadpoles twice a week with boiled lettuce *ad libitum* and changed their water weekly. All tadpoles grew to Gosner stage 21-23 (Gosner 1960) before use in the experiment. The chemical cue stimulus consisted of an extract that I prepared by macerating (rapid crushing ensured that they were instantly killed) 0.2 g of tadpoles (of each species) in 50 ml of water, then diluted in 1 L of water and filtered through filter paper. For controls I used conditioned tap water. I also examined potential intergeneric effects by cross-exposing a myobatrachid species (*Limnodynastes peronii*) and a hylid species (*Litoria dentata*). For logistical reasons I did not cross-expose all study species.

*Experimental equipment and procedures.*—I filled eight clear plastic tanks (each measuring 75 x 75 x 15 cm) with 6 cm (approximately 34 L) of water. I isolated each tank visually from the others by positioning opaque barriers between them. Under each tank I placed a white paper on which I had drawn a grid. Each grid consisted of a hundred squares (each square measuring 7.5 x 7.5 cm) that were visible from

above through the water and the bottom of the tank. I mounted two S1 digital cameras (Canon, Pty Ltd, Sydney, New South Wales, Australia) at a height of 1.55 m above four adjoining tanks to record tadpole behavior. Each camera was equipped with a WC-DC52A, 0.7x wide-angle lens (Canon, Pty Ltd, Sydney, New South Wales, Australia) allowing me to photograph the four adjoining tanks in one frame (i.e., each camera simultaneously recorded a set of four tanks). For a trial, I placed a mixture of 50 kin and non-kin tadpoles in each tank and introduced the stimulus (either control or cues from injured tadpoles) into one corner of each tank via a gravity-fed intravenous infusion set (i.e., a tube connected to a reservoir, as used to perfuse hospital patients: Baxter Healthcare Pty Ltd, Sydney, New South Wales, Australia). Controlling for kinship is important because warning signals might function to warn relatives (Hamilton 1964). I conducted dye trials (using 0.5 ml of food dye per 1 L of water) that confirmed that the stimulus slowly perfused across the tanks. Each treatment had six replicate trials. I set the flow rate such that the one-liter reservoir emptied in six minutes. All treatments took place simultaneously, and I randomized successive trials of each stimulus type by haphazardly shifting the treatments among enclosures and between replicates. After each trial, I rinsed all tanks with dechlorinated tap water and dried them with paper towels before replacing the water containing chemical messengers with clean water. To assure independence of data values, I employed a tadpole only once against a single replicate treatment. I used a WL-DC100 remote control unit (Canon, Pty Ltd, Sydney, New South Wales, Australia) to take one photo with each camera before the addition of the stimulus, and then a photo at successive 5-min intervals for 1 hr. Water temperature was 29°C throughout this period.

*Behavioral variables measured.*—Using the photographs and the grids, I scored three behavioral variables as follows:

(1) *Direction of movement.*—I obtained this score by calculating the numbers of tadpoles within each tank in

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**TABLE 2.** Results of statistical tests of data on behavioral responses of anuran larvae to chemical cues from injured tadpoles of their own or other species. See text for descriptions of how I calculated indices of repulsion, aggregation, and movement. The table shows degrees of freedom (df),  $F$ , and  $P$  values for the overall treatment effect from single-factor repeated-measures ANOVAs comparing responses in the presence vs. absence of chemical cues from injured tadpoles. The first part of the table lists responses to conspecific cues, and the two later parts list responses to heterospecific cues. Boldface font shows significant results ( $P < 0.05$ ).

