Keep the ball rolling: sexual differences in conglobation behavior of a terrestrial isopod under different degrees of perceived predation pressure (#89537)

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Keep the ball rolling: sexual differences in conglobation behavior of a terrestrial isopod under different degrees of perceived predation pressure

Francisco Javier Zamora-Camacho Corresp. [1]

[1] Department of Biogeography and Global Change, Museo Nacional de Ciencias Naturales, Madrid, Spain

Corresponding Author: Francisco Javier Zamora-Camacho
Email address: zamcam@ugr.es

Background. Antipredator behaviors are theoretically subjected to a balance by which their display should be minimized when their benefits do not outweigh their costs. Such costs may be not only energetic, but also entail a reduction in the time available for other fitness-enhancing behaviors. However, these behaviors are only beneficial under predation risk. Therefore, antipredator behaviors are predicted to be maximized under strong predation risk. Moreover, predation pressure can differ among individuals according to traits such as sex or body size, if these traits increase vulnerability. Antipredator behaviors are expected to be maximized in individuals whose traits make them more conspicuous to predators. **Methods.** In this work, I tested these hypotheses in the common pill woodlouse (*Armadillidium vulgare*), which conglobate (i.e., they roll up their bodies almost conforming a sphere that conceals their appendages) in response to predator attacks. Specifically, I tested whether latency to unroll was greater in animals exposed to predator chemical cues, incorporating sex and body mass in the analyses. **Results.** In agreement with my prediction, latency to unroll was greater in individuals exposed to predator chemical cues. In other words, these animals engage in conglobation for longer under perceived predator vicinity. However, this result was only true for males. This sexual dimorphism in antipredator behavior could result from males being under greater predation risk than females, thus having evolved more refined antipredator strategies. Indeed, males of this species are known to actively search for females, which makes them more prone to superficial ground mobility, and likely to being detected by predators. Body size was unrelated to latency to unroll. As a whole, these results support the hypothesis that antipredator behavior is tuned to predator cues in a way consistent with a balance between costs and benefits.

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Francisco Javier Zamora-Camacho

Department of Biogeography and Global Change. Museo Nacional de Ciencias Naturales, (MNCN-CSIC), C/José Gutiérrez Abascal 2, 28006, Madrid, Spain.

Email address: zamcam@mncn.csic.es

Abstract

Background. Antipredator behaviors are theoretically subjected to a balance by which their display should be minimized when their benefits do not outweigh their costs. Such costs may be not only energetic, but also entail a reduction in the time available for other fitness-enhancing behaviors. However, these behaviors are only beneficial under predation risk. Therefore, antipredator behaviors are predicted to be maximized under strong predation risk. Moreover, predation pressure can differ among individuals according to traits such as sex or body size, if these traits increase vulnerability. Antipredator behaviors are expected to be maximized in individuals whose traits make them more conspicuous to predators.

Methods. In this work, I tested these hypotheses in the common pill woodlouse (*Armadillidium vulgare*), which conglobate (i.e., they roll up their bodies almost conforming a sphere that conceals their appendages) in response to predator attacks. Specifically, I tested whether latency to unroll was greater in animals exposed to predator chemical cues, incorporating sex and body mass in the analyses.

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Introduction

Predators erode their prey's fitness in various ways, thus embodying a potent selective pressure on them (Abrams, 2000; Lima, 2002). First and foremost, successful predatory events involve the annihilation of the prey's life, and consequently of any potential future fitness it might have had (Barbosa and Castellanos, 2005; Beauchamp et al., 2007). However, predators also exert non-lethal effects on their prey that are also pivotal in multifarious ways (Lima, 1998; Preisser et al., 2005; Wirsing et al., 2021). After consumption, the gravest damage predators inflict on their prey is probably represented by physical injury following failed attacks (Laha and Mattingly, 2007; Bowerman et al., 2010), which frequently entail infections (Aeby and Santavy, 2006) as well as impaired locomotion, growth, and ultimately fitness (Archie, 2013; Zamora-Camacho and Aragón, 2019; Zamora-Camacho and Calsbeek, 2022). Even in the absence of an actual attack, preys are bound to face the harmful effects of predators. Some animal species innately possess physical (Mukherjee and Heithaus, 2013) or chemical defenses (Glendinning, 2007), occasionally remarkably sophisticated (Zamora-Camacho, 2023), which can dissuade predators

