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Should I stay or should I go? The influence of temperature and sex on predator-induced responses in newts

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Ectotherms compensate for variation in thermal environments in diverse behavioural ways. Despite widely occurring sex differences in morphology and physiology, the sex-specific thermal sensitivity of behavioural traits has received little attention. We tested the interactive influence of sex and environmental temperature on predator-induced responses in the Alpine newt, *Ichthyosaura alpestris*. Escape decisions and the duration of flight were less thermally sensitive in females than in males. Males ran more slowly than females. Both sexes required more stimuli at intermediate temperatures than at other temperatures. We conclude that temperature and sex are important determinants of a newt's defensive repertoire. The sex-specific thermal sensitivity of defensive responses suggests both asymmetric selection regimes and an impact of environmental change in newt populations.

Temperature affects all aspects of organismal biology, from biochemical reactions to fitness components, and thus represents a powerful selective factor in the wild. Many ectotherms cope with immediate variations in environmental temperatures by showing various behavioural responses that may include thermoregulation and compensation for thermal constraints imposed on performance traits. Behavioural thermoregulatory mechanisms range from simple movements across water columns in aquatic organisms (Marek & Gvoždík, 2012; Ward, Hensor, Webster, & Hart, 2010) to various basking postures and shuttling behaviour in thermoregulating lizard taxa (Bauwens, Hertz, & Castilla, 1996; Cadena & Tattersall, 2009). Behavioural compensation depends on the individual ectotherm's cost/benefit ratios (Broom & Ruxton, 2005; Ydenberg & Dill, 1986) and the limits of a particular behavioural response, which are determined by the thermal sensitivity of the underlying physiological machinery (Herrel, James, & Van Damme, 2007).

A typical example of the compensatory response is predator-induced behaviour. Since a seminal paper by Hertz, Huey, and Nevo (1982), it has been demonstrated that many lizard taxa modify their defences depending on their body temperature (de Barros, de Carvalho, Abe, & Kohlsdor, 2010; Crowley & Pietruszka, 1983; Herrel et al., 2007; Mautz, Daniels, & Bennett, 1992). Generally, lizards with high body temperatures flee predators, whereas they defend themselves by biting as their body temperature drops. From a mechanistic basis, the contractile dynamics of lizard limb muscles engaged in sprinting are more thermally sensitive than the maximum force production of jaw muscles (Herrel et al., 2007). However, in other groups, the potentially fatal outcome of predator–prey interactions can lead to the evolution of diverse behavioural responses determined not only by muscle performance, but also by the presence of morphological (aposematic coloration) and chemical (skin secretion) defences (Ruxton, Sherratt, & Speed, 2004). The context dependency of complex defensive strategies remains little understood.

The thermal sensitivity of defensive strategies is further complicated by sex differences. In many taxa, the sexes differ not only in body size but also in hormone levels, body fat, energy metabolism and performance traits (Lailvaux, 2007; Van Damme, Entin, Vanhooydonck, & Herrel, 2008). Accordingly, these differences may influence thermally induced defensive decisions of both sexes in disparate ways. For example, male lizards run faster to escape human predators, whereas slower females rely on crypsis (Lailvaux, Alexander, & Whiting, 2003; see also Shine, Olsson,

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Lemaster, Moore, & Mason, 2000). Surprisingly, the interactive influence of sex and temperature on defensive responses has received limited attention.

In this study, we examined the influence of temperature and sex on predator-induced responses in Alpine newts. Newts and salamanders are suitable for this task because their defensive strategy combines various components such as aposematic postures, defensive secretion, immobility or flight from predators (Brodie, 1977; Ducey & Brodie, 1983). Although there are field observations of antipredator postures and immobility in this species (Brodie, 1977; Kupfer & Teunis, 2001), recent laboratory studies have mainly focused on burst escape responses (Gvoždík & Van Damme, 2006, 2008; Šamajová & Gvoždík, 2010). Field observations are often performed during spring migrations. Since newt running speed decreases at lower body temperatures (Gvoždík & Van Damme, 2008), a possible solution to the posture–running contradiction is the thermal sensitivity of newt defences (Brodie, Ducey, & Lemos-Espinal, 1991; Dodd & Brodie, 1976; Mochida, 2010). In addition, sexual dimorphism may contribute to sex differences in predator-induced responses: as in other newt taxa (Malmgren & Thollesson, 1999), males have longer limbs and a shorter trunk than females (Kalezić, Crnobrnja, Dorović, & Džukić, 1992). Although both sexes run at similar maximum speeds in the laboratory (Šamajová & Gvoždík, 2010), the relatively shorter limbs in females may increase transport costs (Pontzer, 2007), thereby reducing endurance and the duration of the escape response. Accordingly, females should become exhausted sooner and rely on crypsis more frequently than males. In this study, we predicted that (1) at lower body temperatures, newts would respond to the tactile stimulus of a snake predator with an antipredator posture, immobility or defensive secretion, but would flee at higher temperatures; and (2) males would respond to the same stimulus more frequently by fleeing, and their flight episodes would last longer than females'.

