The relationships between toad behaviour, antipredator defences, and spatial and sexual variation in predation pressure

Commented [JBG1]: Overall, I think that this manuscript is look in really good shape. I have inserted a number of – mostly writing style and structure – comments. As such, they are not mandatory, however I think they could be useful in the final polishing of this paper. When you resubmit it, you do not need to worry about a formal response/rebuttal or a tracked changes form. This round of edit is more to just provide one last round of suggestion to further increase the readability and presentation of this work.

A few things I did not stop to edit each time, but you may want to try to adjust as you read through are:

1)The amount of time you start a sentence with a qualifier (e.g., “However”, “Yet”, “Firstly”, “Finally”, “On one hand”, and so on). I understand using qualifiers like this allow the reading to sound more conversational, which can increase readability – but if used to often the opposite effect occurs. I would suggest you work through and try to limit the amount of them here. There are some paragraphs where more than half the sentences have them. A few here and there are good. But try to use them sparsely.
2) One thing I think would help push this manuscript to the next level for readability is double checking each paragraph has a strong and encompassing topic sentence. This way the reader, at the start of each paragraph, has the ideas that are about to be discussed put into context right off the hop. This makes a world of difference. You have some great one already. But some spots could use improvement.
3) I would suggest revising the figure and table captions, right now they are a little bare and if readers are skimming the paper, they do not provide enough detail or clarity.

Francisco Javier Zamora-Camacho[1,2]

[1]Departamento de Sistemas Físicos, Químicos y Naturales. Universidad Pablo de Olavide, Carretera de Utrera km 1, 41080, Seville, Spain.

[2]Departamento de Biodiversidad, Ecología y Evolución, Facultad de Ciencias Biológicas, Universidad Complutense de Madrid, C/José Antonio Novais 12, 28040 Madrid, Spain.

Corresponding Author:
Email address: zamcam@ugr.es

Abstract

Background. Animal behaviour is under strong selection. ~~However, s~~Selection on behaviour, however, might not act in isolation from other fitness-related traits. Since predators represent outstanding selective forces, animal behaviour could covary with antipredator defences, such that individuals better suited against predators could afford facing the costs of riskier behaviours. Moreover, not all individuals undergo equivalent degrees of predation pressure, which can vary across sexes or habitats. Individuals under lower predation pressure might also exhibit riskier behaviours.

Methods. In this work, I tested these hypotheses on natterjack toads (*Epidalea calamita*). Specifically, I gauged activity time, exploratory behaviour, and boldness in standard laboratory conditions, and assessed whether they correlated with body size and antipredator strategies, namely sprint speed, parotoid gland area, and parotoid gland colour contrast. Additionally, I compared these traits between sexes and individuals from an agrosystem and pine grove, since there is evidence that males and agrosystem individuals are subjected to greater predation pressure.

Results. Sprint speed as well as parotoid gland contrast and size appeared unrelated to the behavioural traits studied. In turn, body mass was negatively related to activity time, boldness, and exploration. This trend is consistent with the fact that larger toads could be more detectable to their predators, which are mostly gape unconstrained and could easily consume them. As predicted, females exhibited riskier behaviours. Nonetheless, agrosystem toads did not differ from pine grove toads in the behavioural traits measured, despite being under stronger predation pressure.

Introduction

Animal behaviour is strongly subjected to selection, and thus represents a fundamental component of fitness (*Dingemanse & Réale, 2005*; *Dugatkin, 2020*). Traits such as sexual selection (*Schuett, Tregenza & Dall, 2010*), reproductive success (*Zhao et al., 2016*), productivity (*Biro & Stamps, 2008*), contest outcome (*Briffa, Sneddon & Wilson, 2015*), and even mortality (*Stamps, 2007*), are known to be linked to traits such as boldness (i.e., willingness to engage in activities that involve exposure) or exploratory behaviour (i.e., willingness to investigate a novel environment), although these traits oftentimes exert opposing fitness consequences. Indeed, selection on behaviour can hardly be regarded as directional, since the fitness consequences of behavioural traits are context-dependent (*Smith & Blumstein, 2008*; *MacPherson et al., 2017*). For instance, bold red squirrels (*Sciurus vulgaris*) survive better in food-restrictive habitats, but worse in sites where food supplies are stable, whereas exploratory behaviour has a consistent negative relationship with survivorship and female reproductive success across habitats (*Santicchia et al., 2018*). This case illustrates how the diversity of contexts animals can face could be key in maintaining the enormous variation in behavioural traits documented (*Nettle, 2006*; *Briffa & Sneddon, 2016*; *Roche, Careau & Binning, 2016*). Nevertheless, the contextual components of animal behavioural traits are poorly understood and remain an eminent subject of debate among scientists (*Koski, 2014*; *Weiss, 2018*; *Wilson et al., 2019*).

Particularly relevant are the fitness consequences of behavioural traits that affect mortality. With predators being among the most frequent causes of mortality in many animals (*Lima, 2002*; *Beauchamp, Wahl & Johnson, 2007*), the ~~huge~~ substantial effect of antipredator behaviour on prey fitness comes as no surprise (*Lind & Cresswell, 2005*). Prey's behavioural traits are tuned to, and affected by, predation pressure in intricate ways (*Toscano & Griffen, 2014*; *Belgrad & Griffen, 2016*). On the one hand, predators may trigger plastic behavioural responses in their prey (*Quinn & Cresswell, 2005*; *Dingemanse et al., 2010*). For instance, *Euricea nana* salamanders reduce their activity time in the presence of predators (*Davis & Gabor, 2015*). Likewise, great tits (*Parus major*) exposed to predators have a lower tendency to explore than non-exposed controls (*Abbey-Lee, Mathot & Dingemanse, 2016*), and juvenile *Negaprion brevirostris* sharks that exhibit more exploratory behaviour forage in riskier habitats, but only under low predation pressure (*Dhellemmes et al., 2021*). On the other hand, the relationships between predation risk and behavioural traits may transcend plasticity, as behaviour can be persistent across environments (*Dosmann & Mateo, 2016*). In such a scenario, the ways in which behaviour affects predation risk can be complex. For example, shy *Rutilus rutilus* roaches are more likely to be preyed on by ambushing predators than bold conspecifics (*Blake et al., 2018*), whereas bolder *Panopeus herbstii* crabs experience greater mortality rates (*Belgrad & Griffen, 2018*).

Despite the fact that some trends have been detected, inter-individual variation in antipredator behavioural traits is oftentimes high (*López et al., 2005*; *Brown et al., 2014*; *Cremona et al., 2015*). Such variation could be maintained by spatial differences in predation pressure driving diverging behavioural traits. Supporting this possibility, *Phoxinus phoxinus* minnows from a population under greater predation pressure are bolder but less active than conspecifics from a population where predators are less abundant (*Kortet et al., 2015*). Indeed, how animals make use of space affects their success against predators (*Leblond, Dussault & Ouellet, 2013*). Moreover, whenever the sexes are subjected to differential predation pressures, sexual disparities in behaviour could be expected. For instance, highly active ~~males of~~ *Perca fluviatilis* ~~perches~~ perch males face greater mortality than females (*Yli-Renko, Pettay & Vesakoski, 2018*), and ~~male guppies (~~*Poecilia reticulata*~~)~~ guppy males are significantly bolder than females (*Harris et al., 2010*).

