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Prey metabolic responses to predators depend on predator hunting mode and prey antipredator defenses

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Predation risk causes prey to react in numerous ways, from life history changes to shifts in habitat. These responses give some insight into how different types of predators affect prey, but prey responses are often not comparable across taxa and experimental systems. Metabolism is a ubiquitous trait among living organisms, which offers a way to examine predator effects and create generalizable outcomes. We examined metabolic responses of terrestrial and aquatic prey from three antipredator functional groups under varied contexts of predator cues. We found that constitutively defended prey did not exhibit metabolic responses to any type of predator cues, while deimatic and freeze-flight prey exhibited metabolic responses that were dependent on both the cue type and predator hunting mode. Consistent with previous studies on nonconsumptive effects, we also found that ambush predators elicited a metabolic response while active predators did not. We propose that future work on this topic should continue to take a metabolic approach as a unified, scalable response variable to the sensory ecology of nonconsumptive effects, while identifying additional systems that can evaluate more fully both predator hunting mode and prey antipredator defenses.

Keywords: functional traits, nonconsumptive effects, predation risk, predator cues, predator–prey interactions, sensory ecology

Introduction

Predation risk influences prey behavior and physiology, with subsequent impacts on the structure and dynamics of ecological systems (Werner and Peacor 2003, Peckarsky et al. 2008, Schmitz et al. 2010, Hawlena et al. 2012, Wirsing et al. 2020). Prey are known to shift their habitat use (Turner 1996, Heithaus et al. 2009), foraging activity (Skelly and Werner 1990, Brown 1999), escape morphology (Relyea 2001, Hawlena et al. 2011), metabolic rates (Hawlena and Schmitz 2010a) and body elemental stoichiometry (Hawlena and Schmitz 2010a, Dalton et al. 2018) under perceived predation risk. These so-termed nonconsumptive effects can be as large as consumptive effects (Preisser et al. 2005), and can be an important factor for nutrient and energy flows

through ecosystems (Brown et al. 2004, Schmitz and Price 2011, Gjoni et al. 2020). Yet, predicting the direction and magnitude of nonconsumptive effects remains difficult.

Prey assess risk using predator cues that contain different types of information (Lima and Bednekoff 1999, Mirza et al. 2006, Weissburg et al. 2014). Cues can be visual, olfactory, auditory or mechanosensory (Kats et al. 1998, Munoz and Blumstein 2012, Hettena et al. 2014). Most prey are able to detect several different types of predator cues (Munoz and Blumstein 2012), combining information from multiple sensory modalities to reduce uncertainty and rapidly adjust their physiology or behavior according to a perceived threat (sensu Relyea 2003, Ferrari et al. 2009). At a minimum, a single cue type indicates predator presence, but may also convey information about the predator identity (Iyengar and Harvell 2002), density (Van Buskirk and Arioli 2002) or diet (Schoeppner and Relyea 2005). Multiple cue types can convey additional information including predator location or the relative degree of risk (Fischer et al. 2017). Thus, the specific cues detected by prey can influence how they respond to predators (Bouwma and Hazlett 2001, Weissburg et al. 2014), which would alter predictions made by nonconsumptive effects theory based solely on predator functional identity (Schmitz 2007, 2008); for example, that sit-and-wait predators induce fear and elevated metabolic rates, as an adaptive response to chronic risk (Hawlena and Schmitz 2010a). Our study moves beyond predator identity to examine the effects of different predator cues on prey. However, prey responses to predator cues may also be highly dependent on their antipredator functional type.

Prey under predation risk employ a wide range of antipredator strategies. Prey with constitutive defenses (e.g. armored, defensive or aggressive morphology) may not have any need to alter their physiology or behavior to mitigate risk, regardless of the type of predator cues (Tollrian and Harvell 1999, Stankowich and Campbell 2016, Wirsing et al. 2020). In contrast, prey that employ diematic displays (i.e. startle displays; Maldonado 1970, Murali et al. 2018) or freeze-flight strategies (Sih 1987, Moore and Biewener 2015) are likely to make cue-dependent decisions (Mukherjee and Heithaus 2013). For example, diematic displays are only effective if the predator is in close enough proximity to be startled, while the decision to freeze or flee can depend on the level of predation risk that is perceived by the prey (Lima and Dill 1990, Wirsing et al. 2010, 2020). Given the potential for antipredator functional types to drive cue-dependent responses to predation, both factors need to be examined simultaneously.