Species	Repulsion	Aggregation	Movement
<i>Limnodynastes peronii</i>	$F_{1,10} = 6.22; P = \mathbf{0.01}$	$F_{1,10} = 63.36; P < \mathbf{0.0001}$	$F_{1,10} = 59.02; P < \mathbf{0.0001}$
<i>Litoria dentata</i>	$F_{1,10} = 0.23; P = 0.63$	$F_{1,10} = 1.13; P = 0.29$	$F_{1,10} = 0.31; P = 0.58$
<i>Litoria freycineti</i>	$F_{1,10} = 1.97; P = 0.16$	$F_{1,10} = 22.20; P < \mathbf{0.0001}$	$F_{1,10} = 0.60; P = 0.44$
<i>Litoria infrafrenata</i>	$F_{1,10} = 0.002; P = 0.96$	$F_{1,10} = 4.97; P = \mathbf{0.03}$	$F_{1,10} = 11.37; P = \mathbf{0.001}$
<i>Litoria verreauxii</i>	$F_{1,10} = 0.89; P = 0.35$	$F_{1,10} = 0.38; P = 0.54$	$F_{1,10} = 7.70; P = \mathbf{0.006}$
<i>Pseudophryne coriacea</i>	$F_{1,10} = 5.73; P = \mathbf{0.02}$	$F_{1,10} = 93.96; P < \mathbf{0.0001}$	$F_{1,10} = 19.82; P < \mathbf{0.0001}$
<i>Limnodynastes peronii</i> vs injured <i>Litoria dentata</i>	$F_{1,10} = 18.48; P < \mathbf{0.0001}$	$F_{1,10} = 0.16; P = 0.69$	$F_{1,10} = 1.83; P = 0.18$
<i>Litoria dentata</i> vs injured <i>Limnodynastes peronii</i>	$F_{1,10} = 0.04; P = 0.84$	$F_{1,10} = 2.56; P = 0.11$	$F_{1,10} = 8.06; P = \mathbf{0.005}$

each of 10 zones. Zone 1 consisted of the corner grid-square into which the stimulus was introduced, zone 2 consisted of all squares in contact with zone 1, zone 3 consisted of all squares in contact with zone 2, and so forth to form an expanding array of grid-cells at increasing distances from the stimulus source. I determined the direction of movement by calculating the average number of tadpoles per square in each zone at each 5-min interval and then computing the slope of the linear regression between zone number and mean number of tadpoles per square. A positive regression slope indicates repulsion (i.e., tadpole densities are higher as one moves further from the stimulus); whereas, a negative slope suggests attraction (more tadpoles close to the stimulus source).

(2) *Degree of aggregation.*—I scored aggregation via a standardized Morisita's index and computed this index using PopTools version 2.7.1 (CSIRO Org. Canberra, Australian Capital Territory, Australia. Available from <http://www.cse.csiro.au/poptools> [Accessed 23 June 2008]). This index ranges from -1 to +1 with 95% confidence limits at +0.5 and -0.5. A zero indicates a random distribution with clustering increasing as scores become more positive and overdispersion increasing as scores become more negative. I calculated the index on counts of tadpoles in each grid square.

(3) *Overall movement.*—I scored this variable by calculating changes in numbers of tadpoles within each grid-cell through time, and summing those changes. Hence, the movement score represented the total number of times that tadpoles migrated from one cell to another during the observation period; the score did not take into account the distances moved, nor cases in which tadpole movements in opposite directions cancelled each other out.

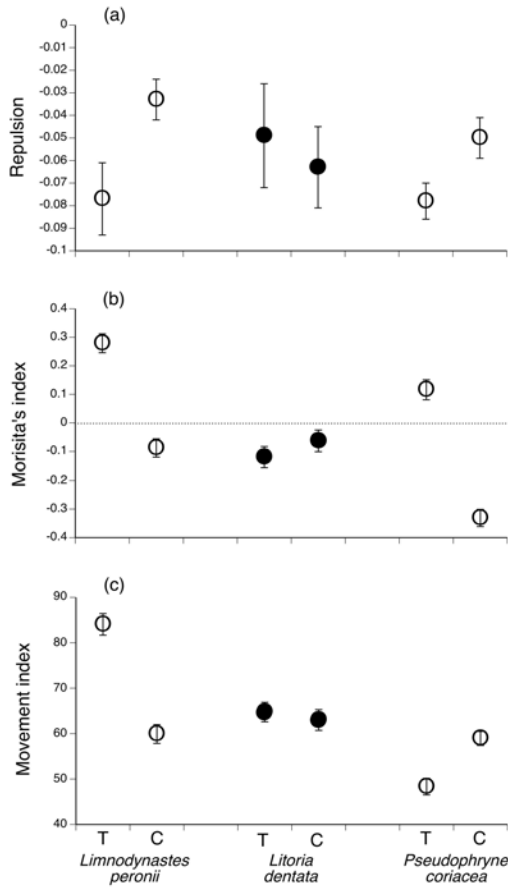
*Statistical analysis.*—For each of the three behavioral variables, I first conducted a two-factor repeated-measures ANOVA with study species and treatment (control vs. conspecific and heterospecific cues) as the factors, and tadpole location in successive 5-minute intervals as the repeated measure. In each case, these analyses revealed highly significant interaction terms, precluding simple interpretation.