~~However, o~~Other potential sources of variation in antipredator behaviour, however, remain underexplored – such as locomotion, chemical deterrents, and colour.

~~For instance, t~~The protection lent by antipredator defences of different kinds could buffer the effects of predation on behavioural traits. One of the most widespread antipredator defences is locomotion, as an active flight can be efficient in avoiding predators (*Watkins, 1996*; *McGee et al., 2009*). Although locomotion has a behavioural component, as animals tune their investment on locomotion according to the benefits it can yield in different situations (*Zamora-Camacho, García-Astilleros & Aragón, 2018*), it may also function as a capability that is dependent on

Commented [JBG2]: In a number of places you shift how you write species names, something the common name followed by the scientific in brackets, while other times the scientific name and a general term. I would suggest trying to remain consistent across the manuscript rather than bouncing back and forth. Consistency lead to easier readability for the audience.

Commented [JBG3]: This is a stronger topic sentence than the previous one, and by moving it up you create a clear segue between this paragraph and the previous one traits other than behaviour (*Zamora-Camacho, 2018*). Other antipredator defences are neatly non-behavioural and passive. Such is the case with chemical deterrents, which are toxic or distasteful substances that repel predator attacks (*Mebs, 2001*; *Brodie, 2009*; *Savitzky et al., 2012*). Aposematic coloration (i.e., conspicuous colours and patterns that potential predators associate with unpalatability and actively avoid) can be frequently found alongside chemical deterrents (*Saporito et al., 2007*; *Zvereva & Kozlov, 2016*; *Ruxton et al., 2018*), thus providing their carriers with additional defence from predators (*Skelhorn & Rowe, 2006*; *Prudic, Skemp & Papaj, 2007*).

Commented [JBG4]: This paragraph is missing a summary sentence this connects the ideas presented back to how these potential sources of variation in antipredator behaviour are underexplored. As it reads now, it sounds like they have been explored – which contradict the previous statement made above.

Like, I see what you are saying, you just need a statement that ties it all together

The relationship between body size and predation is particularly interesting. On the one hand, larger prey could be easier to detect (*Mänd, Tammaru & Mappes, 2007*; *Karpestam, Merilaita & Forsman, 2014*~~).~~), while ~~O~~on the other hand, larger prey can be more difficult to handle (*Díaz & Carrascal, 1993*; *Kalinkat et al., 2013*). In fact, survivorship of *Hyla chrysoscelis* tadpoles to attacks by *Tramea lacerata* dragonfly nymphaea increases with body size (*Semlitsch, 1990*). Similarly, larger grasshoppers are better defended against a wide array of predators (*Whitman & Vincent, 2008*). However, larger predators do not necessarily prefer larger prey (*Tsai, Hsieh & Nakazawa, 2016*), although they can exploit prey of a wider size range (*Radloff & du Toit, 2004*). The influence of the morphology of a predator's mouthparts is also ~~of the essence~~important, with gape-limited predators preferring smaller prey, while gape-unconstrained predators are less dependent on their prey's body size (*Jobe, Montaña & Schalk, 2019*). Furthermore, the role of prey body size may depend on other antipredator strategies. For example, whereas detectability of cryptic prey can appear unrelated to body size, conspicuous prey might be more detectable at larger body sizes (*Mänd et al., 2007*). Moreover, toxins are more efficient antipredator defences in smaller prey (*Smith, Halpin & Rowe, 2016*).

Consequently, antipredator behaviour is not independent from body size, but such relationships are intricate (*Preisser & Orrock, 2012*).

In this work, I studied activity, exploratory behaviour, and boldness in the natterjack toad, *Epidalea calamita*. Specifically, I tested whether these traits co-vary with other antipredator strategies, including locomotion. This toad is cursorial, and uses quick runs to flee from its predators (*Zamora-Camacho, 2018*). I also ~~checked~~ examined the potential effects of body size on these behavioural traits. Additionally, this species has notable parotoid glands, which are a pair of swollen structures located dorsally behind the eyes in many amphibian species. The size of these glands is directly proportional to the quantity of chemical deterrents they are capable of ejecting (*Zechmeister, 1948*; *Llewelyn et al., 2012*). The parotoid glands of *E. calamita* are aposematic~~:~~, with predators avoid plasticine models with highly colour-contrasting parotoid glands (*Zamora-Camacho, 2021*). Therefore, I tested whether the aforementioned traits are correlated with sprint speed and parotoid gland area and colour contrast. In addition, predation pressure is subject~~ed~~ to spatial variation, according to an experiment ~~with~~ where ~~toad~~ plasticine toad models ~~that~~ received more attacks in an agrosystem than in a natural habitat (*Zamora-Camacho, 2021*). Moreover, agrosystem toads are larger than those from a natural habitat (*Zamora-Camacho & Comas, 2017*), which could be an adaptive response to greater predator pressure. Accordingly, I tested whether the aforementioned traits vary between these habitats. Finally, the fact that males in this species are faster (*Zamora-Camacho, 2018*) and have larger parotoid glands than females (*Zamora-Camacho, 2021*) supports the notion that ~~the former~~males are under greater predation pressure than ~~the latter~~females~~, as detected~~ – which aligns with reports from ~~in~~ other related species (e.g., *Frétey et al., 2004*). Therefore, I tested whether the aforementioned traits vary between sexes. I predict that individuals that are better suited against predators (i.e., those that are faster, ~~more massive~~larger in body size, or have larger and more contrasting parotoid glands) will display riskier ~~behaviours~~behavioural traits, including being more active, bolder, and more ~~more~~ exploratory~~ behaviours~~. Similarly, I expect pine grove toads and females to be more active, bolder, and more exploratory, as they are under reduced predation pressure.

Materials & Methods

Study species

Epidalea calamita is a bufonid toad that thrives in diverse habitats, including unaltered as well as human-modified systems, in extensive areas in the ~~centre~~ central and western ~~of~~ Europe (*Gomez-Mestre, 2014*). Owing to the variable climatic conditions throughout this vast area, the phenology of this species is asynchronous, with aestivation being common in hot regions and hibernation occurring in cold climates (*Gomez-Mestre, 2014*). This species is primarily nocturnal, and its activity and reproduction take place during wet and not excessively cold weather, which happens during winter in warmer ~~areas' winters~~regions,~~ but~~ and in the spring in colder regions ~~areas' springs~~ (*Gomez-Mestre, 2014*). Under adverse circumstances, they rest under rocks or logs, or in dens they burrow in loose soils, safe from predators (*Gomez-Mestre, 2014*). These toads are potential prey of a wide array of predators, including snakes (e.g. *Natrix maura* and *Natrix astreptophora*), birds (e.g. *Larus ridibundus* and *Pica pica*) and mammals (e.g. *Meles meles*), among others (see *Gomez-Mestre, 2014*). When under attack, toads use intermittent runs to flee (*Zamora-Camacho, 2018*). ~~However, w~~When escape is not possible, however, they commonly arch their loins and exhibit their parotoid glands, which can release great amounts of toxins (*Stawikowski & Lüddecke, 2019*).

