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Do Bufonids Employ Different Anti-Predator Behaviors Than Ranids? Comparison among Three European Anurans

Krzysztof Kowalski[1], Olga Sawościanik[1], and Leszek Rychlik[1]

European anurans are prey for a variety of predators, against which they have evolved a range of defense behaviors. We investigated defensive behaviors of three European anurans: the Common Toad (*Bufo bufo*), the Common Frog (*Rana temporaria*), and the Edible Frog (*Pelophylax esculentus*) during interactions with a predator (hedgehog) and a control stimulus (rabbit). We hypothesized that (H1) due to small capacity to flee quickly from a predator, *B. bufo* has evolved a more diverse repertoire of behavioral defenses than the ranids, *R. temporaria* and *P. esculentus*. We also hypothesized that (H2) *B. bufo* can minimize the secretion of metabolically costly poison through behavioral control. According to our predictions, the repertoire of defensive behaviors was more complex in *B. bufo* than in the ranids. Also, the number of threatening behaviors was higher in toads than in both frog species. Fleeing was the most common response employed by all tested anurans. We report a new anti-predator behavior in the Common Toad: head hitting. As *B. bufo* released the poison only after squeezing of parotoid macroglands by the predator, we conclude that the Common Toad can minimize poison release through behavioral control. Our data suggest that length of hind-legs and, related to this, mode of locomotion (jumping vs. hopping) can affect anti-predator behavior in anurans.

FOR decades, predator-prey interactions have been among the main issues in ecological studies. Research on predation risk has been fundamental to understanding prey species ecology, as predation affects prey foraging, mating, and reproduction (Otter, 1994; Candolin, 1998; Dill et al., 1999). Additionally, predation may be a selective agent influencing prey traits such as morphology (e.g., spine presence), physiology (e.g., toxins production), or behavior (e.g., development of alarm calling behavior in many bird and mammal species; Abrahams, 1995; Lenzi-Mattos et al., 2005; Hollén and Radford, 2009; Mailho-Fontana et al., 2014).

Predator-prey interactions have been comprehensively examined across taxa including spiders (e.g., Heiling and Herberstein, 2004), insects (e.g., Eisner et al., 2005), fishes (e.g., Wainwright and Turingan, 1997), reptiles (e.g., Roth and Johnson, 2004), birds (e.g., Møller and Ibáñez-Álamo, 2012), and mammals (e.g., Eilam et al., 1999). Amphibian research has been mainly focused on interactions between tadpoles and their predators (Watt et al., 1997; Álvarez and Nicieza, 2009; Bennett et al., 2013; Bennett and Murray, 2014, 2015). The influence of predation on adult amphibians, particularly temperate anurans, has not been well studied.

Animals can adapt morphological, physiological, chemical, or behavioral mechanisms to avoid predation and enhance the chance of survival, even after detection by predators (Langerhans, 2007). Anurans are not typically able to run or jump for a long time or distance, and instead employ various behavioral responses to minimize the probability of predator attack. So far, more than 30 categories of defensive behaviors to predator attack have been described in anurans, primarily from field studies of Neotropical species (Jared et al., 2009; Toledo et al., 2011). Many aspects of temperate-zone anuran ecology have also been investigated (Jørgensen, 1983, 1986; Blaustein et al., 2001; Engeler and Reyer, 2001; Johansson et al., 2005; Sinsch and Schäfer, 2016; Cayuela et al., 2017; Kuczynski et al., 2017), but predator-anuran prey interactions are poorly understood. For instance, the behavioral response repertoire of European anurans is yet to be fully described (Marchisin and Anderson, 1978; Grant, 2001; Nishiumi and Mori, 2015).

The ranid frogs (family Ranidae) and bufonid toads (Bufonidae) are among the most common anurans of temperate Europe (Arnold and Ovenden, 2002; Halliday, 2016). It is well known that amphibians are semi-aquatic and their larval development depends on water. Nonetheless, some species are more terrestrial, whereas others spend more time alongside waterbodies, where they can actively search for food. Habitat strongly influences anuran locomotor performance, foraging, and predator avoidance (Rand, 1952; Nauwelaerts et al., 2007; Petrović et al., 2017). The semi-aquatic frog species are characterized by long hind-limbs and better jumping performance, whereas the terrestrial toads predominantly walk or hop instead of jumping due to their relatively short hind-limbs (Rand, 1952; Gomes et al., 2009; Petrović et al., 2017). Semi-aquatic anurans that sit alongside waterbodies avoid terrestrial attacks by waiting until a predator is relatively close (Juszczyk, 1987), jumping into the water, diving to the bottom, and hiding in the mud (Nauwelaerts et al., 2007). Bufonids are unable to flee quickly and must employ other behavioral responses to enhance their probability of survival.

Additionally, semi-aquatic and terrestrial anurans are prey for different suites of predators. Semi-aquatic species are in danger of being attacked by both aquatic and terrestrial predators. Mustelids and some wading birds are the most common predators preying upon ranids (Kahl, 1987; Sidorovich and Pikulik, 1997; Jakubas and Mioduszewska, 2005). On the other hand, terrestrial anurans (e.g., toads or pelobatids) are predominantly attacked by terrestrial predators including snakes (e.g., grass snakes), hedgehogs, badgers, or raptors (e.g., buzzards; Ewert and Traud, 1979; Juszczyk, 1987; Sidorovich and Pikulik, 1997; Jovanovic et al., 2011). However, during breeding season they may be prey for predators foraging actively alongside watercourses. Furthermore, most ranids are mainly active during the day, whereas the toads are active at night (Renaud and Stevens, 1983; Juszczyk, 1987); thus, the ranids are usually hunted by diurnal predators, while the toads by nocturnal ones.