Thus, I divided the data set by species, and examined each species' response separately, against the concurrently-run controls. To compare overall response levels among species, I calculated the difference between treatment and control responses for each time period for each trial involving conspecific cues. These contrasts were used as the dependent variable in a repeated-measures ANOVA with species as the factor and tadpole location as the repeated measure. For all ANOVAs,  $\alpha = 0.05$ .

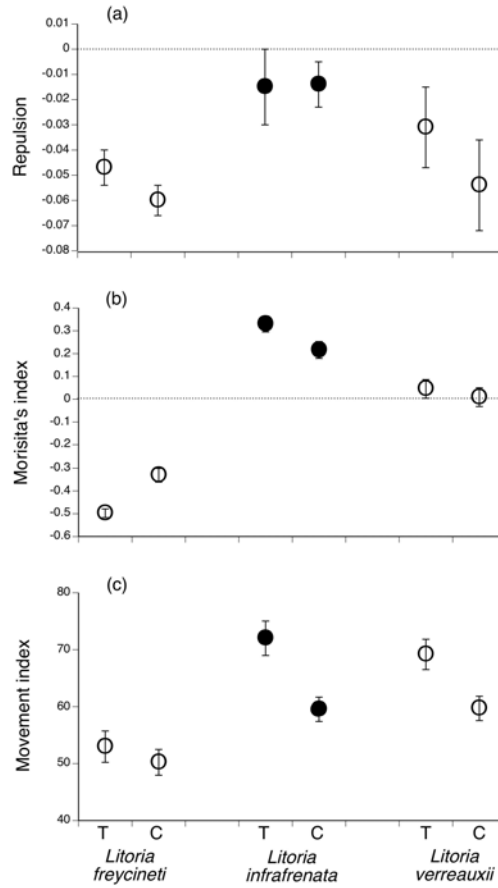
## RESULTS

Of the six species I examined, two (*Limnodynastes peronii* and *Pseudophryne coriacea*) responded significantly in terms of all three of the behavioral variables that I measured. Tadpoles of *L. peronii* were slightly attracted to cues from injured conspecifics and tended to aggregate and move about more (Table 2, Fig. 1). Tadpoles of *P. coriacea* similarly responded with slight attraction and clustering, but decreased rather than increased their movements in response to cues from injured conspecifics (Table 2, Fig. 1). Three of the four hylids that I examined showed significant behavioral responses when exposed to cues from injured conspecifics: *L. freycineti* clustered together (Table 2, Fig. 2b), *L. infrafrenata* became overdispersed (Table 2, Fig. 2b), and both *L. infrafrenata* and *L. verreauxii* exhibited increased levels of movement (Table 2, Fig. 2c). One species, *L. dentata*, showed no significant responses in terms of any of the three behavioural variables that I measured when exposed to cues from injured conspecifics (Table 2). However, when I cross-exposed cues from injured *Limnodynastes peronii* and *Litoria dentata*, both species responded significantly: *L. peronii* tadpoles were attracted to cues from injured *L. dentata* (Table 2, Fig. 3a), while tadpoles of the latter species became more active (Table 2, Fig. 3b).

The repeated-measures ANOVA on contrast scores (treatment minus control for each time period) showed no significant interspecific variation in the degree of repulsion to chemical cues from injured conspecifics ( $F_{5,29} = 0.47, P = 0.79$ ). Species differed significantly in movement patterns ( $F_{5,29} = 6.29, P < 0.0005$ ),



**FIGURE 1.** Behavioral responses (means and standard error of treatment, T, and control, C) of tadpoles of three species of Australian anurans to chemical cues from conspecific tadpoles. The upper graph (a) shows an index of repulsion, calculated as the slope of the linear regression between distance from the stimulus origin and mean number of tadpoles per square. A positive regression slope indicates repulsion (i.e., tadpole densities are higher as one moves further from the stimulus) whereas a negative slope suggests attraction (more tadpoles close to the stimulus source). The middle graph (b) shows an index of aggregation (Morisita's index) and the lower graph (c) an index of movement.