Animal capture and management

Toads were captured in the pine grove “Pinares de Cartaya” (SW Spain: 37º20’ N, 7º09’ W) and in the agrosystem nearby. The forest is an 11 000-ha extension dominated by *Pinus pinea* and an undergrowth of Mediterranean bushes such as *Pistacea lentiscus*, *Cistus ladanifer* and *Rosmarinus officinalis*. ~~Even~~ Although ~~whether~~ this plant assemblage could be considered ~~is~~ autochthonous or introduced in this region ~~is a moot point~~, its predominance dates back at least 4 000 years (*Martínez & Montero, 2004*)~~,~~ and as such ~~so~~ it is deemed a natural ~~whatever the case~~habitat for the purposes of this study ~~(*Martínez & Montero, 2004*)~~. The agrosystem is ~~Around~~ about 5 km away from the pine grove, and is a~~lies a~~ 2 800-ha agricultural area~~,~~ where extensive vegetable crops have gradually given way to intensive orange tree, blueberry, and strawberry fields (among others) ~~fields all~~ throughout the last few decades. In these croplands, landowners apply fertilizers, fungicides, herbicides, and pesticides at their discretion, and artificial watering softens the three-to-four-month-long summer droughts. Animal capture and management was according to permits by the Junta de Andalucía government (Reference AWG/mgd GB-369-20).

Due to the mild local climate, *E. calamita* breeds in the winter there. Accordingly, toad capture was conducted from December 2018 to March 2019. I caught 22 females and 20 males in the agrosystem, plus 21 females and 25 males in the pine grove. Toads were captured by hand while active in nights of suitable weather, then transported to the laboratory in plastic buckets with well-ventilated lids and a substrate of humid earth. When they were in the laboratory, I used their sexual dimorphism in coloration (females have browner backs and greyish throats, whereas males have greener backs and purplish or pinkish throats; *Zamora-Camacho & Comas, 2019*) and the presence of blackish nuptial pads ~~exclusively i~~on male~~s’~~ forelimbs (*Gomez-Mestre, 2014*), to sex them. Next, I allocated them to individual plastic terraria (20 x 13 x 9 cm) with wet peat as a substrate and an opaque plastic shelter. Toads were undisturbed in these terraria at all times, except during the trials. Photos were taken approximately 24 hours after capture (see below). Then, 24 hours after the photos, toad activity trials were recorded (see below). Finally, 24 hours after the activity trials, sprint speed tests were performed (see below). Toads were released at their capture sites shortly afterward.

Measurements of coloration and morphology

I used a ruler to measure toad snout-vent length (hereafter, SVL) to the nearest mm, and a scale (model CDS-100) to weigh them to the nearest 0.01 g. No later than 24 hours after capture, I orthogonally photographed each toad’s back using a photo camera Canon EOS 550D, set at 18 megapixels of resolution, F10 of shutter-aperture, and a focal length fixed at 53 mm. Only exposure time was allowed to be automatically adjusted by the device, to optimize sharpness in each individual photo. The camera was secured to a tripod, which guaranteed perpendicularity, steadiness, and a constant distance of 40 cm from the lens to the photographed area. This area was a square (30 ~~-~~cm-side), white piece of paper that lay horizontal. On both lateral and the rear sides (considering that the tripod was located opposite to the front side), three square (30 ~~-~~cm-side), white pieces of white polyester sat vertically, conforming an incomplete cube which lacked the front (allowing toad handling) and the upper sides (allowing photograph taking). In order to avoid all parasitic lights (i.e., any uncontrolled source of light), photos were taken at night in a completely closed room, where the only sources of light were two 80W white-light bulbs, one next to each lateral side of the cube, externally to it, at a height of 20 cm, so that shades on the photographed area were prevented and the white polyester of the lateral, vertical squares filtered the light. This setting is depicted in Fig. S1 in Supplementary Material. Immediately prior to taking the photos, once the set was fixed as described, I calibrated white balance to a spotless piece of paper, after which I added a standardized colour chart (IMAGE Photographic) for digital calibration of white balance, and a piece of graph paper to calibrate length. Any remainder of humidity and dirt was gently removed from the toads' skins with a disposable napkin before each photo.

Afterwards, these photos were processed with the software Adobe Photoshop CS5. Firstly, I calibrated white balance one more time in each photo by using the tool eyedropper in the white calibration function on the colour chart. Furthermore, colour mode was set to the L*a*b* colour space preconized by the *Commision Internationale d'Eclairage* (CIE) (*Montgomerie, 2006*). This is a three-dimensional colour space were L* quantifies lightness, and varies from 0 (pure black) to 100 (pure white); a* quantifies the green-red axis (positive values represent red and negative values represent green); and b* quantifies the blue-yellow axis (positive values represent yellow and negative values represent blue). I calibrated length using the piece of graph paper, and manually outlined both parotoid glands making use of the lasso tool, which allowed me to calculate the sum of the areas of each. After this, parotoid gland relative area was calculated as the residuals of regression of parotoid gland area against SVL. Next, once the parotoid glands were outlined, I calculated their average colour and, with the histogram tool, retrieved their average values of L*, a*, and b*. Lastly, I followed the same steps to trace the dorsum (excluding the parotoid glands and the limbs) and retrieve its average L*, a* and b* values. The average L*, a*, and b* parotoid gland and dorsum values were used to calculate parotoid gland contrast (ΔE*) as in the CIE formula to assess difference in colour: ΔE* = $(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$ (*Nguyen, Nol & Abraham, 2007*; *Moreno-Rueda et al., 2019*).

Measurements of activity and sprint speed

Starting 24 hours after the photos were taken, toads were recorded for activity and sprint speed trials (see details below), in this order. Videos were filmed with a camera Canon EOS 550D, at 25 frames per second. The camera was attached to a 2.5 ~~-~~m-high tripod, with a 90º angle, at all times. In both trials, only one individual was recorded at a time. To remove the effect that temperature may have on amphibian activity (*Muller, Cade & Schwarzkopf, 2018*) and locomotion (*Preest & Pough, 2003*), the room was at approximately 19ºC at all times. Light conditions were standardized, as the only light source during all trials was a 60W white light bulb 2.5 m high at the centre of the container where the toad was performing the trial in question (see below). All videos were recorded at night (approximately between ~~09pm ~~21:00-~~and ~~02:00 ~~am~~h, local time), when these toads are naturally active (*Gomez-Mestre, 2014*).

Measurement of activity

Activity trials were recorded while these toads were freely moving in a plastic arena (54 x 27 x 40 cm). A grid of 9~~-~~ cm-side squares was painted with non-toxic ink on the bottom of this arena. Prior to the recordings, toads were placed at the centre of the arena, enclosed within a vertical hollow cylinder (50 cm high, 15 cm diameter) open at its lower end. The cylinder was built with a metal mesh (5 mm light), which allowed acclimation to the experimental setting. After two minutes, the cylinder was gently removed in the vertical plane, and the toad’s activity was recorded for 10 minutes (*Chajma, Kopecký & Vojar, 2020*).