(Brown et al., 2016). Moreover, most prey are equipped with sensory systems capable of detecting predator vicinity (Leavell and Bernal, 2019). Such perceived predator proximity oftentimes elicits the expression of inducible morphological or chemical defenses of different types (Kishida et al., 2010; Yamamichi et al., 2019). In either case, whether innate or inducible, these defenses can be costly, given the energy diverted to their production (Hamill et al., 2008; Gilbert, 2013; Hermann et al., 2014, Zvereva et al., 2017), and the fact that the metabolic processes involved in these responses may even trigger oxidative stress (Janssens and Stoks, 2013).

On a different note, prey can also tune their behavior to the threat represented by potential predators (Lima and Dill, 1990; McGhee et al., 2013) and the level of risk involved (Kavaliers and Choleris, 2001). The most immediate antipredator behavior is oftentimes spatial circumvention, which prevents an actual encounter (Palmer et al., 2022; Suraci et al., 2022). Also, prey typically respond to predator proximity by diminishing the conspicuousness of their activities (Moll et al., 2020; Balaban-Feld et al., 2022). When the encounter is imminent, however, prey can decide whether to face or avoid the predator depending on the chances of success of each strategy (Reichmuth et al., 2011; Zhang et al., 2020). A particularly common reaction of prey to such encounters is flight (Møller and Erritzøe, 2014; Basille et al., 2015). In addition, more refined behaviors against predation are likewise common, such as postural strategies that facilitate the deflection of the attack towards a non-vital (Myette et al., 2019) or well protected body region (Crofts and Stankowich, 2021), that make it difficult for the predator to handle and subdue the prey (Kowalski et al., 2018), or that invoke death feigning or thanatosis (Humphreys and Ruxton, 2018). This wide array of antipredator behaviors can coexist in the same individual and be subjected to complex interactions (Lind and Cresswell, 2005).

In any case, antipredator behavior is not devoid of costs. Besides the energy demands of strategies such as flight, which involves a frequently intense muscular exertion (Biewener and Patek, 2018), a cost in terms of fitness is expected given that antipredator behaviors are time-consuming (Lima and Dill, 1990) and thus reduce the time devoted to foraging, mating and reproducing (Langerhans, 2007; Gulsby et al., 2018). The final decision of a prey regarding whether and to which extent to engage in antipredator behaviors should be made considering a balance between their costs and benefits (Herberholz and Marquart, 2012). Indeed, antipredator defenses are expected to be selected against in the absence of predators (Reznick et al., 2008; Palkovacs et al., 2011), at least to a certain extent (Blumstein, 2006), which could release the bearer from the costs associated to such behaviors if they are no longer beneficial. In fact, the success of a given antipredator behavior depends on diverse circumstances, such as the actual predatory pressure and the qualitative and quantitative expressions of the antipredator behaviors adopted by other potential preys (Menezes, 2021).

Indeed, prey are predicted to adjust their antipredator behavior to the actual intensity of predator pressure, responding strongly when predators are an actual threat, but mildly when that threat is lesser (Sih et al., 2000; Ferrari et al., 2009). Also, even at the intraspecific level, some individuals can be at higher risk than others, depending on differences in morphology (Zamora-Camacho, 2022) and personality (Sommer and Schmitz, 2020) that can make some individuals more or less prone to succumb to predator attacks. Given that, probably as a part of their mating strategies, males are often morphologically (Williams and Carroll, 2009) or behaviorally (Schuett et al., 2010) more conspicuous than females, males can be subjected to a stronger predation pressure than females (Husak et al., 2006; Kojima et al., 2014), thus responding with stronger antipredator strategies (Husak and Fox, 2008; Zamora-Camacho, 2022).

In this context, this work aims to contextualize the display of an unusual antipredator behavior, conglobation in common pill woodlice (*Armadillidium vulgare*), as a function of extrinsic factors, such as predator cues, and intrinsic traits, such as body mass and sex. Conglobation is a particular behavior by which these animals coil up into a ball when disturbed, concealing their appendages within their dark grey cuticle (Cazzolla Gatti et al., 2020). This position makes them not only difficult to handle, but also resemble a pebble rather than edible animals (Tuf and Ďurajková, 2022), which has been interpreted as tonic immobility or even as thanatosis (Horvátz et al., 2019; Cazzolla Gatti et al., 2020). Specifically, I studied the time spent by male and female *A. vulgare* in the conglobated position in the presence and the absence of olfactory predator cues. In line with the aforementioned rationale that antipredator behavior is costly, I predict that the conglobated position will be abandoned earlier in the absence of predator cues, when its potential benefits are lower. Also, I expect that, if one of the sexes is under greater predation pressure, this risk will have selected for a stronger reaction to predator cues.