Numerous studies have evaluated prey metabolic responses to predators (reviewed by Glazier et al. 2020), finding negative (Krams et al. 2013, Pettett et al. 2017, Briceño et al. 2018, Robison et al. 2018), null (Stibor and Machacek 1998, Toscano and Monaco 2015, Mathot et al. 2016) and positive (Ward et al. 1996, Hawlena and Schmitz 2010a, Culler et al. 2014, Manzur et al. 2014, Thaler et al. 2014) effects, sometimes all within a single predator–prey pairing (Steiner and Van Buskirk 2009, Glazier et al. 2011,

Okuyama 2015, Antoł et al. 2018). These studies all use a single species approach, with traits that are taxonomically bounded (Heinen 1995, Large et al. 2012, Zaguri et al. 2018, Guiliano and Karr et al. 2020), hindering our ability to synthesize results towards a collective understanding of prey–predator interactions. Recognizing this limitation, we investigated metabolic responses to predation risk, as a single and biotically ubiquitous response. This approach enabled us to perform empirical work across taxa, in both terrestrial and aquatic systems, to understand how prey respond to varied contexts of predation risk.

Using metabolism as a unifying trait, we examined two major sources of variability in predation risk responses: the antipredator strategy of the prey and the cue type of the predators. Informed by the natural history of our terrestrial and aquatic systems, we focused on three antipredator strategies – constitutively defended, deimatically displaying and freeze-fleeing – under two cue types that conveyed information about the relative location of the predator (or immediacy of predation risk). We entered with a number of predictions based on an assumption that prey will optimize their metabolic response to the predation context, within the constraints of their defense strategy. First, constitutively defended prey would not exhibit any changes in metabolic rate because their antipredator response is morphologically static; therefore any metabolic adjustments would fail to produce a decrease in predation risk regardless of the type of cue. Second, deimatic prey would increase their metabolic rate through elevated energy expenditure necessary for defensive displays, which they would perform regardless of the type of predator cue. Third, freeze-flight prey would decrease their metabolic rate (i.e. freezing) when faced with a combination of cue types that signaled the close proximity of the predator, but increase their metabolic rate (i.e. fleeing) when faced with predator cues that indicated sufficient opportunity for escape.

Methods

We measured metabolic responses of seven different prey species, which were aggregated in three functional groups based on their antipredator defenses: constitutively defended prey, deimatic displaying prey (Maldonado 1970, Caro 2014, Skelhorn et al. 2016) and freeze-flight prey (Sih 1987, Moore and Biewener 2015). All seven prey species were measured under two contexts of predator cues: combined olfactory and visual cues, and olfactory-only cues. These two contexts of predator cues are not all-encompassing. We did not create a visual-only treatment because such a situation would not occur in our study systems. We also did not include a vibrational or mechanical cue treatment. Vibrational cues are likely an important cue type in our study systems; however the current respirometry technology involves a series of pumps that would distort vibration treatments or otherwise render unrealistic degrees of vibratory cues.

Natural history

We included predator–prey pairs from both terrestrial and aquatic systems to represent a range of antipredator functional types. We selected six terrestrial predator–prey pairs of arthropods based on the prey's functional antipredator defenses and their habitat domain overlap with spider predators (Fig. 1). The six prey species represented three categories of antipredator defenses: constitutive morphology (woodlice *Oniscus asellus* and *Trachelipus rathkii*), deimatic displays (spiders *Phiddipus* spp. and *Oxyopes salticus*) and freeze-flight behavior (orthoptera *Gryllidae pennsylvanicus* and *Melanoplus femurrubrum*). Both of the constitutive prey (*O. asellus* and *T. rathkii*) and one of the freeze-flight prey (*G. pennsylvanicus*) are ground-dwelling arthropods; we measured their metabolic responses to abundant ground-hunting spider predator *Rabidosa rabida*. Both of the deimatic display prey (*Phiddipus* spp. and *O. salticus*) and one of the freeze-flight prey (*M. femurrubrum*) are largely canopy-dwelling arthropods; we measured their responses under canopy-hunting spider predator *Pisaurina mira*. The two spider predators fall into different functional hunting modes: ground-dwelling *R. rabida* is an active hunter, while canopy-dwelling *P. mira* is a sit-and-wait predator. As a consequence, our experiment did not have a fully factorial design (Fig. 1). The terrestrial species are strongly segregated in the grassland canopy simply due to their morphology – ground-dwelling *R. rabida* does not hunt in the upper canopy (Guiliano et al. 2020), while canopy-dwelling *P. mira* is only ever found in the grassland understory under extreme temperature stress (Rosenblatt et al. 2019). We decided to create predator–prey pairings that shared a coevolutionary history at the expense of a factorial design. We hoped to avoid unrealistic and unreliable results, both due to the conundrum of naïve prey and for any subsequent consideration of energy flows within real ecosystems.