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As the ranids (e.g., the Edible Frog) and bufonids (e.g., the Common Toad) inhabit different habitats, vary in locomotor performance, and face different types of predators, they presumably adapt different behavioral strategies to avoid predation and enhance the chance of survival. Therefore, we hypothesized that (H1) due to small capacity to flee quickly from a predator, *Bufo bufo* has evolved a more diverse repertoire of behavioral defenses than the ranids, *Rana temporaria* and *Pelophylax esculentus*. We predicted that (P1) the number of categories of defensive behaviors and (P2) the number of threatening behaviors would be higher in the Common Toad than in both ranid species, and (P3) both ranids would flee most frequently to avoid predator attack, whereas the Common Toad would remain motionless or employ reactions other than fleeing to avoid predation. In contrast to the tested ranids, *B. bufo* secretes strong toxins from parotoids and other skin glands (Juszczyk, 1987; Hutchinson and Savitzky, 2004). Therefore, toads might display at least some behavioral patterns related to the use of this weapon as an alternative to fleeing. However, since poison production is metabolically costly (McCue, 2006; Nisani et al., 2007), according to the venom optimization hypothesis (Wigger et al., 2002), the poisonous animals should evolve various strategies (e.g., morphological adaptations or behavioral responses) to minimize the toxin expenditure and release the poison frugally (Morgenstern and King, 2013). Therefore, we hypothesized that (H2) the Common Toad can minimize the secretion of metabolically costly poison through behavioral control. We predicted that (P4) at first *B. bufo* would avoid predation through remaining immobile or displaying threatening behaviors, such as body raising, body-tilting, or puffing up the body, and (P5) would release the poison only after a physical predator attack and squeezing of the parotoid macroglands. The aim of our study was to describe the repertoire of behavioral responses to predators of the Common Toad and two ranid species in the context of these hypotheses.

MATERIALS AND METHODS

Trapping procedure and animal housing.—Between March and August 2015 we captured 20 adult Common Toads, *Bufo bufo* (18 males and 2 females), 10 Common Frogs, *Rana temporaria* (8 males and 2 females), and 10 Edible Frogs, *Pelophylax esculentus* (7 males and 3 females). All animals were captured in gardens and parks (toads) or near small waterbodies (frogs) in Poznań (western Poland). To capture toads, 12 plastic buckets (arranged in two lines; 10 L each) were dug into the ground and a 50 cm high fence (made of black plastic) was set between them. The buckets were checked twice a day (early in the morning and after dusk) from March to April. Frogs were captured using a net. Captured animals were placed into plastic transporters (up to 5 animals per transporter; $9 \times 21 \times 28$ cm; 23 L) and carried to a laboratory by car, where their snout–vent length was measured (mean SVL [mm] ± SEM: *B. bufo*: 64.92±1.13; *R. temporaria*: 83.16±4.94; *P. esculentus*: 76.47±3.24). Next, they were placed into large ($46 \times 30 \times 28$ cm; 39 L) aqua-terraria (up to 4 animals per terrarium) equipped with bedding (a mixture of peat and sand). The terraria were regularly irrigated to maintain adequate humidity. Each terrarium contained a shelter (flowerpot) and a water bowl to allow amphibians to submerge in the water. Food (mealworms and crickets) and water were provided *ad libitum*. The anurans were kept (up to 3 weeks) in the animal room under standard laboratory conditions (temperature: 19±1°C; humidity: 65–70%; artificial photoperiod: 12L:12D) and after behavioral trials, were released at the site of capture. One toad died after the experiment as a result of injuries (see Results).

Hedgehogs (8 specimens of the Northern White-breasted Hedgehog, *Erinaceus roumanicus,* and 2 specimens of the West European Hedgehog, *E. europaeus*) were captured by hand in parks and gardens in Poznań, then placed into transporters ($25 \times 32 \times 41$ cm; 33 L), carried to the laboratory by car, weighed (mean body mass [g] ± SEM: *E. roumanicus*: 804.37±11.85; *E. europaeus*: 799.0±4.24) and placed individually into large ($80 \times 50 \times 120$ cm; 480 L) boxes equipped with bedding (a mixture of peat, sand, and hay). Food (mealworms, earthworms, and veal) and water were provided *ad libitum*. To ensure the same starvation level of hedgehogs, we fed them once a day and 24 h before the experiment the following day. The hedgehogs were kept (up to 7 days) in the animal room under the same standard conditions as anurans, and after behavioral trials were released at the site of capture. Among the ten tested hedgehogs only five hunted anurans, none of them showed symptoms of poisoning, irritation, or paralysis after biting toad parotoid glands and licking the poison, and none of them died after the experiment.

To perform control tests, one Domestic Rabbit, *Oryctolagus cuniculus* f. *domesticus* (wild coloration, body mass similar to those of the tested hedgehogs: 773.0 g), was purchased from a pet shop. The rabbit was kept in a large ($80 \times 50 \times 100$ cm; 400 L) cage equipped with bedding (a mixture of sawdust, sand, and hay). Food (carrots, sunflower seeds, pelleted food, and fresh, green leaves of the Dandelion, *Taraxacum officinale*) and water were provided *ad libitum*. The rabbit spent four weeks (2 weeks in early spring and 2 in summer) in the animal room under the same conditions as described above.