Videos were then analysed with the program Tracker v. 4.92. I measured several variables as surrogates of different traits of alleged relevance in the behaviour of animals in general (*Réale et al., 2007*) and of amphibians in particular (*Kelleher, Silla & Byrne, 2018*). Activity time was the amount of time (~~in~~ s) the toad spent moving (*Chajma et al., 2020*). Exploration behaviour was estimated as the number of squares visited (excluding squares that had been visited before; *Chajma et al., 2020*) and the number of square visits (counting the number of times any square was visited, including repeated visits; *Carlson & Langkilde, 2013*). These measures differ in the fact that the former assumes that the individual distinguishes and keeps track of the areas that have already been visited, whereas the latter assumes the opposite (*Carlson & Langkilde, 2013*). Time until the first move (i.e., latency) was also recorded, as a surrogate of the shyness/boldness gradient, as bolder individuals are expected to start moving sooner (*Chajma et al., 2020*). However, the use of space is also widely considered a surrogate of the shyness/boldness gradient, in amphibians (*Réale et al., 2007*; *Carlson & Langkilde, 2013*; *Chajma et al., 2020*) and other taxa (*Burns, 2008*; *Harris, D'Eath & Healy, 2009*). Specifically, thigmotaxis, the tendency for some individuals to remain in the periphery of their enclosures next to the walls rather than in the open areas, has been regarded as an anxiety-like, predator-avoidance behaviour as opposed to the boldness subjacent to the use of open areas (*Harris et al., 2009*; *Carlson & Langkilde 2013*; *Chajma et al., 2020*). Therefore, I also estimated the shyness/boldness gradient by counting independently the number of external and internal squares visited (excluding squares that had been visited before) and the number of external and internal square visits (counting the number of times squares of these types were visited, including repeated visits). Then, I divided the number of external squares visited by the total number of squares visited (external squares visited ratio), as well as the number of external square visits by the total number of square visits (external square visit ratio). Both ratios have a direct relationship with boldness. These measurements are relevant in an ecological context, as laboratory surrogates of animal behaviour mirror actual behaviour in the wild (*Herborn et al., 2010*).

Measurement of sprint speed

Prior to conducting and recording ~~of~~ the sprint speed trials, I emptied toad bladders by firmly - but gently - pressing their lower abdomens, which eliminates any potential effect of different bladder water burden by reducing it to zero (*Preest & Pough, 1989*; *Walvoord, 2003*; *Prates et al., 2013*). Next, I allowed toads to rest in their terraria for one hour. After that, they were recorded (with the same camera already described) while running along a brownish cardboard linear raceway (200 x 15 x 15 cm). On its bottom, one transversal white stripe of insulating tape was placed every 10 cm, so that the raceway was longitudinally divided into 10-cm stretches delimited by contrasting-colour stripes easy to visualize in the videos. Locomotor performance may depend on the substrate where the race takes place (*Vanhooydonck et al., 2015*): cardboard provided a surface rough enough to facilitate an appropriate traction. I also set a dark background at one end of the raceway, which could be viewed as a shelter and encourage toads' moving forward (*Zamora-Camacho, 2018*; *González-Morales et al., 2021*). Individuals were placed at the opposite end of the raceway, and continuously pursued as a way of encouraging running, until they covered the raceway. Once these trials were completed, toads were released at their capture sites within 24 hours. No visible damage was inflicted on toads because of this investigation.

The footages produced were analysed with the program Tracker v. 4.92, which allows frame-by-frame video handling. For each toad, I registered the time (to the nearest 0.01 s) needed to cover each stretch in the raceway, which equals the time elapsed between the moments when the snout of a toad surpassed two consecutive white stripes (*Martín & López, 2001*; *Zamora-Camacho et al., 2014*; *Zamora-Camacho, 2018*). As the distance covered was 10 cm in all cases, I calculated the speed (in cm/s) reached in each stretch by dividing 10 cm by the time (~~in~~ s) it took for the toad to cover it. I considered each individual's sprint speed as its highest speed value. Finally, relative speed was calculated as the residuals of regression of sprint speed against SVL.

Statistics

Firstly, I built two correlation matrices, one including the behavioural traits measured (number of squares visited, number of square visits, external squares visited ratio, external square visits ratio, activity time, and time until the first move) and another including the antipredator defences measured (body mass, parotoid gland contrast, parotoid gland relative area, and relative sprint speed). The aim of these matrices was to detect collinearity between both sets of data. Most behavioural traits measured were highly correlated, except for time until the first move (Table S1 in Supplementary Material). On the contrary, the antipredator defences measured were mostly uncorrelated, except for relative sprint speed and parotoid gland relative area, which were positively and significantly correlated (Table S2 in Supplementary Material).

Then, to condense the correlated variables into fewer, uncorrelated variables, and solve the limitation caused by the high collinearity among the variables measured, I conducted a Principal Component Analysis (PCA; *Jongman, Braak & Tongeren, 1995*) including the behavioural traits measured that were correlated (number of squares visited, number of square visits, external squares visited ratio, external square visits ratio, and activity time; Table S3a in Supplementary Material) and another PCA including the antipredator defences measured that were correlated (parotoid gland relative area and relative sprint speed; Table S3b in Supplementary Material). In both cases, only Principal Components (PC) with an eigenvalue greater than 1 were selected, according to the Guttmann-Kaiser Criterion (*Yeomans & Golder, 1982*).

Then, I conducted three separate ANCOVAs, where habitat, sex and their interaction were included as factors, and all PC with an eigenvalue greater than 1 in the second PCA (namely, PC1, see Results below), as well as body mass and parotoid gland contrast, were included as covariates. The response variable of the first ANCOVA was time until the first move. In the second and the third ANCOVAs, the variable responses were each PC with an eigenvalue greater than 1 in the first PCA (namely, PCa and PCb, see Results below). Stepwise backward selection was applied to these ANCOVAs. Tests were conducted with the package “*nlme*” (*Pinheiro et al., 2012*) in the software R (*R Development Core Team, 2012*). Before conducting parametric statistical analyses, I checked that the data met the criteria of homoscedasticity and residual normality (*Quinn & Keough, 2002*). Since no transformation could make body mass homoscedastic, I implemented the function “*varIdent*” (*Zuur et al., 2009*).

Results

Variable grouping according to PCAs

In the PCA including the behavioural traits, the only two PC with eigenvalues greater than 1, named PCa and PCb, explained jointly 88.99% of the total variance. PCa was strongly and positively correlated with the number of squares visited, the number of square visits, and activity time, whereas its correlations with the external squares visited ratio and the external square visits ratio were strong and negative (Table 1). Therefore, PCa was positively correlated with exploration behaviour, activity, and boldness. In turn, PCb was negatively correlated with all behavioural traits measured (Table 1). Therefore, PCb was negatively correlated with exploration behaviour and activity, and positively correlated with boldness.

In the PCA including parotoid gland relative area and relative sprint speed, the only PC with an eigenvalue greater than 1, named PC1, explained 68.20% of the total variance. PC1 was strongly and positively correlated with relative sprint speed and relative parotoid gland area (Table 2).

ANCOVAs

After stepwise backward selection was applied to the ANCOVA including PCa as the response variable, sex had a significant effect, with PCa being greater in females than in males (Mean ± SE; females: 0.387 ± 0.220; males: -0.349 ± 0.300; $X^2_{1, 85} = 3.910$; $P = 0.048$; Fig. 1). Moreover, body mass had a negative, significant relationship with PCa ($X^2_{1, 85} = 8.122$; $\beta = -0.045$; $P = 0.004$).

After stepwise backward selection was applied to the two ANCOVAs including either PCb or time until the first move as response variables, no factor or covariate appeared significant in either case.

Discussion

These results support some of the predictions formulated, but not others. For example, relative speed as well as parotoid gland contrast and size were not related to the traits studied. In contrast

Commented [JBG5]: I would suggest inserting a stronger and more direct topic sentence here. It should be full and encompassing, and clearly state the general trend found.

It is always best to not rely on the readers following the Results sections with the same clarity as you see it. As such, the opening line of the Discussion should act to cue them in on what the main point is.