Materials & Methods

Study species

The common pill woodlouse (*A. vulgare*) is a terrestrial isopod, native to the Mediterranean region but introduced worldwide (Schmalfuss, 2003), that occupies a variety of temperate habitats. It shows a preference for a certain degree of moisture (Bonuti et al., 2021), which can determine some extent of small-scale seasonal migration in search of sufficient yet not excessive humidity (Paris, 1963). Reproduction takes place in the summer in cold regions (Dangerfield and Hassall, 1992), but in spring in more temperate areas (Sorensen and Burkett, 1977). Females possess a ventral pouch where eggs are deposited until hatching (Suzuki, 2001; Suzuki and

Futami, 2018). As a macrodecomposer, it feeds on a variety of dead organic matter sources (Paris, 1963) which it selects according to its quality (Tuck and Hassall, 2005). In turn, a wide array of invertebrates, amphibians and reptiles have been cited as predators of this species (Paris, 1963).

Animal capture and management

Fieldwork took place in Pinares de Cartaya (SW Spain; 37º 21'N, 7º 11'O), an 11,000-ha *Pinus pinea* grove with an undergrowth dominated by *Rosmarinus officinalis*, *Pistacia lentiscus* and *Cistus ladanifer*. In this forest, I collected 43 adult *A. vulgare* (19 females and 24 males) by hand, searching under rocks, decaying logs, and other potential refugia at appropriate sites. However, to diminish the chances of capturing genetically related individuals, only one specimen was caught at a given site, and at least 50 m were left among sites (Horváth et al., 2019; Beveridge et al., 2022). Sampling took place in February 2022, immediately before the onset of the mating season, because parental care can affect antipredator behavior in females, involving a difficulty in the adoption of the conglobated position, which could affect the results (Suzuki and Futami, 2018).

The animals captured were transferred to the laboratory, where they were assigned an ID number, weighed to the nearest 0.01 g with a CDS-100 scale, and individually housed in cylindric plastic terraria (6 cm diameter x 15 cm height) with wet peat as a substrate, a piece of fresh carrot as nourishment, and a wet cotton disk (4 cm diameter x 1 mm thick) above it as a shelter. The terraria were randomly set in a shelve in the laboratory, and their position was changed every 24 hours. A window let daylight in, which permitted the adjustment of circadian rhythms. Room temperature was not manipulated, and fluctuated naturally between 10 ºC at night and 20ºC during the day.

The behavioral tests began 24 hours after capture. These tests were conducted in individual cylindric plastic terraria (4 cm diameter x 10 cm height) with a cotton disk lining (4 cm diameter x 1 mm thick) at the bottom. Because this species interprets chemical cues to identify potential mates (Beauché and Richard, 2013) and predators (Pniewski, 2014), and tunes its conglobation behavior can to diverse environmental factors (Horváth et al., 2019), I used different chemical cues (or the absence thereof) in three separate tests. In the experimental tests, the cotton disk at the bottom of the terrarium was soaked with a 1-mL aliquot extracted from a preparation of 0.5L of distilled water where 50g of a mix of fresh feces from 2 male and 2 female adult common toads (*Bufo spinosus*), captured in the same habitat as the woodlice, had been diluted. These toads are abundant and widespread generalist predators of invertebrates, including isopods (Ortiz-Santaliestra, 2014). In the control tests, the cotton disk at the bottom of the terrarium was soaked with a 1-mL aliquot extracted from a preparation of 0.5L of distilled water where 50g of a mix of fresh feces from 4 different European rabbit (*Oryctolagus cuniculus*) latrines (separated by at least 600 m) from the same habitat as the woodlice, had been diluted. These rabbits are abundant and widespread generalist herbivores (Gálvez-Bravo, 2017). In the manipulation control tests, the cotton disk at the bottom of the terrarium was soaked with 1 mL of distilled water. In this way, humidity was constant across tests, which avoided a potential effect of moisture on conglobation behavior, as conglobation can also be a behavioral strategy against water loss in these animals (Smigel and Gibbs, 2008).