**FIGURE 2.** Behavioral responses (means and standard error of treatment, T, and control, C) of tadpoles of three species of Australian anurans to chemical cues from conspecific tadpoles. The upper graph (a) shows an index of repulsion, calculated as the slope of the linear regression between distance from the stimulus origin and mean number of tadpoles per square. A positive regression slope indicates repulsion (i.e., tadpole densities are higher as one moves further from the stimulus) whereas a negative slope suggests attraction (more tadpoles close to the stimulus source). The middle graph (b) shows an index of aggregation (Morisita's index) and the lower graph (c) an index of movement.

however. Post-hoc PLSD tests (Fisher's PLSD) showed that conspecific cues elicited more movement by tadpoles of *Limnodynastes peronii* than by *Litoria dentata*, *L. freycineti*, *L. infrafrenata* or *Pseudophryne coriacea*. The latter species also moved significantly less than did either *L. infrafrenata* or *L. verreauxii* (post-hoc tests, all  $P < 0.05$ ). The degree of aggregation also differed among species ( $F_{5,29} = 4.96$ ,  $P < 0.003$ ), with post-hoc tests showing that conspecific cues induced higher levels of aggregation in tadpoles of *Limnodynastes peronii* than those of *Litoria dentata*, *L. freycineti*, or *L. verreauxii*. Tadpoles of *P. coriacea* also aggregated significantly less in response to chemical cues than did those of *L. dentata*, *L. freycineti*, *L. infrafrenata* or *L. verreauxii*.

## DISCUSSION

Tadpoles in my study displayed significant behavioral responses to chemical cues from injured conspecifics, and in some cases at least, to heterospecifics (logistical difficulties prevented me from testing for cross-reactions between all my study species). Although the responses that I elicited varied widely among species, the adaptive significance of those responses remains unclear.

Overall, my results suggest that water-body attributes may influence the probability of chemoreception evolving in tadpoles: two species that frequently inhabit murky waters showed significant responses across all behavioural variables that I measured; whereas, clear-water species were less

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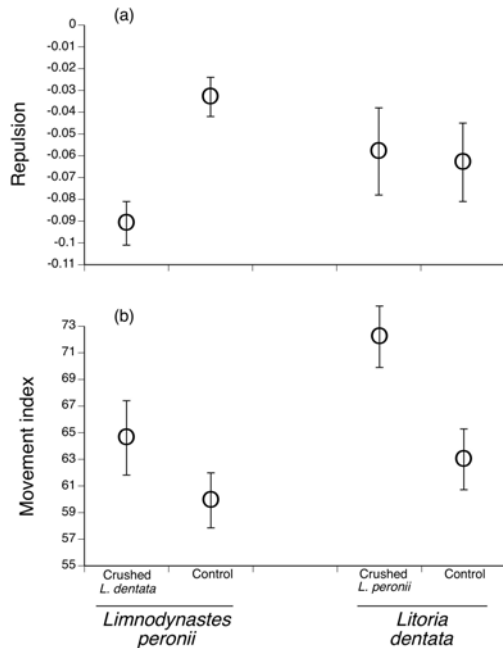
TABLE 3. Behavioral responses of anuran tadpoles to chemical cues from other tadpoles.