Something like “My findings saw that the antipredator responses of *E. calamita was….”*

with other bufonids, such as *Rhinella marina*, whose jumping distance is directly proportional to body length (*Hudson et al., 2020*), sprint speed of *E. calamita* is unrelated to body size (*Zamora-Camacho, 2018*), which allows for a separate evaluation of both parameters. The absence of correlations between speed and the behavioural traits tested as a part of this study is aligned with findings on *Zootoca vivipara* (*Le Galliard et al., 2013*) and *Phrinocephalus vlangalii* lizards (*Chen et al., 2019*), but not with findings on *Myotomis unisulcatus* (*Agnani et al., 2020*) and *Tamias striatus* rodents (*Newar & Careau, 2018*). It is worth mentioning that all toads in this sample engaged in flight behaviour. However, the tonic immobility observed in other cases, especially in taxa that rely on their toxins against attacks, can also be considered an expression of boldness (*Edelaar et al., 2012*; *Hudson, Brown & Shine, 2017*). In turn, relationships between overall coloration and different behavioural traits have been described in taxa as disparate as tortoises (*Mafli, Wakamatsu & Roulin, 2011*), fish (*Schweitzer, Motreuil & Dechaume-Moncharmont, 2015*) and birds (*Costanzo et al., 2018*). However, there is a lack of such studies on amphibians (reviewed in *Kelleher et al., 2018*) and, to the best of my knowledge, the potential relationship between aposematism and behaviour at the individual level remains unexplored thus far. Nonetheless, according to these results, the degree of aposematism is unrelated to behaviour in these toads. A decoupling between colour and behaviour, albeit in a reproductive context, has also been described in phrysonomatid lizards (*Wiens, 2000*). These mismatches between some antipredator defences and behaviour could suggest that the success of these traits is independent of each other, or simply that they have evolved separately.

Similarly, parotoid gland size was not associated with a more exploratory and bolder behaviour nor increased activity time. These are considered risky ~~behaviours~~ behavioural traits that increase conspicuousness to predators (*Hall et al., 2015*; *Reader, 2015*). Therefore, this finding does not support the prediction that more extensive parotoid glands, capable of releasing greater amounts of toxins (*Zechmeister, 1948*; *Llewelyn et al., 2012*), could better protect their bearers against predators, thus reducing the potential costs of risky behaviours with regard to their benefits (*Smith & Blumstein, 2008*; *Niemelä, Lattenkamp & Dingemanse, 2015*). However, the amount of toxin contained in the glands at the moment of the trials could not be assessed. This could represent a limitation of the experimental design, as closely-related *R. marina* toads adjust their antipredator behaviour after parotoid gland toxin depletion (*Blennerhassett et al., 2019*). On the contrary, body mass was negatively related to exploratory behaviour, boldness, and activity time. Although larger prey individuals could be better suited against gape-limited predators (*Turesson, Persson & Brönmark, 2002*; *Urban, 2007*), this is not necessarily true when predators are non-gape-limited (*Jobe et al., 2019*; *Stretz, Andersson & Burkhart, 2019*). Indeed, gape unconstrained predators such as mammals (*Owen-Smith & Mills, 2008*) or birds (*Comay & Dayan, 2018*) can and do handle remarkably voluminous prey. Although some local snakes (mainly *Natrix astreptophora* and ~~*Natrix*~~ *N. maura*; *Gomez-Mestre, 2014*), which are gape-limited, have been described as predators of these toads, their activity seldom overlap, as those snakes are mainly diurnal and hibernate in the winter (*Santos, 2015*; *Pleguezuelos, 2018*), whereas *E. calamita* ~~natterjack~~ toads are primarily active in winter nights (*Gomez-Mestre, 2014*). Therefore, the most likely predators of *E. calamita* adults are birds and mammals (*Gomez-Mestre, 2014*), to which larger toads could be more conspicuous, but not less vulnerable. In this context, the less risky behaviour of larger toads could be advantageous against their main predators. Variation in the relative pressure exerted by dissimilar predator preferences on body size might underlie the apparently contradictory relationships between body size and behaviour found among this and other studies~~:~~. For example, whereas bold ~~male~~ *Lacerta monticola* lizard males are smaller (*López et al., 2005*), there is a positive relationship between body size and boldness in juvenile *Tropidonophis mairii* snakes (*Mayer, Shine & Brown, 2016*) and between body size and exploratory behaviour in *Pseudophryne corroboree* frogs (*Kelleher et al., 2017*).

Moreover, females exhibited a bolder behaviour than ~~did~~ males. This finding is aligned with the assumption that female toads are under milder predation pressure (*Frétey et al., 2004*), and can thus afford riskier behaviours. Indeed, *E. calamita* males in this system are faster (*Zamora-Camacho, 2018*), brighter (*Zamora-Camacho & Comas, 2019*), and have larger parotoid glands than females (*Zamora-Camacho, 2021*), which could be interpreted as antipredator defences triggered by a harsher predation pressure. Remarkably, other toads, such as *R. marina* (*Gruber et al., 2018*) or *Sclerophrys gutturalis* (*Baxter-Gilbert, Riley & Measey, 2021*) do not appear to differ in these behavioural traits. Sexual differences in boldness, moreover, vary notably in other taxa: male dogs (*Starling et al., 2013*) and *Brachyraphis episcopi* fish (*Brown, Jones & Braithwaite, 2007*) are bolder than females, female *Diomeda exulans* albatross are bolder than males (*Patrick, Charmantier & Weimerskirch, 2013*), and *Diploptera punctata* male and female cockroaches do not diverge in boldness (*Stanley, Mettke-Hofmann & Preziosi, 2017*).

Lastly, habitat did not affect the behavioural traits measured, despite the fact that this species is under greater predation pressure in agrosystem than in pine grove (*Zamora-Camacho, 2021*). Other traits seem to be aligned with this spatial pattern of predation pressure: agrosystem toads have a more intermittent locomotion mode (*Zamora-Camacho, 2018*), are brighter (*Zamora-Camacho & Comas, 2019*), and have larger and more contrasting parotoid glands than pine grove conspecifics (*Zamora-Camacho, 2021*), which could signify greater antipredator defences, likely triggered by more intense predation pressure. Remarkably, while habitat alone does not innately affect boldness behaviour of *S. gutturalis* tadpoles (*Mühlenhaupt et al., 2022*), predation pressure can explain spatial divergence in behavioural and morphological traits of other anurans, such as *Bombina variegata* toads (*Kang et al., 2017*). Spatial differences in boldness may have implications at other levels.

Conclusions

Commented [JBG6]: One thing I am thinking is missing is some sort of direction on how these findings should be used to inform future work. I would suggest trying to work in some ideas for how this work could guide the next study, based on what you found.

~~To conclude, r~~Relative speed as well as parotoid gland contrast and size appeared unrelated to the behavioural traits studied. In turn, body mass was negatively related to activity time, boldness, and exploration. This trend is consistent with the fact that most predators of this species are gape unconstrained and could more easily find and hunt larger toads. Females were bolder, which matches the assumptions that males and agrosystem toads are under harsher predation pressure. Nonetheless, the behavioural traits measured did not vary between habitats, which is not aligned with previous findings that agrosystem toads are under greater predation pressure. Jointly, these results partly support the predictions that behaviour is tuned to antipredator defences and to differential predation pressure in this toad.