In addition to the terrestrial predator–prey pairs, we selected an aquatic pair of tadpole *Rana sylvatica* prey and dragonfly larvae *Aeshna* spp. predators. Wood frogs are freeze-flight prey and dragonfly larave are sit-and-wait predators (Chovanec 1992, Hirvonen and Ranta 1996), but depending on prey density and mobility, they may shift into an active hunting mode and stalk their prey over long distances (Rowe 1987, Johansson 1991). Details on animal collection, laboratory holding conditions and respirometry setup can be found in the Supporting information.

Respiration rates for terrestrial prey

We measured the respiration rates of individual prey across three predator cue contexts: 1) no predation cue, 2) an olfactory cue and 3) an olfactory + visual cue. We simulated an olfactory cue by placing the corresponding spider predator within the measurement chamber with a mesh piece for a cue deposition period of 25 min (sensu Storm and Lima 2008). After the 25 min period, we removed the spider predator from the measurement chamber and immediately added the prey individual. We simulated a visual cue by placing a spider predator within an adjacent but unattached measurement chamber and covered both chambers with the translucent cloth. We recognize the limitations of this approach to simulating cues; with our flow-through respiration system we could not precisely control the concentration of the olfactory cue, and the visual cues were potentially distorted by the glass of the measurement chambers. To minimize variability across all prey measurements, we used the same two *P. mira* and the same two *R. rabida* spiders, similar in size, fed on identical diets of leaf hoppers, with identical holding conditions, to
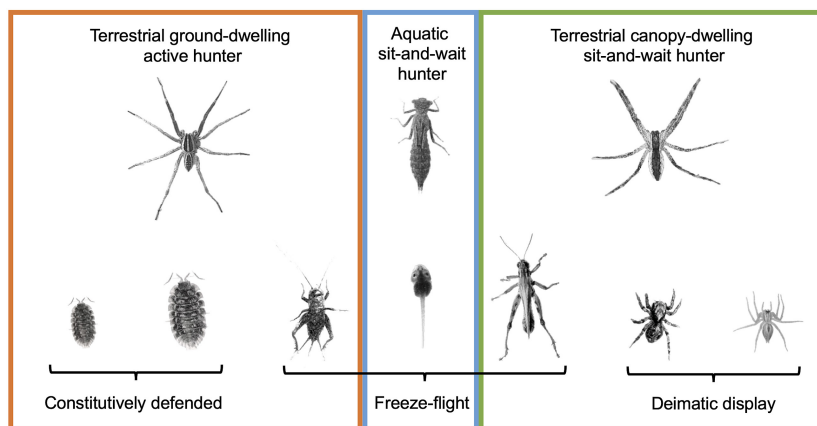

Figure 1. Predator–prey pairings for metabolic measurements were based on the natural history for each functional group. The predator species are in the top row. From left to right: ground-dwelling active hunter *Rabidosa rabida*, aquatic sit-and-wait hunter *Aeshna* spp., and canopy-dwelling sit-and-wait hunter *Pisaurina mira*. The prey species are in the lower row. From left to right: *Trachelipus rathkii*, *Oniscus asellus*, *Gryllidae pennsylvanicus*, *Rana sylvatica*, *Melanoplus femurrubrum*, *Phiddipus* spp. and *Oxyopes salticus*. Prey species were measured under the predator type that corresponds to their habitat use, hence the experimental design is not fully factorial. It would be ecologically meaningless to measure prey metabolic rate under predators they have no overlapping evoluationary history with (i.e. naïve prey). We tested three terrestrial prey under a ground-dwelling predator, three terrestrial prey under a sit-and-wait predator and one aquatic prey under an aquatic predator.

facilitate cue deposition prior to the metabolic measurement. This overaching approach of using actual predators to create cues was taken in order to simulate signal intensity at a level typically produced by that predator; if signal intensity was variable, it was accurate to the cue produced by the predator type. Our goal was to assess how prey would respond metabolically to information about predator type, rather than to hold signal intensity constant.