Taxa	Response to crushed conspecifics	Response to crushed congeners	Reference
<b>Bufo</b>			
<i>Bufo americanus</i>	Avoid	Approach, feeding response	Petranka 1989
<i>Bufo boreas</i>	Avoid, increase activity	None	Hews and Blaustein 1985; Hews 1988
<i>Bufo bufo</i>	Avoid	-	Eibl-Eibesfeldt 1949
<i>Bufo bufo</i>	Flight	None, Avoidance	Hrbacek 1950
<i>Bufo bufo</i>	Swim to bottom	-	Kulzer 1954
<i>Bufo bufo</i>	Reduce activity	None	Pfeiffer 1966
<i>Bufo bufo</i>	Reduce activity	-	Marquis et al. 2004
<i>Bufo bufo</i>	Avoid	-	Manteifel 2001
<i>Bufo calamita</i>	Avoid, swim to the bottom	None	Pfeiffer 1966
<i>Bufo maculatus</i>	Reduce activity, Aggregation	None	Spieler and Linsenmair 1999
<i>Bufo marinus</i>	Reduce activity, swim to bottom	None	Summey and Mathis 1998
<i>Bufo marinus</i>	Avoid	-	Hagman and Shine 2008a
<i>Bufo marinus</i>	Avoid	-	Hagman and Shine 2008b
<i>Bufo terrestris</i>	None	-	Lefcort 1998
<b>Discoglossidae</b>			
<i>Alytes obstetricans</i>	None	-	Pfeiffer 1966
<i>Bombina variegata</i>	None	None	Pfeiffer 1966
<i>Discoglossus pictus</i>	Flight	-	Eibl-Eibesfeldt 1962
<b>Hylidae</b>			
<i>Hyla arborea</i>	None	-	Pfeiffer 1966
<i>Hyla regilla</i>	Reduce activity	-	In: Summey & Mathis 1998
<i>Litoria dentata</i>	None	Increase activity	Present study
<i>Litoria dentata</i>	-	Aggregation	Hagman and Shine 2008b
<i>Litoria freycineti</i>	Aggregate	-	Present study
<i>Litoria freycineti</i>	-	Avoid	Hagman and Shine 2008b
<i>Litoria infrafrenata</i>	Disperse, Increase activity	-	Present study
<i>Litoria infrafrenata</i>	-	Aggregation	Hagman and Shine 2008b
<i>Litoria verreauxii</i>	Increase activity	-	Present study
<i>Litoria verreauxii</i>	-	None	Hagman and Shine 2008b
<i>Smilisca cyanosticta</i>	None	None	Summey and Mathis 1998
<b>Myobatrachidae</b>			
<i>Limnodynastes peronii</i>	Approach, Aggregate, Increase activity	Approach	Present study
<i>Limnodynastes peronii</i>	-	None	Hagman and Shine 2008b
<i>Pseudophryne coriacea</i>	Approach, Aggregate, Decrease activity	-	Present study
<i>Pseudophryne coriacea</i>	-	Avoid	Hagman and Shine 2008b
<b>Pipidae</b>			
<i>Xenopus laevis</i>	None	-	Eibl-Eibesfeldt 1962
<i>Xenopus laevis</i>	None	-	Pfeiffer 1966
<b>Ranidae</b>			
<i>Rana aurora</i>	Reduce activity	-	Wilson and Lefcort 1993
<i>Rana brownorum</i>	None	None	Summey & Mathis 1998
<i>Rana cascadae</i>	Increase activity	None	Hews and Blaustein 1985
<i>Rana esculenta</i>	None	-	Pfeiffer 1966
<i>Rana heckscheri</i>	None	-	Altig and Christensen 1981
<i>Rana pipiens</i>	None	-	Pfeiffer 1966
<i>Rana temporaria</i>	None	-	Pfeiffer 1966
<i>Rana temporaria</i>	Reduce activity	-	Marquis et al. 2004
<i>Rana utricularia</i>	None	None	Lefcort 1996

responsive to chemical stimulus. However, this ecological comparison is confounded by phylogeny: the clear-water species belong to the family Hylidae, which suggests a phylogenetic constraint on the ability to respond to chemical stimuli (Relyea 2007).

Although the ability of anuran larvae to produce and detect chemical signals may be an important facet of their biology, data on this topic are few for the diverse

Australian frog fauna. Instead, most published studies of chemical communication in anurans have involved only three anuran families from America and Europe (Table 3). This bias is highlighted in a recent review, which also calls for more studies on a broader range of species (Relyea 2007).



**FIGURE 3.** Behavioural responses (means and standard error of treatment, T, and control, C) of tadpoles of two species of Australian anurans exposed to heterospecific scent cues. The upper graph (a) shows an index of repulsion, calculated as the slope of the linear regression between distance from the stimulus origin and mean number of tadpoles per square. A positive regression slope indicates repulsion (i.e., tadpole densities are higher as one moves further from the stimulus) whereas a negative slope suggests attraction (more tadpoles close to the stimulus source). The lower graph (b) shows an index of movement.