Acknowledgements

Gregorio Moreno-Rueda and Mar Comas kindly provided their logistic support. Comments by Max Mühlenhaupt, Cameron Hudson and an anonymous reviewer improved the manuscript.

References

Abbey-Lee RN, Mathot KJ, Dingemanse N (2016) Behavioral and morphological responses to perceived predation risk: a field experiment in passerines. Behav Ecol 27:857-864.

Agnani P, Thomson J, Schradin C, Careau V (2020) The fast and the curious II: performance, personality, and metabolism in Karoo bush rats. Behav Ecol Sociobiol 74:123.

Baxter-Gilbert J, Riley JL, Measey J (2021) Fortune favors the bold toad: urban-derived behavioral traits may provide advantages for invasive amphibian populations. Behav Ecol Sociobiol 75: 130.

Beauchamp DA, Wahl D, Johnson BM (2007) Predator–prey interactions. In: Guy CS, Brown MJ, editors. Analysis and interpretation of inland fisheries data. Bethesda, MD: American Fisheries Society. p. 765-842.

Belgrad BA, Griffen BD (2016) Predator-prey interactions mediated by prey personality and predator hunting mode. Proc R Soc B 283:20160408.

Biro PA, Stamps JA (2008) Are animal personality traits linked to life-history productivity? Trend Ecol Evol 23:361-368.

Blake CA, Andersson ML, Hulthén K, Nilsson PA, Brönmark C (2018) Conspecific boldness and predator species determine predation-risk consequences of prey personality. Behav Ecol Sociobiol 72:133.

Blennerhassett RA, Bell-Anderson K, Shine R, Brown GP (2019) The cost of chemical defence: the impact of toxin depletion on growth and behaviour of cane toads (*Rhinella marina*). Proc R Soc B 286:20190867.

Briffa M, Sneddon LU (2016) Proximate mechanisms of among-individual behavioural variation in animals. Behaviour 153:1509-1515.

Briffa M, Sneddon LU, Wilson AJ (2015) Animal personality as a cause and consequence of contest behaviour. Biol Lett 11:20141007.

Brodie ED III (2009) Toxins and venoms. Curr Biol 19:R931-R935.

Brown C, Jones F, Braithwaite WA (2007) Correlation between boldness and body mass in natural populations of the poecilid (*Brachyraphis episcopi*). J Fish Biol 71:1590-1601.

Brown GE, Elvidge CK, Ramnarine I, Chivers DP, Ferrari MCO (2014) Personality and the response to predation risk: effects of information quantity and quality. Anim Cogn 17:1063-1069.

Burns JG (2008) The validity of three tests of temperament in guppies (*Poecilia reticulata*). J Comp Psychol 122:344-356.

Carlson BE, Langkilde T (2013) Personality traits are expressed in bullfrog tadpoles during open-field trials. J Herpetol 47:378-383.

Chajma P, Kopecký O, Vojar J (2020) Individual consistency in exploration and shyness but not activity in smooth newts (*Lissotriton vulgaris*): the effect of habituation? J Zool 311:269-276.

Chen J, Qi Y, Wu Y, Wang X, Tang Y (2019) Covariations between personality behaviors and metabolic/performance traits in an Asian agamid lizard (*Phrynocephalus vlangalii*). PeerJ 7:e7205.

Comay O, Dayan T (2018) What determines prey selection in owls? Roles of prey traits, prey class, environmental variables, and taxonomic specialization. Ecol Evol 8:3382-3392.

Costanzo A, Romano A, Ambrosini R, Parolini M, Bubolini D, Caprioli M, Corti M, Canova L, Saino N (2018) Barn swallow antipredator behavior covaries with melanic coloration and predicts survival. Behav Ecol 29:1472-1480.

Cremona T, Mella VSA, Webb JK, Crowther MS (2015) Do individual differences in behavior influence wild rodents more than predation risk? J Mammal 96:1337-1343.

Davis DR, Gabor CR (2015) Behavioral and physiological antipredator responses of the San Marcos salamander, *Eurycea nana*. Physiol Behav 139:145-149.

Dhellemmes F, Smukall MJ, Guttridge TL, Krause J, Hussey NE (2021) Predator abundance drives the association between exploratory personality and foraging habitat risk in a wild marine meso-predator. Funct Ecol 35:1972-7984.

Díaz JA, Carrascal LM (1993) Variation in the effect of profitability on prey size selection by the lacertid lizard *Psammodromus algirus*. Oecologia 94:23-29.

Dingemanse NJ, Kazem AJN, Réale D, Wrigth J (2010) Behavioural reaction norms: animal personality meets individual plasticity. Trend Ecol Evol 25:81-89.

Dingemanse NJ, Réale D (2005) Natural selection and animal personality. Behaviour 142:1165-1190.

Dosmann A, Mateo JM (2014) Food, sex and predators: animal personality persists with multidimensional plasticity across complex environments. Anim Behav 90:109-116.

Dugatkin LA (2020) Principles of animal behavior. University of Chicago Press.

Edelaar P, Serrano D, Carrete M, Blas J, Potti J, Tella JL (2012) Tonic immobility is a measure of boldness toward predators: an application of Bayesian structural equation modeling. Behav Ecol 23:619-626.

Frétey T, Cam E, Le Garff B, Monnat JY (2004) Adult survival and temporary emigration in the common toad. Can J Zool 82:859-872.

Gomez-Mestre I (2014) Sapo corredor – *Epidalea calamita* (Laurenti, 1768). In: Salvador A, Marco A, editors. Enciclopedia Virtual de los Vertebrados Españoles. Museo Nacional de Ciencias Naturales, Madrid. See http://www.vertebradosibericos.org

González-Morales JC, Rivera-Rea J, Moreno-Rueda G, Bastiaans E, Castro-López M, Fajardo V (2021) Fast and dark: the case of Mezquite lizards at extreme altitude. J Therm Biol 102:103115.

Gruber J, Brown G, Whiting MJ, Shine R (2018) Behavioural divergence during biological invasions: a study of cane toads (*Rhinella marina*) from contrasting environments in Hawai'i. R Soc Open Sci 5:180197.

Hall ML, van Asten T, Katsis AC, Dingemanse NJ, Magrath MJL, Mulder RA (2015) Animal personality and pace-of-life syndromes: do fast-exploring fairy-wrens die young? Front Ecol Evol 3:28.

Harris AP, D'Eath RB, Healy SD (2009) Environmental enrichment enhances spatial cognition in rats by reducing thigmotaxis (wall hugging) during testing. Anim Behav 77:1459-1464.

Harris S, Ramnarine IW, Smith HG, Pettersson LB (2010) Picking personalities apart: estimating the influence of predation, sex and body size on boldness in the guppy *Poecilia reticulata*. Oikos 119:1711-1718.

Herborn KA, Macleod R, Miles WTS, Schofield ANB, Alexander L, Arnold KE (2010) Personality in captivity reflects personality in the wild. Anim Behav 79:835-843.

Hudson CM, Brown GP, Shine R (2017) Evolutionary shifts in anti-predator responses of invasive cane toads (*Rhinella marina*). Behav Ecol Sociobiol 71:134.

Hudson CM, Vidal-García M, Murray TG, Shine R (2020) The accelerating anuran: evolution of locomotor performance in cane toads (*Rhinella marina*) at an invasion front. Proc R Soc B 287:20201964.