For these tests, each individual was placed alone in one arena as described above. After 5 minutes for habituation, I gently poked the animal with a wooden stick until it adopted the fully conglobated position. The test ended when the individual abandoned this position. All individuals underwent all three tests, with a 24-h resting period in between. Every time, the cotton disks were replaced and the arenas were rinsed thoroughly. Conglobation behavior in these animals is affected by previous experience (Matsuno and Moriyama, 2012). For that reason, the sequence in which the tests involving the different stimuli were conducted was random for each individual.

All tests were recorded with a Canon EOS 550D video camera. The resulting footages were then studied using the software Tracker v 6.0.8, which allows frame-by-frame analyses. Specifically, I measured latency to unroll as the time each individual spent in the conglobated position, by recording the time elapsed since the frame in which this position was adopted until it was abandoned. After the tests, the woodlice were sexed, based on the presence of the abdominal pouch in females, and released in the same habitat where they had been captured.

Statistics

Latency to unroll needed to be ln-transformed in order to meet the assumptions of homoscedasticity and residual normality needed for parametric statistics (Quinn and Keough, 2002). After that, a mixed model was conducted where latency to unroll (ln-transformed) was the response variable, sex, treatment and their interactions were included as factors, body mass was included as a covariate, and ID was a random factor. Sum of squares was type III. A Tukey post-hoc test was applied on the interaction term. These tests were conducted with the package *lmerTest* (Kuznetsova et al., 2017) in the software R v. 4.1.2 (R Core Team, 2021).

Results

Body mass had no significant effect on latency to unroll ($F_{1,\,122} = 0.698$; $\beta = -2.843$; $P = 0.409$). The effect of sex on latency to unroll was non-significant ($F_{1,\,122} = 0.073$; $P = 0.789$), but that of treatment was significant ($F_{2,\,122} = 5.823$; $P = 0.004$). According to the Tukey post-hoc test applied on the marginally non-significant Sex×Treatment interaction ($F_{2,\,122} = 2.786$; $P = 0.068$), males exposed to toad scent had greater latency to unroll than males exposed to rabbit scent and to water, and than females exposed to water, with every other pairwise comparison being non-significant (Table 1; Fig. 1).

Discussion

Some of these results were in agreement with my predictions. In the first place, latency to unroll was greater in the presence of predator chemical cues. According to theory, predator vicinity can trigger a fear response on the prey, which is not devoid of costs (Wang and Zou, 2018; Qiao et al., 2019; Tripathi et al., 2022). Previous research supports that, in behavioral terms, most prey reduce their susceptibility to predators by diminishing their activity rates when threatened (Brodin and Johansson, 2004; Laurila et al., 2006), even resorting to total immobility (Brooks et al., 2009) and death feigning (Konishi et al., 2020). However, by engaging in such antipredator behavior, prey inevitably reduce the amount of time available for other fitness-enhancing activities, such as mating, feeding, and territory defense (Persons et al., 2002; Lind and Cresswell, 2005), which may entail negative effects, for example on growth (Brodin and Johansson, 2004; Laurila et al., 2006) and reproduction (Persons et al., 2002; Kempraj et al., 2020). Thus, such antipredator behaviors are allegedly subjected to a balance between these costs and their benefits, namely predator avoidance. In this context, prey are expected to minimize antipredator behaviors when their benefits are scarce, i.e. under low predation risk (Ferrari et al.

2008; Supekar and Gramapurohit, 2020; Batabyal et al., 2022). This prediction is supported by these results, as latency to unroll was greater in the presence of predator chemical cues presented in the short term. Such a result contrasts with a previous study on this species, according to which long-term contact with predatory ants relates to a shift in flight behavior, which does not happen after short-term exposure (Hegarty and Kight, 2014).