METHODS

Study Species and Maintenance

The Alpine newt, *Ichthyosaura* (formerly *Triturus*) *alpestris*, is an approximately 10 cm long (total length) amphibian distributed widely from northwest to southeast Europe. It usually has a biphasic lifestyle with a predominantly aquatic period from April to June and a terrestrial period during the rest of the year. Habitat use is not strictly separated, as aquatic individuals frequently leave water and vice versa (Kopecký, Vojar, & Denoël, 2010; L. Gvoždík, personal observation). Adults feed on various invertebrates, mostly chironomid larvae. The newts' predator, a grass snake, *Natrix natrix*, frequently occurs syntopically with Alpine newts. Various newt taxa, including the species studied, provide 15–30% of the snake's diet (Kabisch, 1999).

Adult newts (snout–vent length: 40–56 mm; body mass: 1.8–4.9 g; $N_{\text{males}} = 13$; $N_{\text{females}} = 14$) originated from a population located near Jihlava, Czech Republic. In April 2011, reproductive newts were captured by hand or netting and transported (45 min drive) in plastic aquaria (40 × 25 cm and 30 cm high; 5–6 newts per aquarium) filled with water (10 litres) to the research facility. The car air-conditioning system was set to 18 °C to avoid excessive increases in aquarium water temperatures. Transport had no obvious effect on the health and mortality of newts. Pairs of newts (one male and one female) were placed in aquaria (50 × 30 cm and 18 cm high) filled with 18 litres of tap water and equipped with water plants and a piece of Styrofoam that allowed newts to leave the water. Aquaria were placed in a temperature-controlled room with diel temperature fluctuations between 12 and 22 °C and a natural light period. Temperature controllers were programmed to increase the temperature gradually to a daily maximum at 1500 hours, followed by a gradual decline to the minimum at 0500 hours. This covered the majority of temperatures that newts experience in the field and prefer in laboratory thermal gradients (Marek & Gvoždík, 2012; Šamajová & Gvoždík, 2010). Newts were fed with live chironomid larvae, *Tubifex* worms or plankton once or twice per week. Water (half volume) was replaced regularly at weekly intervals.

The grass snake (total length: 593 mm; male) was caught by hand from near the research facility (Studenec, Czech Republic) 2 weeks before the experiments. The snake was transported in a cloth bag and within a few minutes housed in a terrarium (50 × 30 cm and 18 cm high). The terrarium was equipped with a paper substrate, a shelter, a heating stone and a bowl with tap water. It was placed in a temperature-controlled room (12–22 °C, heated spot at 35 °C) with a natural light period. The snake was fed with thawed fish and thawed baby mice (obtained from a local pet dealer) twice weekly.

All experimental procedures (for newts and the snake) were approved by the Expert Committee for Animal Conservation of the Institute of Vertebrate Biology AS CR (research protocol no. 113/2009). The Agency for Nature Conservation and Landscape Protection of the Czech Republic issued permission to capture the newts (1154/ZV/2008). After the experiments (September 2011), all animals (newts and the snake) were healthy and in good condition, and thus, in line with our permit conditions, were released at the site of their capture.

Predator Trials

The newts' responses to tactical stimuli of the snake were recorded in a circular arena (diameter 50 cm) with the bottom covered by moistened filter paper. Uniform light (ca. 1000 lx) was provided by a series of fluorescent tubes on the ceiling of the room. Trials were performed at three operative temperatures of newts: 12, 17 and 22 ± 0.5 °C (see above for their ecological relevance) maintained by a regulated cooling/heating system. Operative temperatures were continuously measured inside copper cylinders covered with moist paper using a calibrated digital thermometer (HH 22, Omega Engineering, Stamford, CA, U.S.A.). Previous experiments confirmed that these temperatures approach the equilibrium body temperatures of anaesthetized newts (L. Gvoždík, unpublished data). The copper cylinder was placed in the experimental room for the entire duration of the measurement period. The order of temperatures was randomized for individual newts. All trials were recorded using a digital camcorder (50 Hz; Panasonic NV-GS500, Matsushita Electric Industrial, Osaka, Japan) mounted perpendicularly above the arena.

Newts were placed individually in bowls (0.5 litres) with water from their aquaria and left undisturbed in the experimental room until the water temperature in the bowl equilibrated with the operative temperature (30–60 min). A randomly chosen newt was placed in the middle of the arena and covered with a plastic bowl 60 s before a trial for resting. The bowl was slowly removed so as not to disturb the animal. A snake held by hand, with its head and anterior length of body (ca. 10 cm) free, was slowly moved towards the newt to allow the tip of the snake's tongue to touch the posterior part of the newt's tail. This tactile stimulus has been successfully used to elicit antipredator responses in other caudate amphibians (Ducey & Brodie, 1983). Since Ducey and Brodie found no differences in a salamander's response to the snake's tongue when snakes were either free-moving or hand-held, we considered this stimulus to be an ecologically realistic predator tactile cue. We aimed the tactile stimulus towards the newt's tail, because we assumed that this body part is touched first by a scent-tracking snake. If a newt did not respond to the first tongue touch, the stimulus was repeated up to five times in four series, separated by 20 s of rest (total 20 stimuli). A newt not responding to these stimuli was considered immobile. If a moving individual stopped, it