Jobe KL, Montaña CG, Schalk CM (2019) Emergent patterns between salamander prey and their predators. Food Webs 21:e00128.

Jongman RHG, Braak CJF, Tongeren OFR (1995) Data analysis in community and landscape ecology. Cambridge: Cambridge University Press.

Kalinkat G, Schneider FD, Digel C, Guill C, Rall BC, Brose U (2013) Body masses, functional responses and predator-prey stability. Ecol Lett 16:1126-1134.

Kang C, Sherratt TN, Kim YE, Shin Y, Moon J, Song U, Kang JY, Kim K, Jang Y (2017) Differential predation drives the geographical divergence in multiple traits in aposematic frogs. Behav Ecol 28:1122-1130.

Karpestam E, Merilaita S, Forsman A (2014) Body size influences differently the detectabilities of colour morphs of cryptic prey. Biol J Linn Soc 113:112-122.

Kelleher SR, Silla AJ, Byrne PG (2018) Animal personality and behavioral syndromes in amphibians: a review of the evidence, experimental approaches, and implications for conservation. Behav Ecol Sociobiol 72:79.

Kelleher SR, Silla AJ, Dingemanse NJ, Byrne PG (2017) Body size predicts between-individual differences in exploration behaviour in the southern corroboree frog. Anim Behav 129:161-170.

Kortet R, Sirkka I, Lai YT, Vainikka A, Kekäläinen J (2015) Personality differences in two minnow populations that differ in their parasitism and predation risk. Front Ecol Evol 3:9.

Koski SE (2014) Broader horizons for animal personality research. Front Ecol Evol 2:70.

Le Galliard JF, Paquet M, Cisel M, Montes-Poloni L (2013) Personality and the pace-of-life syndrome: variation and selection on exploration, metabolism and locomotor performances. Funct Ecol 27:136-144.

Leblond M, Dussault C, Ouellet JP (2013) Impacts of human disturbance on large prey species: do behavioral reactions translate to fitness consequences? PLoS ONE 8:e73695.

Lima SL (2002) Putting predators back into behavioral predator–prey interactions. Trend Ecol Evol 17:70-75.

Lind J, Cresswell W (2005) Determining the fitness consequences of antipredator behavior. Behav Ecol 16:945-956.

Llewelyn J, Bell K, Schwarzkopf L, Alford RA, Shine R (2012) Ontogenetic shifts in a prey's chemical defences influence feeding responses of a snake predator. Oecologia 169:965-973.

López P, Hawlena D, Polo V, Amo L, Martín J (2005) Sources of individual shy-bold variations in antipredator behaviour of male Iberian rock lizards. Anim Behav 69:1-9.

MacPherson B, Mashayekhi M, Gras R, Scott R (2017) Exploring the connection between emergent animal personality and fitness using a novel individual-based model and decision tree approach. Ecol Inform 40:81-92.

Mafli A, Wakamatsu K, Roulin A (2011) Melanin-based coloration predicts aggressiveness and boldness in captive eastern Hermann's tortoises. Anim Behav 81:859-863.

Mänd T, Tammaru T, Mappes J (2007) Size dependent predation risk in cryptic and conspicuous insects. Evol Ecol 21:485.

Martín J, López P (2001) Hindlimb asymmetry reduces escape performance in the lizard *Psammodromus algirus*. Physiol Biochem Zool 74:619-624.

Martínez F, Montero G (2004) The *Pinus pinea* L. woodlands along the coast of South-western Spain: data for a new geobotanical interpretation. Plant Ecol 175:1-18.

Mayer M, Shine R, Brown GP (2016) Bigger babies are bolder: effects of body size on personality of hatching snakes. Behaviour 153:313-323.

McGee MR, Julius ML, Vajda AM, Norris DO, Barber LB, Schoenfuss HL (2009) Predator avoidance performance of larval fathead minnows (*Pimephales promelas*) following short-term exposure to estrogen mixtures. Aquat Toxicol 91:355-361.

Mebs D (2001) Toxicity in animals. Trends in evolution? Toxicon 39:87-96.

Montgomerie R (2006) Analyzing colors. In: Hill GE, McGraw KJ, editors. Bird Coloration Volume I: mechanisms and measurements. Cambridge MA; Harvard University Press. p 90-140.

Moreno-Rueda G, González-Granda LG, Reguera S, Zamora-Camacho FJ, Melero E (2019) Crypsis decreases with elevation in a lizard. Diversity 11:236.

Mühlenhaupt M, Baxter-Gilbert J, Makhubo BG, Riley JL, Measey J (2022) No evidence for innate differences in tadpole behavior between natural, urbanized, and invasive populations. Behav Ecol Sociobiol 76:11.

Muller BJ, Cade BS, Schwarzkopf L (2018) Effects of environmental variables on invasive amphibian activity: using model selection on quantiles for counts. Ecosphere 9:e02067.

Nettle D (2006) The evolution of personality variation in humans and other animals. Am Psychol 61:622-631.

Newar SL, Careau V (2018) The fast and the curious: locomotor performance and exploratory behaviour in eastern chipmunks. Behav Ecol Sociobiol 72:27.

Nguyen LP, Nol E, Abraham KF (2007) Using digital photographs to evaluate the effectiveness of plover egg crypsis. J Wildl Manage 71:2084-2089.

Niemelä PT, Lattenkamp EZ, Dingemanse NJ (2015) Personality-related survival and sampling bias in wild cricket nymphs. Behav Ecol 26:936-946.

Owen-Smith N, Mills MGL (2008) Predator-prey size relationships in African large-mammal food web. J Anim Ecol 77:173-183.

Patrick SC, Charmantier A, Weimerskirch H (2013) Differences in boldness are repeatable and heritable in a long-lived marine predator. Ecol Evol 3:4291-4299.

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Development Core Team (2012) nlme: linear and nonlinear mixed effects models. R package version 3.1-103. Vienna: R Foundation for Statistical Computing.

Pleguezuelos JM (2018) Culebra de collar mediterránea – *Natrix astreptophora*. In: Sanz JJ, Martínez-Freiría F., editors. Enciclopedia Virtual de los Vertebrados Españoles. Museo Nacional de Ciencias Naturales, Madrid. See http://www.vertebradosibericos.org

Prates I, Angilleta MJ, Wilson RS, Niehaus AC, Navas CA (2013) Dehydration hardly slows hopping toads (*Rhinella granulosa*) from xeric and mesic environments. Physiol Biochem Zool 86:451-457.

Preest MR, Pough FH (1989) Interaction of temperature and hydration on locomotion of toads. Funct Ecol 3:693-699.

Preest MR, Pough FH (2003) Effects of body temperature and hydration state on organismal performance of toads, *Bufo americanus*. Physiol Biochem Zool 76:229-239.

Preisser EL, Orrock JL (2012) The allometry of fear: interspecific relationships between body size and response to predation risk. Ecosphere 3:77.

Prudic KL, Skemp AK, Papaj DR (2007) Aposematic coloration, luminance contrast, and the benefits of conspicuousness. Behav Ecol 18:41-46.

Quinn GP, Keough MJ (2002) Experimental design and data analysis for biologists. Cambridge: Cambridge University Press.

Quinn JL, Cresswell W (2005) Personality, anti-predation behaviour and behavioural plasticity in the chaffinch *Fringilla coelebs*. Behaviour 142:1377-1402.

R Development Core Team (2012) R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing.

