Adult frogs are sensitive to the predation risks of olfactory communication

Rowena Hamer1, Francis L. Lemckert2 and Peter B. Banks1,*

1Evolution and Ecology Research Centre, School of Biological, Earth and Environmental Sciences, University of New South Wales, Kensington, NSW 2052, Australia
2Forest Science Centre, Industry and Investment NSW, PO Box 100, Beecroft, NSW 2119, Australia
*Author for correspondence (p.banks@unsw.edu.au).

Olfaction is a common sensory mode of communication in much of the Vertebrata, although its use by adult frogs remains poorly studied. Being part of an open signalling system, odour cues can be exploited by ‘eavesdropping’ predators that hunt by smell, making association with odour a high-risk behaviour for prey. Here, we show that adult great barred frogs (*Mixophyes fasciolatus*) are highly attracted to odour cues of conspecifics and those of sympatric striped marsh frogs (*Limnodynastes peronii*). This attraction decreased significantly with the addition of odours of a scent-hunting predator, the red-bellied black snake (*Pseudochis porphyriacus*), indicating that frogs perceived predation risks from associating with frog odours. Male frogs, however, maintained some attraction to unfamiliar conspecific scents even with predator odours present, suggesting that they perceived benefits of odour communication despite the risk. Our results indicate that adult frogs can identify species and individuals from their odours and assess the associated predation risk, revealing a complexity in olfactory communication previously unknown in adult anurans.

Keywords: olfaction; anuran; scent-hunting; eavesdropping; signal-exploitation

1. INTRODUCTION

Well-developed olfactory systems are a common feature of the Vertebrata, used in foraging, homing, species recognition, intra- and interspecific communication and predator avoidance [1]. As with all open communication systems, olfactory signals are at risk of being intercepted by ‘eavesdropping’ predators. This risk is likely to be higher for potential prey species that make frequent use of olfactory communication, because high rates of depositing and investigating scent marks create a close relationship between odour and the occurrence of prey. For the same reason, chemical cues are thought to be most useful for detecting slow-moving organisms, or those restricted to a specific area [2]. For such prey, strategies to reduce detection and the risk of attracting scent-hunting predators are vital.

Adult frogs fit the profile of prey that are vulnerable to signal exploitation by predators as they are relatively sedentary, often returning to the same breeding or shelter sites [3]. Whether adult frogs use olfaction in conspecific communication is less clear, as the olfactory capabilities of post-metamorphic frogs have been largely ignored in favour of their more obvious vocal signals [4]. The sophisticated use of scent by salamanders and tadpoles (reviewed in [5]) suggests that odour may be more important to adult anurans than is currently appreciated (see [4,6]).

We used great barred frogs (*Mixophyes fasciolatus*) from southeastern Australia to examine whether adult frogs perceive predation risks of olfactory communication. Adult *M. fasciolatus* show spatially restricted activity, and spend extended periods in one location (R. Hamer 2009, unpublished data), a pattern of movement that would inevitably lead to odour accumulations in shelter and calling sites [7]. The resulting strong association with these odours would give considerable spatial predictability of their occurrence. This tight relationship between odour cue and prey occurrence meets the requirement for exploitation by foraging predators.

We used choice trials to determine (i) whether individuals were attracted to the odours of themselves or other frogs, and therefore, if these frogs are likely to use odours in social or territorial contexts and (ii) if individuals were sensitive to the predation risks of associating with such odours.

2. MATERIAL AND METHODS

The perceived risks of associating with odour were measured in odour preference trials using captive adult *M. fasciolatus* (*n = 25*) caught in Chaelundi SF (northern NSW, Australia) and striped marsh frogs (*Limnodynastes peronii*) (*n = 24*) collected in Sydney. Both are large (greater than 60 mm), ground-dwelling frogs, frequently found at the same breeding sites [8] and are therefore likely to interact in the natural environment. *L. peronii* would represent a potential competitor of *M. fasciolatus* for food and/or shelter sites, particularly within male breeding aggregations. Being slightly smaller, *L. peronii* also represent potential prey for larger *M. fasciolatus* individuals, which are known to eat other frogs [9].

Frogs were presented with a choice between two shelters placed at either end of a rectangular 150 l plastic tub (50 x 72 x 42 cm), following several other studies [4,10,11]. Shelters were plastic containers (12 x 17.5 x 4.5 cm) filled with 250 ml clean sand, into which frogs readily burrowed. Adult frogs (10 males and 10 females) were exposed to four different treatments in random order: (i) ‘self’ scented versus control (unscented); (ii) conspecific (same sex) versus control; (iii) heterospecific (*L. peronii*) versus control; and (iv) self versus conspecific shelters. Each treatment was repeated at two predation levels: high risk, where the scent of a scent-hunting predator (red-bellied black snake, *Pseudochis porphyriacus*) was added to the centre of the experimental arena, or low risk, where dechlorinated tap water was substituted for scent. *Pseudochis porphyriacus* was chosen as they are a predator of both species [12], they use scent cues when hunting [13] and are a common predator where the frogs were collected (R. Hamer & F. Lemckert 2009, personal observation).