Experimental design.—In total 80 behavioral tests were carried out. At first, 40 tests with the Common Toad were performed: 20 with a living predator (hedgehog) and 20 with a rabbit (control tests). As the rabbit is an herbivorous mammal, we expected that it would not be interested in hunting and biting toads, but ignore them. And similarly, the toads, in the presence of the rabbit, were not expected to display such threatening behaviors as body raising, puffing up the body, body-tilting, or poison releasing. Next, 40 tests with the ranids were carried out: 20 tests with the hedgehogs (10 tests with the Common Frog, 10 with the Edible Frog) and 20 control tests with the rabbit. Each anuran prey was tested only once with the hedgehog and once with the rabbit. All ten hedgehogs captured in the field were involved in the experiment. Each hedgehog was chosen randomly and tested a maximum of four times (usually 3) for each type of anuran prey. Because of similar ecology and diet composition (occasionally including anurans; Pucek, 1981; Corbet, 1988), as well as their co-occurrence in Poznań, both species of hedgehogs were considered as one type of predator.

All tests were performed in an empty laboratory on a closed arena (diameter: 1 m, height of walls: 40 cm) with a white floor. To allow hedgehogs to hunt anurans (as well as prevent toads and ranids from hiding, digging, or diving), the arena was not equipped with shelters, bedding, or water tanks. In tests with ranids, the arena was covered with a transparent lid to prevent frogs from jumping out of the arena. Anuran prey and predator (hedgehog) or control stimulus (rabbit) were placed into the arena simultaneously. Initially, to prevent them from seeing each other, they were separated in different

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halves of the arena with a non-transparent wall. After five minutes (given to animals for habituation), the wall was removed and the experiment began. Interactions between animals were recorded with a digital video-camera (Sony HDR-PJ780) for 30 min. The arena was illuminated by a light bulb (25 W); however, the light was not directed towards the tested animals. Each experiment was performed in the presence of an observer invisible to the animals who simultaneously noted observed reactions of anurans on a paper sheet. The observer was sitting motionless on a chair behind the video-recorder in a corner of the laboratory at the distance of 1 m from the arena. He did not interfere in the interaction between tested animals. After each test, the arena floor was cleaned with ethanol to remove the scents left by the tested animals. The following test began ca. 15–25 minutes after the end of the previous one. We usually conducted up to five tests a day. All of them were performed under standard laboratory conditions (temperature: 19±1°C; humidity: 65–70%).

Data analysis.—Behavioral responses of anurans have been named in accordance with the terminology proposed by Toledo et al. (2011). However, as the Common Toad is poisonous (not venomous, according to the definitions of Mebs, 2002), we replaced the term 'venom releasing' by 'toxin (or poison) releasing'. We distinguished three classes of behaviors (Table 1): escape (including only a few ways of fleeing), freezing (immobility and crouching down), and threatening behaviors (others). Defensive behaviors of anurans were registered while the prey was being approached or attacked by a predator (or the rabbit in control tests). We recorded (1) diversity of defensive reactions (number of different categories displayed by tested anurans) and (2) frequency of behavioral responses employed by anurans to avoid predation. As regards frequency, the results of observations of a given category among all observed defensive behaviors of toads and frogs are presented in percentages. Differences in categories number and frequency of behaviors employed by the tested anurans were analyzed by performing Chi-Square Goodness of Fit Test, while differences in the mean number of reactions displayed per 1 test (with hedgehog vs. rabbit) by Wilcoxon Signed-Rank Test. Differences in body sizes (snout–vent lengths) between toads and frogs were compared by Mann-Whitney *U*-test. All statistical analyses were carried out using R software (R Core Team, 2015). Differences were considered as statistically significant for P values less than 0.05.

RESULTS

Behavioral responses of the Common Toad.—In the tests with the hedgehogs, we distinguished 11 categories of behavioral responses of the Common Toad to predator. Fleeing followed by immobility were employed by the toads most frequently (52.85% and 30.95%, respectively; Fig. 1A). In 3% of these reactions, fleeing was accompanied by cloacal discharge, which occurred rarely and constituted 1.43% of all the recorded reactions. Moreover, in spite of discharging of the cloaca, the toads were unable to avoid predator attack. We observed repeatedly that fleeing was not a good strategy to avoid predation because the hedgehogs responded mainly to the toads' movement. As soon as a toad began to flee, a hedgehog started to follow and chase it. During hunting the hedgehog sniffed intensively and its motion became more vigorous. Among threatening behaviors, puffing up the body, body raising, and body-tilting were the most common responses employed by the toads to avoid predation. However, in comparison to fleeing and immobility, the three mentioned categories were rare (2.62%, 1.66%, and 1.66%, respectively; Fig. 1A). Puffing up the body always consisted of filling the lungs with air and usually was coupled with body raising with legs stretched vertically. However, quite frequently (36.37% responses) we observed puffing up the body without body raising. The toads usually remained immobile with legs stretched laterally and filled their lungs with air. Similar to puffing up the body, body-tilting commonly co-occurred with body raising. It always involved tilting the dorsum and parotoid glands towards the predator. Body raising was usually coupled with chin-tucking (90% of observations) and sometimes with defensive vocalization (distress calls; 15.71%).