Radloff FGT, du Toit JT (2004) Large predators and their prey in a southern African savanna: a predator's size determines its prey size range. J Anim Ecol 73:410-423.

Reader SM (2015) Causes of individual differences in animal exploration and search. Topics Cogn. Sci 7:451-468.

Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ (2007) Integrating animal temperament within ecology and evolution. Biol Rev 82:291-318.

Roche DG, Careau V, Binning SA (2016) Demystifying animal 'personality' (or not): why individual variation matters to experimental biologists. J Exp Biol 219:3832-3843.

Ruxton GD, Allen WL, Sherratt TN, Speed MP (2018) Avoiding attack: the evolutionary ecology of crypsis, aposematism, and mimicry. New York: Oxford University Press.

Santicchia F, Gagnaison C, Bisi F, Martinoli A, Matthysen E, Bertolino S, Wauters LA (2018) Habitat-dependent effects of personality on survival and reproduction in red squirrels. Behav Ecol Sociobiol 72:134.

Santos X (2015) Culebra viperine – *Natrix maura*. In: Salvador A, Marco A, editors. Enciclopedia Virtual de los Vertebrados Españoles. Museo Nacional de Ciencias Naturales, Madrid. See http://www.vertebradosibericos.org

Saporito RA, Zuercher R, Roberts M, Gerow KG, Donnelly MA (2007) Experimental evidence for aposematism in the Dendrobatid poison frog *Oophaga pumilio*. Copeia 2007:1006-1011.

Savitzky AH, Mori A, Hutchinson DA, Saporito RA, Burghardt GM, Lillywhite HB, Meinwald J (2012) Sequestered defensive toxins in tetrapod vertebrates: Principles, patterns, and prospects for future studies. Chemoecology 22:141-158.

Schuett W, Tregenza T, Dall SRX (2010) Sexual selection and animal personality. Biol Rev 85:217-246.

Schweitzer C, Motreuil S, Dechaume-Moncharmont FX (2015) Coloration reflects behavioural types in the convict cichlid, *Amatitlania siquia*. Anim Behav 105:201-209.

Semlitsch RD (1990) Effects of body size, sibship, and tail injury on the susceptibility of tadpoles to dragonfly predation. Can J Zool 68:1027-1030.

Skelhorn J, Rowe C (2006) Avian predators taste-reject aposematic prey on the basis of their chemical defence. Biol Lett 2:348-350.

Smith BR, Blumstein DT (2008) Fitness consequences of personality: a meta-analysis. Behav Ecol 19:448-455.

Smith KE, Halpin CG, Rowe C (2016) The benefits of being toxic to deter predators depends on prey body size. Behav Ecol 27:1650-1655.

Stamps JA (2007) Growth-mortality tradeoffs and ‘personality traits’ in animals. Ecol Lett 10:355-363.

Stanley CR, Mettke-Hofmann C, Preziosi RF (2017) Personality in the cockroach Diploptera punctata: evidence for stability across developmental stages despite age effects on boldness. PLoS ONE 12:e0176564.

Starling MJ, Branson N, Thomson PC, McGreevy PD (2013) Age, sex and reproductive status affect boldness in dogs. Vet J 197:868-872.

Stawikowski R, Lüddecke T (2019) Description of defensive postures of the natterjack toad *Epidalea calamita* (Laurenti 1768) and notes on the release of toxic secretions. Herpetol Notes 12:443-445.

Stretz P, Andersson TL, Burkhart JJ (2019) Macroinvertebrate foraging on larval *Ambystoma maculatum* across ontogeny. Copeia 107:244-249.

Toscano BJ, Griffen BD (2014) Trait-mediated functional responses: predator behavioural type mediates prey consumption. J Anim Ecol 83:1469-1477.

Tsai CH, Hsieh C, Nakazawa T (2016) Predator-prey mass ratio revisited: does preference of relative prey body size depend on individual predator size? Funct Ecol 30:1979-1987.

Turesson H, Persson A, Brönmark C (2002) Prey size selection in piscivorous pikeperch (*Stizostedion lucioperca*) includes active prey choice. Ecol Freshw Fish 11:223-233.

Urban MC (2007) The growth-predation risk trade-off under a growing gape-limited predation threat. Ecology 88:2587-2597.

Vanhooydonck B, Measey J, Edwards S, Makhubo B, Tolley KA, Herrel A (2015) The effects of substratum on locomotor performance in lacertid lizards. Biol J Linn Soc 115:869-881.

Walvoord ME (2003) Cricket frogs maintain body hydration and temperature near levels allowing maximum jump performance. Physiol Biochem Zool 76:825-835.

Watkins TB (1996) Predator-mediated selection on burst swimming performance in tadpoles of the Pacific tree frog, *Pseudacris regilla*. Physiol Zool 69:154-167.

Weiss A (2018) Personality traits: a view from the animal kingdom. J Pers 86:12-22.

Whitman DW, Vincent S (2008) Large size as an antipredator defense in an insect. J Orthoptera Res 17:353-371.

Wiens JJ (2000) Decoupled evolution of display morphology and display behaviour in phrysonomatid lizards. Biol J Linn Soc 70:597-612.

Wilson V, Guenther A, Øverli Ø, Seltmann MW, Altschul D (2019) Future directions for personality research: contributing new insights to the understanding of animal behavior. Animals 9:240.

Yeomans KA, Golder PA (1982) The Guttman-Kaiser criterion as a predictor of the number of common factors. Statistician 31:221-229.

Yli-Renko M, Pettay JE, Vesakoski O (2018) Sex and size matters: selection on personality in natural prey-predator interactions. Behav Proc 148:20-26.

Zamora-Camacho FJ (2018) Locomotor performance in a running toad: roles of morphology, sex and agrosystem versus natural habitat. Biol J Linn Soc 123:411-421.

Zamora-Camacho FJ (2021) Sex and habitat differences in size and coloration of an amphibian's poison glands match differential predator pressures. Integr Zool in press.

Zamora-Camacho FJ, Comas M (2017) Greater reproductive investment, but shorter lifespan, in agrosystem than in natural-habitat toads. PeerJ 5:e3791.

Zamora-Camacho FJ, Comas M (2019) Beyond sexual dimorphism and habitat boundaries: coloration correlates with morphology, age, and locomotor performance in a toad. Evol Biol 46:60-70.

Zamora-Camacho FJ, García-Astilleros J, Aragón, P (2018) Does predation risk outweigh the costs of lost feeding opportunities or does it generate a behavioural trade-off? A case study with Iberian ribbed newt larvae. Biol J Linn Soc 125:741-749.

Zamora-Camacho FJ, Reguera S, Rubiño-Hispán MV, Moreno-Rueda G (2014) Effects of limb length, body mass, gender, gravidity, and elevation on escape speed in the lizard *Psammodromus algirus*. Evol Biol. 41:509-517.

Zechmeister L (1948) Progress in the Chemistry of Organic Natural Products. Springer.

Zhao QS, Hu YB, Liu PF, Chen LJ, Sun YH (2016) Nest site choice: a potential pathway linking personality and reproductive success. Anim Behav 118:97-103.

Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) Mixed effects models and extensions in ecology with R, Statistics for biology and health. Springer Science + Business Media, LLC New York, USA.

Zvereva EL, Kozlov MV (2016) The costs and effectiveness of chemical defenses in herbivorous insects: a meta-analysis. Ecol Monogr. 86:107-124.