Twenty individuals of each of the six prey species were measured three times in each of the three cue contexts for a total of 1080 observations. Twelve hours prior to respiratory measurements, individuals were removed from their food source to put them into a post-absorptive state. The order in which each individual was exposed to the predation cue was randomized and each individual was measured consecutively. For every cue, each individual was given 10 min to acclimate to the measurement chamber with the standard incurrent air-flow rate, followed by three measurement cycles of a 200 s measurement and a 150 s rest period. All individuals were measured in the same sized measurement chamber under the same light and temperature conditions.

Terrestrial analysis

We standardized the CO_2 analyzer readings for temperature and individual mass. Mass-specific respiration rate was calculated as:

$$\mu l\, CO_2\, / \left(g \times min \right)$$

where live mass (g) of each individual was measured immediately following respiration measurements and µl CO_2 was adjusted based on the volume of the respirometer, barometric pressure and temperature (Hawlena and Schmitz 2010a, Rosenblatt et al. 2016, Sommer and Schmitz 2020). We then corrected mass-specific respiration rates by subtracting blank runs corresponding to each day of measurement. Because our measurements occurred in a closed system with CO_2 accumulating over time, we also checked the R^2 for each of the 1080 observations to detect any possible errors with the respirometer or any abnormal activity level of individuals within the measurement chamber. We fit repeated measures mixed effect models in lme4 with metabolic rate as the response variable (ver. 1.1.21, Bates et al. 2015) and assessed significance of the main effect of predation cue treatments by computing 95% confidence intervals from 1000 bootstrap replicates in package parameters (ver. 0.5.0, Makowski et al. 2019).

The terrestrial respirometer does not allow for a true resting, or no-activity measurement because preventing animal movement would likely induce stress. However, excessive activity could reduce the precision of our measurements, so we ran our terrestrial analyses with and without the measurements with a low R^2 for CO_2 accumulation. There were no differences in the qualitative result, so we removed measurements with an $R^2 < 0.25$ and a negative respiration rate

(which implied the leak rate of the system was higher than the mass-specific accumulation rate). Removing these observations brought our sample size for *O. salticus* down to n = 16 from 20, but did not affect any other species.

Metabolic rates for aquatic prey

As in the terrestrial system, we included a non-predator cue (water) as a control and two predator cue treatments to simulate two different risk contexts. To create an olfactory-only context, we added aliquots of olfactory predator cue to water cycling through the baths. To add a visual cue, we placed live predators in the baths adjacent to the respirometer chambers. Unlike the repeated measures in the terrestrial system, larvae were exposed to either olfactory cue or olfactory + visual cue to avoid order effects. Per trial, larvae were divided into two groups: a group that received olfactory cue only, and a group that received both olfactory and visual cue. This design resulted in 36 individuals in total being subjected to olfactory and visual cue, and 27 subjected to olfactory cue only. At the end of the trials, tadpoles were euthanized, preserved in 70% ethanol and deposited with the Vertebrate Zoology Division of the Yale Peabody Museum of Natural History, catalog numbers available upon request.

Analysis

We estimated oxygen consumption rates ($\dot{V}_{O_2}$) following Lighton (2008):

$$\dot{V}_{O_2} = \Delta O_2 \times V_w$$

where ΔO_2 is the decrease in oxygen concentration in water (C_{wO_2}) during the measurement phase of each cycle, estimated by fitting linear models to C_{wO_2} by time and extracting the slope coefficients and V_w is the water volume in the closed system including the chamber and recirculation pump but excluding the volume of the tadpole (which we estimated from live-mass, assuming a density of 1 ml g^{-1} as is common for neutrally buoyant aquatic animals (Clark et al. 2013)). We estimated C_{wO_2} with respect to local barometric pressure, temperature (recorded each second in the water bath) and negligible salinity. In order to compare oxygen consumption across individuals, we calculated mass-specific oxygen consumption rates as:

$$\dot{M}_{O_2} = \dot{V}_{O_2} / M_l$$

where M_l is the live-mass of the larvae.