Poison releasing was among the rarest responses displayed by the Common Toad (1% of observations; Fig. 1A). In our experiment, it was always accompanied by puffing up the body. We never observed toxin release coupled with crouching down. Toxic secretions were released by the toads only after the hedgehog attack, i.e., after squeezing of parotoid macroglands. It was always accompanied by the release of poison from the glands covering the whole toad dorsum. However, secretion of toxins from dorsal glands occurred even after the cessation of attack by the hedgehog and did not require squeezing. Poison releasing was still unable to prevent the toad's death. Firstly, biting of inflated prey was easier because such prey was not able to move and flee quickly. Quite frequently (63.62% reactions) we observed the hedgehogs hunting and biting inflated toads. The bufonids were usually bitten in limbs, dorsum, head, and the postorbital region, where the parotoid macroglands are located. We observed no salivation, poisoning, or paralysis in hedgehogs licking the toxins from toad dorsums. Instead, we observed the hedgehogs licking their spines with the toad poison.

Head hitting was exhibited only once by the Common Toad (Fig. 1A); it was initiated when the hedgehog was less than 10 cm from the toad. Initially, the toad remained motionless (Fig. 2A), but when the hedgehog approached the toad and started to sniff it (Fig. 2B), the toad hit the predator with its head once (Fig. 2C, 2D; video in Supplementary Materials, see Data Accessibility). While the hedgehog continued to sniff the prey, the toad remained immobile and crouched down, filling its lungs with air and gently tilting its dorsum towards the hedgehog (Fig. 2E). The toad remained in this position for approximately 40 seconds (Fig. 2F) and then fled.

Eight toads were attacked and frequently bitten by the hedgehogs, but in seven cases we did not observe any injuries, such as bleeding or wounds. Only one toad released a lot of mucous and toxic secretions from parotoid and dorsum glands; its whole body was bloated. The toad was unable to move and died briefly after the experiment.

In control tests, we recorded five defensive reactions of Common Toads to the rabbit. The most common reaction was immobility (64.65% of all the recorded behaviors) followed by fleeing and crouching down (22.22% and 11.11%, respectively). The other two behavioral responses, i.e., defensive vocalization (distress calls) and chin-tucking, were rare (1.01% in both cases; Fig. 1A). In tests with the rabbit, we did not observe poison releasing and threatening behaviors such as body raising, body-tilting, or puffing up the body (Fig. 1A).

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Table 1. Anti-predator behaviors employed by anurans to avoid predation (named in accordance with the terminology proposed by Toledo et al., 2011).

Behavior category	Description	Possible function
Escape		
Fleeing	walking, jumping, withdrawing to move away quickly from a predator	escape quickly
Freezing		
Immobility	remaining motionless with holding the same posture before being approached by a predator	avoid detection by a predator
Crouching down	lowering the habitual sitting position, ranging from a slightly lowered posture to a full crouch with the chin touching the ground	avoid detection by a predator avoid subjugation
Threatening behaviors		
Body raising	stretching the legs vertically (or laterally) and keeping the snout close to, or touching, the ground surface, or fully stretching the legs and arms and lifting the belly and snout off the ground	avoid subjugation intimidate predator
Body-tilting	tilting the dorsum towards a predator	avoid subjugation intimidate predator
Puffing up the body	filling the lungs with air to enlarge the body size	avoid subjugation intimidate predator
Chin-tucking	pulling the chin toward the pectoral region and flexing the head towards the belly with closed eyes in some cases	avoid subjugation
Cloacal discharge	discharging excretions from the cloaca	avoid subjugation
Defensive vocalization	calling to intimidate a predator or alarm other animals	avoid subjugation
Head hitting	hitting a predator with the head	avoid subjugation intimidate predator
Poison releasing	releasing toxic secretions after approaching by a predator, toxins might be released voluntarily or while anuran is seized by a predator	avoid subjugation avoid ingestion/digestion

In the presence of the rabbit, Common Toads employed fleeing significantly less frequently (mean ± SEM: 1.1±0.2 reaction per 1 test) than in tests with the hedgehogs (11.1±1.8; Wilcoxon Test: $W = 136.0$, $P = 0.0004$). Also, immobility was displayed less frequently in control trials (3.2±0.38 reactions per 1 test) than in tests with the hedgehogs (6.5±2.84; $W = 181.0$, $P = 0.005$). There was no difference in the mean number of crouching down reactions when toads confronted by the hedgehogs and the rabbit were compared ($W = 53.5$, $P = 0.26$).

Behavioral responses of the ranid frogs.—In the tests with the hedgehogs, we observed six categories of behavioral responses of the Common Frog to predator. The most common was fleeing followed by immobility (75.76% and 18.37%, respectively), whereas the rarest were body raising and puffing up the body (0.54% in both cases; Fig. 1B). Body raising was usually accompanied by chin-tucking. In the tests with the rabbit, the Common Frogs displayed only four behavioral categories. Again, the most common was fleeing followed by immobility (65.31% and 32.65%, respectively), whereas crouching down and cloacal discharge were rare (1.02% in both cases; Fig. 1B). Cloacal discharge always co-occurred with fleeing. In the presence of the rabbit, Common Frogs employed fleeing less frequently (mean ± SEM: 6.4±1.45 reactions per 1 test) than in the tests with the hedgehogs (14.2±2.30; $W = 44.0$, $P = 0.01$). The mean numbers of immobility and crouching down reactions did not differ between the tests with the hedgehogs and the rabbit (immobility: $W = 28.0$, $P = 1.0$; crouching down: $W = 2.0$, $P = 1.0$).