What is the ecological significance of the responses that I elicited? The responses broadly resemble those recorded from previous studies (Table 3), although differences in experimental protocols make it difficult to draw direct comparisons. For example, strong repulsion or attraction is likely to result in aggregation in small containers, because all tadpoles that flee or approach the stimulus end up concentrated in one corner of the enclosure (Hagman and Shine 2008a). Thus, laboratory studies may detect aggregation as well as repulsion, even in cases where field studies on much larger natural waterbodies report only repulsion (Hagman and Shine 2008a). The ability to produce and detect chemical signals is common in many anuran lineages (Table 3). Such chemicals presumably induce behaviors that reduce the risk of being eaten by a predator, and thus have adaptive value (Petranka 1989). Although tadpoles in my study were not noticeably alarmed by cues from injured conspecifics, the responses I observed may simply reflect alternative strategies for predator avoidance rather than lack of it. For example, tadpoles of *P. coriacea* may reduce their activity to avoid detection by a visually orientated predator. Alternatively, decreasing activity levels in response to a scent cue that signals predation may reduce the risk of encountering the predator in murky water. Such responses may vary depending on environmental conditions (e.g., flight response in clear

water vs. decreased activity in murky water): this possibility warrants further testing. Two of my study species, *Litoria peronii* and *L. infrafrenata*, tended to cluster together and move around as a group when exposed to chemical cues from injured tadpoles, suggesting a form of anti-predator behavior (e.g., safety in numbers, selfish herd: Hamilton 1971). Perhaps chemicals are the most efficient way of transferring information in aquatic environments and in large groups? This theory predicts that chemoreception should be more widespread among aquatic organisms that live in schools. At least one species in my study can significantly change both body shape and time to metamorphosis in response to predation (Kraft et al. 2005, 2006). This ability suggests that responses to predator cues might be manifested in phenotypic plasticity rather than behaviour.

An alternative explanation is that the tadpoles in my study were attracted to a potential food source, rather than being repulsed by a cue that predicts predator danger (Fig. 1-3). High levels of movement might, for example, be used by feeding tadpoles to stir up food particles from the bottom of ponds (Richmond 1947; Bragg 1954; Spieler and Linsenmair 1999). In support of this deduction, many tadpoles feed on dead conspecifics (Tyler 1999; Mattias Hagman, pers. obs.). However, I emphasise that my study does not show that Australian tadpoles lack behavioural responses to predators. For example, it is possible that additional stimuli, such as visual evidence of a predator's presence and cues from injured conspecifics, might be needed to elicit antipredator responses in my study species.

Why do some species respond to chemical cues from other species? The most plausible scenario is that such interspecific communication is an evolutionary response to predation, under which individuals that respond to other species' alarm signals will suffer lower rates of mortality. Thus, if a common predator preys upon two or more species, then it should be adaptive for those species to respond to each other's alarm signals. Results from behavioural studies on a wide range of typical "prey-species" accord well with this theory. For example, interspecific communication is common in mixed herds of antelope (Bertram 1978) as well as in mixed flocks of birds (Goodale and Kotagama 2005), and schools of multiple fish species (Mathis and Smith 1993; Ward et al. 2003). The results of my study suggest that such interspecific sociality occurs in anuran larvae, also. Chemically based interspecific communication of predators is a poorly studied topic in larval ecology. Cross-exposing multiple species to each other's alarm pheromones might therefore be worthwhile.

Finally, understanding why species should evolve to respond to alarm signals seems straightforward, but why do individuals produce those signals? There is no obvious value in warning conspecifics about a

predator, particularly if the sender of the signal is the one that is falling prey (Mathis et al. 1995). Likewise, sending a warning signal at the sight of a predator might expose the sender of the signal and attract the attention of the predator (Harvey and Greenwood 1978; Mathis et al. 1995). Perhaps relatedness among group members can explain the evolution of this seemingly non-adaptive behavior. It makes sense in evolutionary terms that the sender of an alarm signal would warn relatives about potential dangers (kin selection: Hamilton 1964; Smith 1977). According to this theory, alarm signalling should evolve in species that associate with relatives. Tadpoles of the genus *Bufo* meet these criteria well; most species live in large sibling groups (Lever 2001; Duellman and Trueb 1986) and consistently flee when exposed to chemical cues from injured conspecifics.

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**MATTIAS HAGMAN** is a Postdoctoral Research Fellow in The Department of Zoology at Stockholm University, Sweden. He received his PhD in 2007 from The University of Sydney, where he was a student in Rick Shine's laboratory. Mattias's research on the evolutionary ecology of reptiles and amphibians has included studies of Australian death adders (*Acanthophis praelongus*), cane toads, and frogs (e.g. Dendrobatid frogs and various Australian species). He likes to travel and has conducted much of his research in the wet tropics of Australia and Latin America.