Frog scent was obtained by placing shelters in enclosures with the frogs overnight (16–20 h), where they accumulated odours from the integumentary and waste products. *Pseudochis porphyriacus* scent was obtained by collecting the paper towel lining from the enclosures of four individuals every 3 days, cutting and freezing it in 5 x 5 cm squares to prevent breakdown of the scent. Scent allocation was randomized, ensuring that frogs did not encounter the same scent twice. Trials ran for 1 h, during which the position of the frog was monitored with infrared video cameras to score the amount of time frogs spent at each shelter.

The amount of time spent at shelter A (the scented shelter, or in the case of treatment iv, the conspecific shelter) and time spent at shelter B (unscented or ‘self’ for treatment iv) was initially compared...
analysis revealed a significant reduction in preference of predator odour reduced the overall attraction (mean preference for scented side (±1 s.e.m.). Asterisk (*) denotes significant (α = 0.05) preference based on separate paired t-tests.

Preferences (differences in time spent on shelters A and B) were then compared across treatments with repeated-measures ANOVA (RM ANOVA). Data were non-normal for the RM ANOVA analysis (Levene’s test p < 0.05), however, the RM ANOVA is robust to small deviations in normality especially when, as here, the sphericity assumption is met (Mauchly’s criterion p > 0.05; [14]). Frogs did not move in 39 of 160 trials; this was interpreted as a lack of response to the experiment rather than an equal preference for both shelters; these results were removed from analyses.

3. RESULTS

Mixophes fasciolatus were attracted to all frog scents, generally preferring to spend more time in the scented shelter compared with the control, but only under low predation risk. Under high predation risk, male frogs still showed greater attraction to conspecific scents compared with the control but no attraction to self or heterospecific scents (figure 1a). Females showed a similar trend although there was no significant attraction or repulsion for any scent type under either predation risk (figure 1b).

The RM ANOVA (table 1) showed that the presence of predator odour reduced the overall attraction to scent for all scent types. However, there was also a predator × scent type interaction. A posteriori contrast analysis revealed a significant reduction in preference for self, conspecific and heterospecific odours (treatments i, ii and iv) under high predation risk when compared with low predation risk (figure 1), but no change in their preference for conspecific scent over self scent (treatment iv). Males had a greater overall attraction to all scents than females, but sex did not interact with other factors.

4. DISCUSSION

We predicted that M. fasciolatus should be sensitive to the risks from scent-hunting predators when selecting shelter sites, because of their high site fidelity and because they adopt a passive defence response when threatened, becoming immobile rather than attempting escape ([15], F. Lemckert & R. Hamer 2009, personal observation), increasing the importance of escaping initial detection by predators. As these frogs are preyed upon by scent-hunting predators, such as the red-bellied black snake, these risks should include the risk of associating with accumulations of frog odours. Such anti-predator behaviour, applied early in the predator:prey encounter, is predicted to be most effective in evading predators but is poorly understood for most prey [16].

This prediction was supported by our results, as both sexes decreased their overall association with odours in the high predation risk treatments (figure 1). Association with self, conspecific and heterospecific scents was strongly reduced in the presence of predator scent, indicating that any benefits of associating with or investigating these scents are outweighed by the increased risks of attracting predators. For males, there was also a preference for conspecific odours over their own odour, even under high-risk treatments (figure 1). In this case, both shelters carried the same relative concentration of scent (and presumably likelihood of predator attraction), and associating with a conspecific may dilute the risk to the individual frog: given their habit of site fidelity, the other frog is likely to be closely associated with their scent.

Male frogs, however, also maintained a significant preference for conspecific scent over a blank shelter under high predation risk, even though this would not only increase the scent concentration but also make it the only point of scent concentration in the arena. This suggests that there are benefits, at least for males, in investigating conspecific scents that

<table>
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Table 1. RM ANOVA results for M. fasciolatus. MS, mean squares from the ANOVA. Bold text indicates statistically significant values (p < 0.05).
outweigh the associated predation risk, possibly related to gathering information about potential competition for mates and mating sites.

The greater overall attraction of male frogs to scented areas compared with females, regardless of the presence of snake odours, suggests that there may be sex-specific benefits in olfactory communication. Males spend most of the breeding season in dense aggregations of calling males around ponds (F. Lemckert 2009, personal observation). Favourable calling sites, access to females and other resources may be contested in such breeding aggregations [17], and male combat has been observed in this species [18]. In such situations, odour may be a useful means of assessing the status of potential competitors before engaging them in physical combat, thus decreasing the physical risk associated with such competition (e.g. [19]). Females, which in the breeding season are found in isolation much further from breeding ponds (R. Hamer 2009, unpublished data), are much less likely to encounter other frogs and therefore perhaps gain less from investigating odour cues.

Use of olfactory cues in communication by adult frogs has only rarely been demonstrated, although the sophisticated use of olfaction by larval anurans and by the closely related salamanders suggests that such use is likely to be more widespread than currently realized [4]. Our results indicate that male *M. fasciolatus* at least showed the ability to discriminate between odours of different conspecifics and different species, and the ability to weigh up the potential predation risks of visiting these odours. These subtle and complex responses parallel those only recently discovered in rodents [20], which are the most prolific and well-studied users of olfaction among vertebrates. Anurans are, therefore, a valuable alternate model to study the costs and consequences of olfactory communication.

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