Edible Frogs displayed only three categories of behavioral responses (fleeing, immobility, and crouching down) in the tests with the hedgehogs, and the most frequent was fleeing, which constituted 88.52% of all the recorded reactions (Fig. 1C). In control tests, we observed four categories. The most common was fleeing (62.22%), followed by crouching down and immobility (18.89% and 17.78%, respectively), whereas cloacal discharge was rare (1.11% of all the recorded reactions; Fig. 1C). Similar to the Common Frog, cloacal discharge in the Edible Frog was coupled with fleeing. In the presence of the rabbit, Edible Frogs employed fleeing significantly less frequently (5.6±2.06 reactions per 1 test) than in the tests with the hedgehogs (21.6±3.99; $W = 55.0$, $P = 0.005$). However, the mean numbers of immobility and crouching down reactions did not differ between the tests with the hedgehogs and the rabbit (immobility: $W = 29.5$, $P = 0.43$; crouching down: $W = 3.0$, $P = 0.27$).

The tested ranids were neither injured nor killed by the hedgehogs, and none of them were bitten. The ranids were extremely active, jumping from one place to another. Their motions were very vigorous, and leaps were long enough to avoid predation. We observed hedgehogs trying to follow and chase the frogs, sniffing a lot and tracking the ranids, but they were unable to approach and seize the prey.

Interspecific differences in defensive behavior.—The number of behavioral responses displayed by the Common Toad (11 categories) was higher than the number exhibited by the ranids (6 categories in the Common Frog and 3 in the Edible Frog). This difference was significant when the Common Toad was compared with the Edible Frog ($\chi^2 = 4.57$, df = 1, $P = 0.03$). The Common Toad displayed more threatening behaviors (8 categories) than the ranids (3 categories in the Common Frog and 0 in the Edible Frog). Again, the results were significant when responses of the Common Toad and the Edible Frog were compared ($\chi^2 = 8.0$, df = 1, $P = 0.004$). Both frog species employed fleeing more often than the Common Toad (*R. temporaria* vs. *B. bufo*: $\chi^2 = 4.41$, df = 1, $P = 0.03$; *P. esculentus* vs. *B. bufo*: $\chi^2 = 8.99$, df = 1, $P = 0.003$). On the other hand, the Common Toad remained motionless more often than the ranids. This difference was significant

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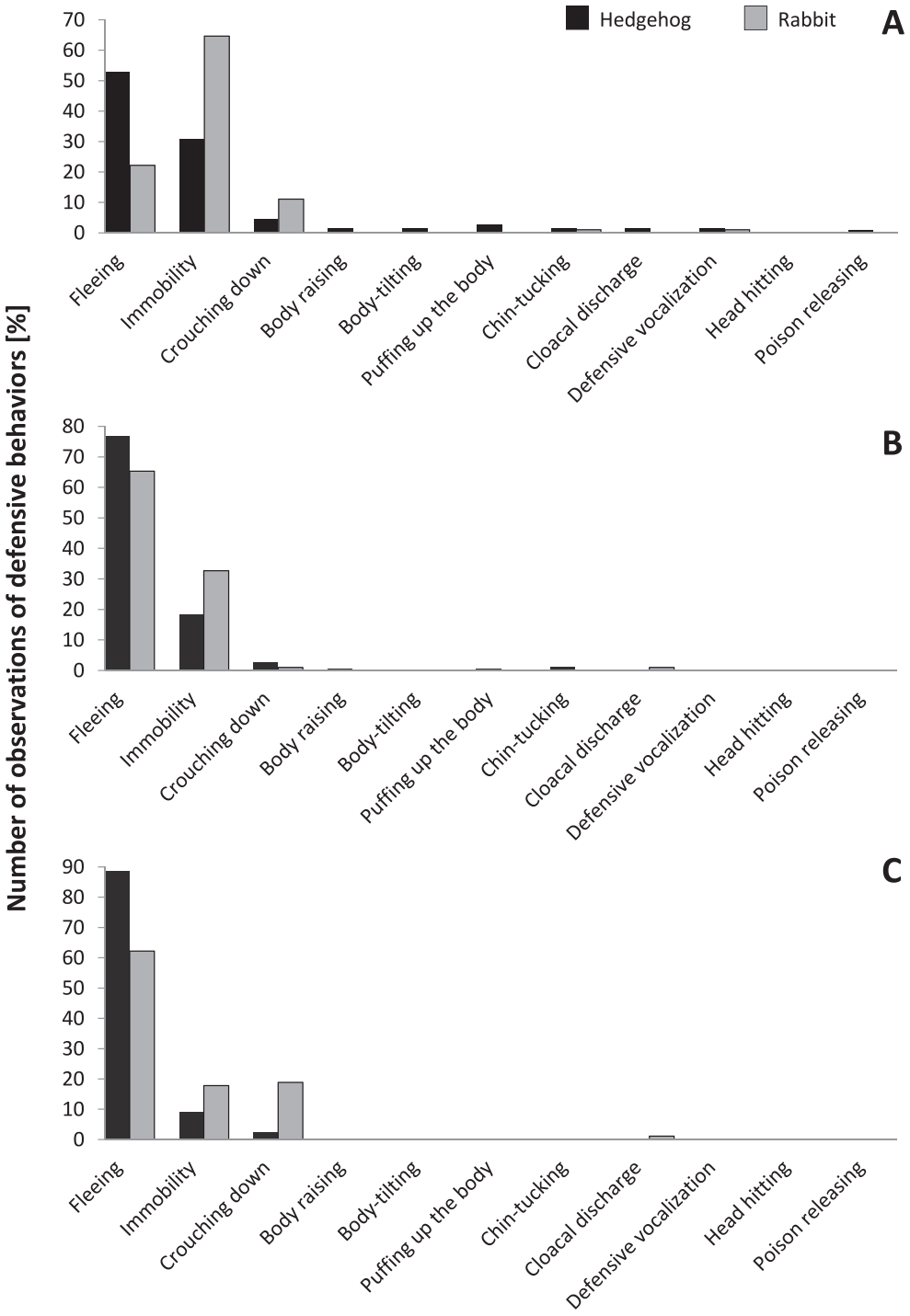