Nonetheless, this greater latency to unroll in the presence of predator chemical cues was only observed in males, whereas females did not respond to these cues with an increase in time to unroll. While the possibility that females lack the ability to recognize predator chemical cues cannot be discarded, a greater response of males as a result of a male-biased predation risk could be a more plausible explanation. In circumstances where both sexes are under equivalent risk, their response to predator cues might not differ (David et al., 2014; Kempraj et al., 2020; Saavedra et al., 2022). However, whenever one sex is under greater risk than the other, it is expected to evolve more efficient antipredator responses (Curio et al., 1983). Although in some species females have been found to face greater predation risk (Post and Götmark, 2006) and to respond with greater intensity to predator pressure (Pärssinen et al., 2021; Woodrow et al., 2021), in most cases males are more conspicuous to predators as a result of more active behaviors (Tobler et al., 2008), such as territory defense (Gwynne and O’Neill, 1980), female pursuit (Fišer et al., 2019) and courtship (Whitaker et al., 2021). Accordingly, males display a stronger behavioral response to predation risk in taxa as disparate as mammals (Grignolio et al., 2019), birds (van den Bemt et al., 2021), reptiles (Bohórquez Alonso et al., 2010), snails (Donelan and Trussell, 2020), insects (Schultz, 1981), spiders (Krupa and Sih, 1998) or crabs (Jennions et al., 2003).

In the specific case of *A. vulgare*, different lines of evidence suggest that males could be more active, and thus more detectable by predators, which could favor a greater investment in antipredator behavior. In the first place, genetic analyses have revealed that females are philopatric whereas males are not, which is compatible with males being more prone to dispersal and, allegedly, to be intercepted by predators (Durand et al., 2019). Moreover, males are known to actively search for females based on chemical cues (Beauché and Richard, 2013) and to compete for access to them given their multiple paternity scheme (Verne, 2007; Valette et al., 2016). Also, male presence can stimulate female receptiveness (Lefebvre and Caubet, 1999). These features could be accompanied by behavioral displays that might increase male conspicuousness to predators. Indeed, males are more active in the ground surface, whereas females tend to make a greater use of underground shelters, which is a probable consequence of the former actively competing and searching for the latter (Dangerfield and Hassall, 1994).

In correspondence with previous studies on this species (Beveridge et al., 2022), body mass was uncorrelated with latency to unroll, as well as with other antipredator behaviors (Cazzolla Gatti et al., 2020). This finding contrasts with research that indicates that antipredator behavior depends on body size on other taxa, both vertebrates (Hoare et al., 2000; Roth and Johnson, 2004) and invertebrates (Johnson et al., 2017; Gavini et al., 2020), including larger crustaceans (Wahle, 1992). In this case, the relatively small size of the focal species might make variation in body size irrelevant for most potential predators, thus not selecting for differential antipredator strategies at varying sizes. In any case, conglobation behavior is known to be repeatable in this species (Cornwell et al., 2023), which advocates for consistency in the patterns described herein.

Conclusions

To conclude, latency to unroll was greater in individuals exposed to predator chemical cues, which supports the prediction that *A. vulgare* can detect these cues and react accordingly. Moreover, this finding concurs with the theoretical assumption that antipredator behaviors are subjected to a cost-benefit balance, by which they should be minimized when their benefits do not outweigh their costs. Antipredator behaviors are only beneficial under predation risk, which could be the reason why these animals engage in conglobation for longer under perceived predator vicinity. However, this result was only true for males. This sexual dimorphism in antipredator behavior could indicate that males are under greater predation risk than females, thus having evolved more refined antipredator strategies. Indeed, males of this species are known to actively search for females, which makes them more prone to superficial ground mobility, and likely to being detected by predators.

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Figure 1

Differences in latency to unroll according to sex and treatment.

Sample sizes are indicated. Vertical whiskers represent standard errors.

