

Antipredator pheromones in amphibians: a review

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ABSTRACT: Specific chemosignals (pheromones) have an important role in the antipredator behaviour in amphibians and other vertebrates. However, relatively little is known about the occurrence of chemical alarm cues just in amphibians. The site of chemosignals perception is vomeronasal system. The presence of the vomeronasal system in aquatic amphibians indicates that it did not arise as an adaptation to terrestrial life. Predators may inhibit mate search of some species, and male newts probably take greater risks during the breeding season. Field tests demonstrated different responses to male newt extract – probably trade-off that incorporates risk and resource sensitivity. Response to chemical alarm signals has been documented for tadpoles of frog and for several species of salamander. The response of tadpoles to predator includes morphological modifications and influence of coloration, growth and development retardation. Tadpoles of *Rana aurora* release a chemical that provides conspecifics with an early warning of predator presence. *Bufo boreas* tadpoles living in the presence of conspecific alarm cues and chemosignals of specific predators reduce the time of metamorphosis in order to reduce the time in the presence of its predators. Presence of conspecific alarm substances in water and predators' waste products have an important role in the chemical detection of predators by tadpoles of *Rana temporaria* and *Bufo bufo*. Tadpoles of *Rana utricularia* significantly decreased the growth and increased the mortality of *Hyla cinerea* tadpoles on the basis of behavioral and chemical interference. *Rana utricularia* tadpoles apparently use both chemical interference and aggressive behavior in securing a competitive advantage over *H. cinerea* tadpoles. The response of tadpoles of *Rana aurora* to tadpoles of *Taricha granulosa* appear be similar to their response to tadpole extract in eliciting alarm, while insect-fed newts would have less of an effect since predators consuming other species may be less of a threat. In some cases (e.g. in *Bufo bufo* and *B. calamita*) chemosignals released in response to threat by predators (direct attack or detection of the predator scents) exert their effects across species.

Keywords: infochemicals; vomeronasal system; toad; newt; salamander; frog; tadpole; predator

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1. Introduction

The knowledge of biological features of all groups of terraristic animals (including amphibians) is very important for their successful breeding and veterinary care. Many animals use chemical signals (pheromones) as source of information about the world around them. Infochemicals have an impor-

tant role in the antipredator behaviour in many species of aquatic vertebrates, including amphibians. However, relatively little is known about the occurrence of chemical alarm cues in this animal class. The chemosignal communications in animals are both at the intraspecific level (especially in the sexual relationships) and at the interspecific level (e.g. between predator and its prey). The observa-

tion that predator-induced defenses can result from non-contact cues associated with the presence of a feeding predator is an example of the latter case. Amphibians are an understudied group, and the area of hormone-behavior relationships is inspired by testable hypotheses based on studies of other terrestrial vertebrates. Chemosignals (pheromones) are an important source of information for many animals about the other organisms around them. One of most frequent case of pheromone function is the information about the predator imminency.

2. Amphibians vomeronasal system – the site of chemosignals perception

The amphibians olfactory system and sensory organs of other vertebrates were characterized by Eisthen (1996): the structure and function of the olfactory system are consistent across vertebrates. The presence of the vomeronasal system in aquatic amphibians indicates that the vomeronasal system did not arise as an adaptation to terrestrial life. The vomeronasal system is involved in both foraging and reproductive behavior in reptiles and probably in some pheromonally mediated types of behavior in mammals. The relative functions of the tetrapod olfactory and vomeronasal systems are unclear. The members of the olfactory receptor family can be separated into two groups: for transduction of air-borne and water-borne odorant substances. Both groups occur in aquatic amphibians, although the receptors for air-borne substances have no obvious function in these animals. The presumptive odorant receptors from the vomeronasal epithelium share little sequence similarity with those from the olfactory epithelium. These receptors may have been independently co-opted from the larger family of seven transmembrane domain receptors for use in odour transduction. The microvillar olfactory receptor cells are widespread among vertebrates and are not present only in aquatic animals or in the vomeronasal epithelium of tetrapods. All microvillar receptor cells are not specialized for the detection of pheromones. The frog *Xenopus laevis* is used as very frequent amphibian laboratory model. The expression of the vomeronasal receptor genes in this species was analyzed and the genes of the *Xenopus* V2R receptor family were identified (Hagino-Yamagishi et al., 2004).

The role of inositol 1,4,5-trisphosphate (IP3) in isolated frog vomeronasal microvillar receptor neurones was tested by Gjerstad et al. (2003). The results indicate that local release of IP3 in the terminal vesicle of the vomeronasal neurones triggers transient depolarizations and induces action potentials and that IP3 might be a second messenger in the vomeronasal microvillar receptor neurones. The presence and content of serotonin and catecholamines in the vomeronasal organ of frog was tested by means of high-performance liquid chromatography. Measurable amounts of serotonin, adrenaline and noradrenaline were found in the vomeronasal organ of adult individuals of frog (and in mice also). The amine content varied with sex of adult animals (Zancanaro et al., 1997).