Fig. 1. Percentages of observed behavioral responses of (A) the Common Toad (sample size, the total number of recorded reactions, n_H = 423 with hedgehogs and n_R = 99 with rabbit), (B) the Common Frog (n_H = 185 and n_R = 98), and (C) the Edible Frog (n_H = 244, n_R = 90) in experiments with hedgehog and in control tests with rabbit.

when the Common Toad was compared with the Edible Frog ($\chi^2 = 12.04$, df = 1, $P = 0.0005$) and was almost significant in comparison to the Common Frog ($\chi^2 = 3.21$, df = 1, $P = 0.07$). There were no interspecific differences in the frequency of displaying crouching down, body raising, puffing up the body, and chin-tucking ($P > 0.05$ in all cases).

DISCUSSION

Our results support the prediction that the repertoire of behavioral responses of the Common Toad is more diverse than those of both ranid species, and that the number of threatening behaviors was higher in the Common Toad than in frogs. The third prediction was supported only partially because all three anuran species most frequently employed fleeing to avoid predation. Previous studies have shown that fleeing is one of the most common behavioral responses displayed by many animals to avoid or minimize the risk of predation (e.g., Kramer and Bonenfant, 1997; Eilam, 2005). It is not surprising that the ranids employed mainly fleeing to avoid predator attack as they usually live alongside waterbodies (Juszczyk, 1987; Berger, 2000) into which they jump to hide in the substrate or under floating vegetation (Nauwelaerts et al., 2007). On the other hand, bufonids including the Common Toad, which are more terrestrial than ranids (Juszczyk, 1987; Berger, 2000) and unable to perform long jumps or escape under the water, display certain strategies in addition to fleeing to enhance survival. For

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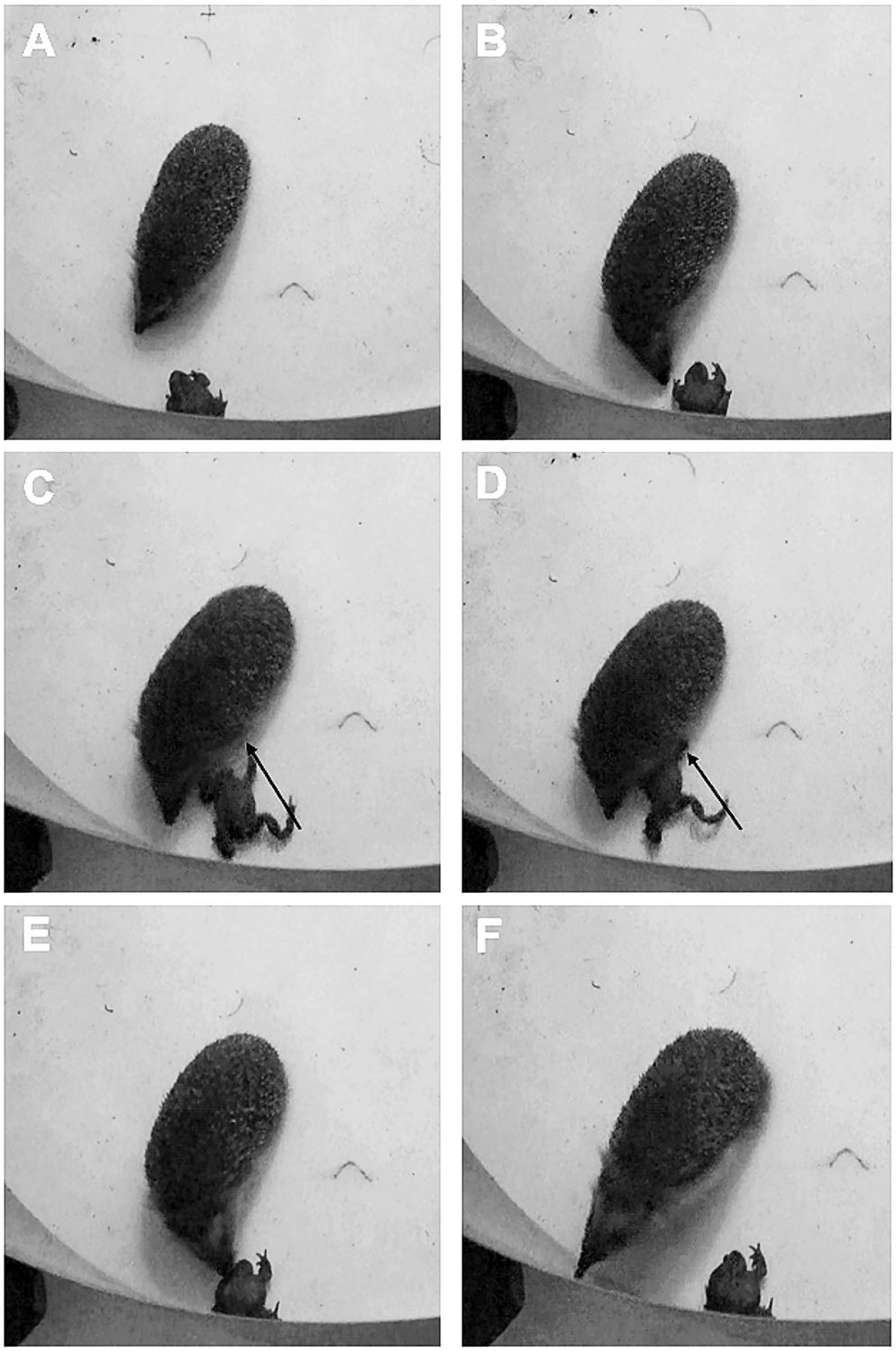