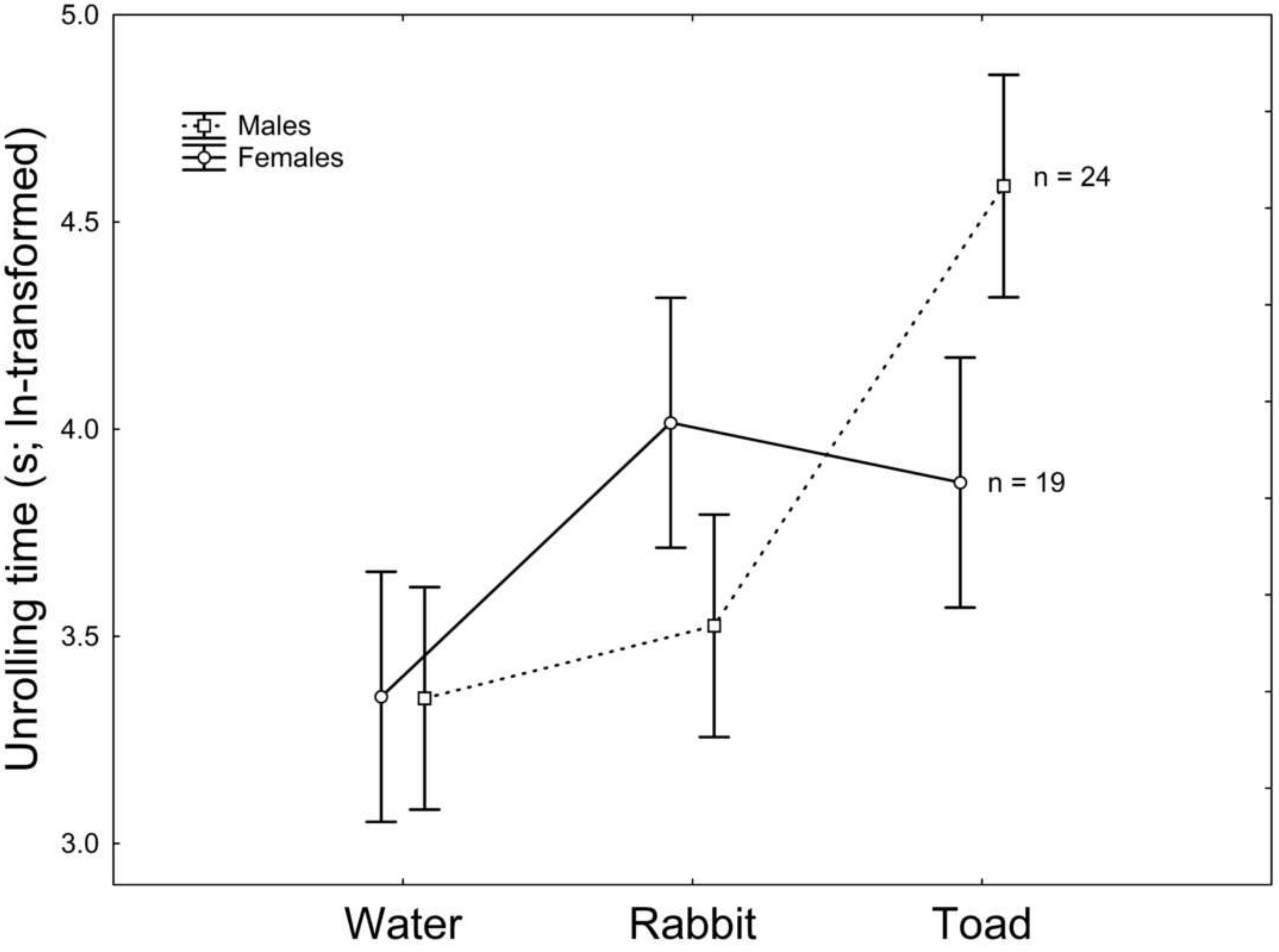

Table 1(on next page)

Tukey post-hoc test performed on the Sex×Treatment interaction.

t- and *P*-values for each pairwise comparison are indicated. Significant results are in bold.

Pairwise Comparison	*t*-value	*P*-value
Female Rabbit vs Male Rabbit	1.213	0.830
Female Rabbit vs Female Toad	0.376	0.999
Female Rabbit vs Male Toad	-1.414	0.719
Female Rabbit vs Female Water	1.722	0.521
Female Rabbit vs Male Water	1.646	0.570
Male Rabbit vs Female Toad	-0.856	0.956
Male Rabbit vs Male Toad	**-3.107**	**0.030**
Male Rabbit vs Female Water	0.424	0.998
Male Rabbit vs Male Water	0.512	0.996
Female Toad vs Male Toad	-1.771	0.489
Female Toad vs Female Water	1.347	0.758
Female Toad vs Male Water	1.289	0.791
Male Toad vs Female Water	**3.050**	**0.033**
Male Toad vs Male Water	**3.619**	**0.007**
Female Water vs Male Water	0.009	1.000