The pheromonal role of progesterone, and probably of prostaglandin E₂ (PGE₂), stimulatory role in inducing progesterone release in crested newt *Triturus cristatus* was investigated. The pheromonal activity of prostaglandin F₂-alpha (PGF₂-alpha) and PGE₂ cannot be excluded. The increase of PGF₂-alpha-dependent estradiol at the end of reproduction could be interpreted as a mechanism for interruption of the abdominal gland activity (Cobbetti and Zerani, 1992).

The amphibian pheromones are secreted especially in skin glands. The significance of these glands as producers of pheromones was described for example by Quay (1977).

3. Antipredator chemosignals in adults

The attraction to conspecific chemical cues in nonlarval anurans was first demonstrated in the toad *Bufo cognatus*. Postmetamorphic toads of this species formed aggregations and were attracted to areas that had been previously occupied by postmetamorphic conspecifics. This suggests that orientation to chemical cues is involved in the aggregation response. There is a hypothesis that aggregation may be an anti-predator strategy of this species (Graves et al., 1993). Responses to alarm infochemicals from injured prey may influence predation risk and foraging success of receivers and senders, while learning can influence the strength of these responses. Red-spotted newts (*Notophthalmus viridescens*) do not produce alarm chemicals until late in larval development, but can respond to predation-related chemical cues soon after hatching (Rohr et al., 2002). Two tested species of salaman-

der, fire-bellied newts (*Cynops pyrrhogaster*) and red-spotted newts (*Notophthalmus viridescens*) avoided chemical alarm cues from a conspecific damaged skin extract. *Notophthalmus viridescens* avoided also chemical cues from *C. pyrrhogaster* skin extract, but not *vice versa*. Both species did not avoid chemical cues from a conspecific viscera extract, conspecific heated skin extract and plethodontid salamander (*Desmognathus* and *Plethodon*) skin extracts. These facts suggest that the avoidance behaviour is an alarm response to chemical cues released from damaged newt skin and not a general response to chemical cues from damaged salamander tissue (Marvin and Hutchison, 1995). An avoidance response to chemical cues from damaged conspecific skin has an adaptive value in predator avoidance probably in terrestrial as well as aquatic vertebrates. Salamander *Desmognathus ochrophaeus* did not avoid denatured (heated) conspecific skin, fresh conspecific viscera, fresh mealworm (*Tenebrio molitor*), or fresh *Plethodon richmondi* (as heterospecific salamander) skin extracts. These results indicate that chemical alarm cues are present in the skin of tested salamander species, but are not present in meal-worm or the viscera of the experimental animal used (Lutterschmidt et al., 1994).

The black-bellied salamander (*Desmognathus quadramaculatus*) defends its territory (which is repeatedly used), from which it excludes conspecifics through a probable combination of a pheromonal signal and aggressive behaviour (including cannibalism as an ultimate aggressive response) (Camp and Lee, 1996).

In laboratory experiments with red-spotted newts (*Notophthalmus viridescens*), males of this species decreased their activity in response to macerated male newt extract and showed an intermediate attraction to gravid female odour paired with said chemosignal, which indicates that predators may inhibit mate search, and that male newts probably take greater risks during the breeding season. Field tests provided in ponds demonstrated different responses to male newt extract (the same reaction as in laboratory experiments, and no significant avoidance of male newt extract for either sex) – probably trade-off that incorporates risk and resource sensitivity (Rohr and Madison, 2001).

The effect of chemical cues from distressed conspecifics was studied in Ozark zigzag salamanders, *Plethodon angusticlavius*. Simultaneous influence of other chemical cues (in this case kairomones)

from predatory ringneck snakes, *Diadophis punctatus* (and for comparison physical attacks, too) were also investigated. Stressed individuals showed lower foraging success than unstressed salamanders. The physical attack did not result in a more intense response than the other treatments (Watson et al., 2004).

The first report of a repelling function of pheromones in vertebrates, described in red-spotted newts (*Notophthalmus viridescens*), is very interesting. Sexually motivated males of this species produce a pheromone with functions to repel other approaching conspecific males. These feromones may probably increase both the sender's and receiver's mating success when the sex ratio is male biased (Park and Propper, 2001).

The pheromone system of amphibians can be a potential target of chemical environmental contaminants. Experiments on red-spotted newt (*Notophthalmus viridescens*) females demonstrated that the pheromone system is highly susceptible to low concentrations of the insecticide endosulfan. The impairment of the pheromonal functions directly led to a disrupted mate choice and lowered breeding success. No other physiologic or behavioural changes were found by the experimental newts at the endosulfan concentrations administered. Thus this insecticide (and probably other contaminants), introduced into the environment by human activity, can affect survivorship and reproduction function of amphibians (Park et al., 2001). This suggests a possible influence of various other chemicals used in terraristic on the breeding success and welfare of these organisms.