Fig. 2. Head hitting displayed by the Common Toad against the predator, a hedgehog: (A) an immobile toad; (B) the immobile toad sniffed by a hedgehog; (C) and (D) the toad hitting the hedgehog with its head (arrows indicate the attack direction); (E) after retreat, the toad is employing crouching down coupled with filling the lungs with air and gentle tilting the dorsum towards the hedgehog; (F) the toad remaining in this position after the attack cessation by hedgehog.

instance, Marchisin and Anderson (1978) found that immobility was the most common reaction employed by toads (81.4%) to avoid snake attack, usually coupled with crouching down. Immobility accompanied by crouching down and cryptic coloration seem to be efficient ways to minimize the probability of detection by a predator. In contrast to Marchisin and Anderson (1978), in our study fleeing was the most common behavior displayed by the Common Toad. Possibly despite the fact that *B. bufo* is characterized by short hindlimbs and cannot perform long leaps, it frequently employs small hops, which could increase the possibility of changing direction and consequently increase maneuverability (Nauwelaerts et al., 2007). A sudden change of direction may confuse a predator and enhance the probability of escaping. On the other hand, the anurans we tested were not provided with water tanks, shelters, or other structures; unable to dive or hide, they were often approached by the hedgehogs.

As predicted, the Common Toad displayed more threatening behaviors (8 categories) than both ranid species (3 categories in the Common Frog and 0 in the Edible Frog). But, in comparison to fleeing and immobility, threatening behaviors were rarely employed by the tested anurans. Our results indicate that for the Common Toad, such a type of defense is not an efficient strategy to avoid a predator in the

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size of a hedgehog or larger. Body raising and puffing up help to enlarge the size of prey, and puffing up the body makes prey difficult to swallow by snakes (Toledo et al., 2011). However, even some small predator species like grass snakes (*Natrix natrix*) are able to overcome inflated prey by biting the hind part of the prey's body and forcing exhalation (Ewert and Traud, 1979). According to our observations, puffing up the body was not a good strategy to avoid an attack by the hedgehog. As hedgehogs are unable to swallow the whole prey, they must subdue it. Biting of inflated prey is easier because such prey is not able to move and flee quickly (Toledo et al., 2011). The ranids displayed body raising and puffing up rarely. After being approached by the hedgehog, Common Frogs usually performed long jumps to avoid predator attack. Although the hedgehogs tried to chase the frogs, they were unable to catch and seize them because of the frogs' long hind-limbs and ability to jump far distances (Rand, 1952; Juszczyk, 1987; Gomes et al., 2009; Petrović et al., 2017), making fleeing the most efficient predator-avoidance strategy for frogs. Morphological traits, such as hind-limb length, body mass, snout–vent length (SVL), or locomotion mode (Rand, 1952; Nauwelaerts et al., 2007; Gomes et al., 2009) can influence the diversity of anuran behavioral responses. In our study, the frogs were bigger than toads (significant differences in mean SVL; Mann-Whitney *U*-test: *B. bufo* vs. *R. temporaria*: $U = 22.0$, $P = 0.0005$; *B. bufo* vs. *P. esculentus*: $U = 40.0$, $P = 0.008$). Body size influences other morphological traits (e.g., length of hind-limbs) and jumping performance; therefore, larger frogs with longer hind-limbs can jump further, whereas smaller anurans should perform shorter leaps (Rand, 1952; Nauwelaerts et al., 2007), as observed in our experiments. We suggest that the length of hind-limbs, the locomotion mode, and the ability to jump can determine the repertoire of anuran defensive behaviors.

Body-tilting may startle or force a predator to abandon attack (Ewert and Traud, 1979). Prey that tilts the body towards a predator may seem to be larger, and if a predator decided to attack the prey, the first part of the prey body that the predator would bite is the head with parotoid macroglands. Consequently, the poison could be squeezed from the glands immediately into the predator's mouth, which could be distasteful or could cause irritation or paralysis (Toledo and Jared, 1995; Toledo et al., 2011). We found body-tilting only in the Common Toad, as did Ewert and Traud (1979). This behavior has also been reported in other bufonid species, e.g., in *Rhinella ornata* inhabiting the tropical forests of South America (Toledo, 2004). However, in our study body-tilting was never followed by poison releasing and did not prevent hedgehogs from attacking Common Toads.