4. Antipredator chemosignals in larval phase

Response to chemical alarm signals has been documented for some larval stage – tadpoles of frog and for several species of salamander, e.g. newt *Notophthalmus viridescens*. The hypothesis that *Notophthalmus viridescens louisianensis* can learn to recognize unfamiliar potential predator (smallmouth bass – *Micropterus dolomieu*) through association of predator signal with chemical alarm signals was also tested (Woody and Mathis, 1998). Experiments with the tadpoles of pinewoods tree frog (*Hyla femoralis*) exposed to dragonfly larvae (*Anax junius*) showed, that the response of tadpoles to predator included morphological modifications

with respect to the body and tail development. Bodies and tails were both deeper and shorter, and the overall body size was smaller. An influence on coloration was also found: a more pronounced orange tail fin coloration and black tail outline. Growth and development were slowed in these tadpoles. The triggers of morphological anti-predator response are both the metabolites of digestion and alarm cue. The joint action of both these cues resulted in a greater response (LaFiandra and Babbitt, 2004).

The results of interesting experiments with chemosignals on red-legged frog (*Rana aurora*) tadpoles suggest that these tadpoles release a chemical that provides conspecifics with an early warning of predator presence, and that ammonium (NH_4^+) may be a component of the disturbance signal. Disturbed animals increased ammonium (their main metabolic waste) excretion relative to undisturbed individuals. The tadpoles responded by antipredator behaviour when exposed to chemical cues of disturbed conspecifics (or to low-level ammonium concentrations, 1 mg NH_4^+ /litre), but not when exposed to chemical cues of control (undisturbed) conspecifics (Kiesecker et al., 1999).

The conspecific alarm cues released from injured individuals of the same species, chemical substance of predatory invertebrates (backswimmers *Notonecta* spp.) and related nonpredatory invertebrates (water boatman – Corixidae) were tested in tadpoles of western toad (*Bufo boreas*). Tadpoles living in the presence of conspecific alarm cues and chemosignals of specific predators reduced the time of metamorphosis and, consequently, reduced the time in the presence of its predators (Chivers et al., 1999). The presence of conspecific alarm substances in water and predators' waste products may have an important role in the chemical detection of predators by tadpoles of two anuran species, the common frog, *Rana temporaria*, and the common toad, *Bufo bufo*, for which a slight but non significant reduction in swimming behaviour was found in presence of chemical cues from starved sympatric predators (larval spotted salamander, *Salamandra salamandra*; larval dragonfly, *Anax imperator*). Swimming activity of both species tadpoles was also found to be significantly decreased in response to chemical cues released by crushed conspecifics, but test solutions from starved crawfish *Astacus leptodactylus*, a recently introduced predator, produced no change at all in behaviour (Marquis et al., 2004).

Interactions between tadpoles of the southern leopard frog (*Rana utricularia*) significantly decreased the growth and increased the mortality of the green treefrog (*Hyla cinerea*) tadpoles. There is a competition involving both behavior reaction and chemical interference between these two species. *Rana utricularia* tadpoles apparently use both chemical interference and aggressive behavior in securing a competitive advantage over *H. cinerea* tadpoles, who suffer from these interactions. In this way, *R. utricularia* tadpoles may increase their chance of survival and metamorphosis in natural conditions. By contrast, *H. cinerea* tadpoles, which avoid interactions both with conspecifics and with *R. utricularia* tadpoles, may increase their own chances of survival and metamorphosis (Faragher and Jaeger, 1998).

The response of tadpoles of red-legged frog (*Rana aurora*) to tadpole-fed newts (*Taricha granulosa*) appears to be similar to their response to tadpole extract in eliciting alarm, while insect-fed newts probably have less of an effect since predators consuming other species may be less of a threat. The behaviour of tadpoles was studied in response to an extract of crushed tadpoles, both alone and in combination with newts (*Taricha granulosa*). Newts were fed a diet of either *R. aurora* tadpoles or insect larvae (Wilson and Lefcort, 1993).

It is known that tadpoles manufacture, and release in water, alarm pheromones designed to control the behaviour of other tadpoles in the same locality. These chemosignals are released by tadpoles threatened by predators (direct attack or detection of the predator scents). In some cases these "killer" pheromones exert their effects across species. This was found for in the Common Toad, *Bufo bufo*, and the Natterjack, *B. calamita* (Allen, 1993). The same or similar "killer" pheromones do not represent any problem for these species, because the two species live in the different habitats.

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