Similar to body-tilting, cloacal discharge did not prevent predator attack, perhaps because the liquid secretion released from the cloaca is not unpalatable to hedgehogs. A few times we recorded cloacal discharge accompanied by fleeing in frogs in control tests, suggesting that cloacal discharge is employed to reduce body weight and allow quicker fleeing.

Interestingly, we observed a new anti-predator behavior in the Common Toad: head hitting. According to our knowledge, this reaction has never before been reported in *B. bufo*. It is known for some leptodactylids, but presented only by females guarding tadpoles (Toledo et al., 2011). This reaction has also been observed in another bufonid species (*Rhinella rubescens*) and, similar to our results, it was not related to parental care (Prado et al., 2000; Toledo et al., 2011). In other experiments testing whether the Water Shrew (*Neomys fodiens*) can hunt *B. bufo*, we observed that sometimes the Common Toad could hit a predator with its head up to three times during one encounter (Kowalski and Rychlik, 2018).

In contrast to the ranids, the Common Toad can secrete toxins from parotoid macroglands (Juszczyk, 1987; Hutchinson and Savitzky, 2004), which can be effective in three ways: (1) the poison can be distasteful or irritating to the predator (particularly after getting into the mouth or eyes), (2) it might cause poisoning, paralysis, cardiac arrhythmia, or (3) even the death of the predator (Toledo and Jared, 1995; Abdel-Rahman et al., 2010; Gao et al., 2010). However, as the production of protein-rich toxin (as well as of venom) is metabolically costly (McCue, 2006; Nisani et al., 2007), according to the venom optimization hypothesis (Wigger et al., 2002), toxic and venomous animals should evolve various strategies (e.g., morphological adaptations or behavioral responses) to minimize venom expenditure and release the poison (or venom) frugally (Morgenstern and King, 2013). In our experiments, consistent with our predictions, Common Toads at first displayed threatening behaviors to startle the predator and released toxins only after the predator attack and squeezing of the parotoid glands, supporting our hypothesis that the Common Toad is able to minimize the use of poison, applying first alternative behavioral reactions. Releasing toxins from parotoids was always coupled with secretion of poison from dorsal glands, which was displayed even after the predator stopped biting the toad. Additionally, our results indicate that releasing toxins by the Common Toad is ineffective in repelling the hedgehogs. Hedgehogs, like some snakes (e.g., *Natrix natrix*; Gregory and Isaac, 2004), may be able to digest toad toxins without being poisoned. Moreover, we repeatedly observed hedgehogs anointing their spines with the toad poison, a behavior that has been previously reported (Brodie, 1977; Crump, 2009). Hedgehogs may employ toad toxins as a defensive weapon against their predators (Brodie, 1977; Ewert and Traud, 1979; Crump, 2009). As a consequence, they could prefer to hunt toads to stimulate them to secrete the poison and then anoint their spines with toxins. Therefore, we recommend performing experiments with different prey to analyze hedgehog prey preferences.

To verify hypothesis H2, we had to enable the hedgehogs to hunt and bite the anurans, particularly the Common Toad; therefore, we did not outfit the arena with shelters and water tanks in which prey could hide. However, providing anurans with water tanks and shelters such as flowerpots, logs, or bedding (sand, sawdust, or moss) could result in a higher number of behavioral responses of anurans, particularly of the toads. For instance, hiding and burying have been previously reported in toads and frogs (Marchisin and Anderson, 1978).

Also, stress can affect the animal behavior (Clark et al., 1997; Dwyer, 2004). In our experiments, a small closed arena could increase the prey-predator contact frequency, which could result in higher stress levels in anurans and indirectly affect anuran behavior. Threatening behaviors were displayed by the anurans mainly in the tests with the hedgehogs because only these predators hunted and bit the bufonids. These results indicate that only the hedgehogs were considered a serious threat. The rabbit usually ignored the Common Toad and both ranids. Cloacal discharge, always accompanied by fleeing, was the only threatening behavior employed by the frogs in control tests with the rabbit. It has been reported that anurans can employ fleeing or immobility not only in response to a live predator but also

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to inanimate objects (Ewert, 1970). However, to display threatening behaviors, such as body-tilting or puffing up the body, they often require approach by a live predator.

In conclusion, our results enrich the knowledge on behavioral responses of three European anurans to predators. We show that the repertoire of the behavioral responses of the Common Toad is more diverse than those of the Common Frog and the Edible Frog. While ranids employ mainly fleeing to avoid predation, toads additionally display threatening behaviors to discourage predator attack. As *B. bufo* releases the poison only after predator attack and squeezing of parotoid macroglands, we conclude that the toad can minimize the secretion of metabolically costly poison through behavioral control. We suggest that the length of hind-legs and, related to this, the mode of locomotion (jumping vs. hopping) are the most important characteristics affecting anti-predator behavior of the tested anurans. As many responses evolved against various predators (such as snakes, badgers, or otters), we plan to carry out further experiments with different types of predators. Additionally, providing anurans with shelters (e.g., water bowls, bedding, or flowerpots) during experiments could elicit additional behavioral responses, such as diving into water, burying, or hiding, thus enabling us to better understand the complex nature of predator-anuran interactions.

DATA ACCESSIBILITY

Supplemental material is available at http://www.copeiajournal.org/ce-16-567.

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