We corrected for background respiration by assuming a linear rate of accumulation between pre- and post-trial measurements and subtracting this from our estimates. In addition to background respiration, there are two main sources of error when estimating linear rates of C_{wO_2} decline in aquatic respirometry: sensor noise, and aberrant readings from animal

movement or sensor issues (Steffensen 1989, Clark et al. 2013, Rodgers et al. 2016). High-frequency noise resulting from the sensitivity of the sensor and incomplete mixing of water in the chamber do not greatly impact the main trend of C_{wO_2} decline. However, spontaneous activity and air bubbles or the animal's body interfering with the sensor can result in non-linear trends of C_{wO_2} decline. To detect and exclude slopes from measurement periods with problematic error, we fit loess models that isolate the main trend from sensor noise and compare the loess fit to the linear fit. Linear slopes in disagreement with loess fits, measured as the difference in root mean squared error (RMSE) above the 95th percentile, were excluded.

We corrected for changes in temperature and used the lowest 50% of $\dot{M}_{O_2}$ values, resulting in 1008 observations, in total. We estimated the effect of predation treatments compared to baseline $\dot{M}_{O_2}$ by fitting repeated measures mixed effect models in lme4 (ver. 1.1.21, Bates et al. 2015), and assessed significance of the main effect of predation treatments by computing 95% confidence intervals from 1000 bootstrap replicates in package parameters (ver. 0.5.0, Makowski et al. 2019).

Results

For the terrestrial predator–prey trials, temperatures during measurments varied by 0.02°C on average (SD = 0.04°C). Among the predator–prey pairs, we found no effects of either olfactory-only or olfactory + visual cues from the active hunting spider *R. rabida* on constitutively-defended prey *O. asellus* and *T. rathkii*, nor on freeze-flight prey *G. pennsylvanicus* (Supporting information, Fig. 2). There was no effect of sit-and-wait spider *P. mira* on deimatic prey *O. salticus* (Supporting information, Fig. 2). Under olfactory-only cues from sit-and-wait *P. mira*, deimatic prey *Phiddipus* spp increased CO_2 production from 6.24 to 8.56 µl CO_2 g^{-1} min^{-1} (37.2% increase). Also under olfactory-only cues from sit-and-wait spider *P. mira*, freeze-flight prey *M. femurrubrum* suppressed CO_2 production from 11.8 to 10.8 µl CO_2 g^{-1} min^{-1} (8.5% reduction) but this effect was not statistically signifcant (p = 0.062).

For the aquatic predator–prey trials, temperatures during measurments varied by 0.3°C on average (SD = 0.08°C), but did not have a significant association with $\dot{M}_{O_2}$. After accounting for temperature, baseline $\dot{M}_{O_2}$ for wood frog tadpoles was 0.075 mg$_{O_2}$ h^{-1} g^{-1}, on par with other estimates for anuran larvae (Feder and Burggen 1992). The addition of olfactory predator cue had no measurable effect on $\dot{M}_{O_2}$ (Supporting information, Fig. 2). However, the combination of visual and olfactory predator cues resulted in significant suppression of $\dot{M}_{O_2}$ by 0.004 mg$_{O_2}$ h^{-1} g$_{tadpole}^{-1}$ (6% reduction; Supporting information, Fig. 2). This result is consistent with the metabolic suppression of terrestrial freeze-flight prey under a sit-and-wait predator. However in the terrestrial predator–prey pairing, metabolic suppression occurred only under olfactory cues, whereas in this aquatic pairing, metabolic suppression occurred under the combination of olfactory and visual cues (Fig. 2).

Discussion

We evaluated how predator cues interacted with prey antipredator types to influence metabolic responses, using three different antipredator types across terrestrial and aquatic systems from seven different prey taxa. We found that constitutively defended prey did not exhibit metabolic responses to predator cues, while deimatic and freeze-flight prey exhibited metabolic responses that were dependent on both cue type and predator hunting mode. More generally, cues from active hunting predators did not induce metabolic responses different from baseline metabolism, while the ambush predator had cue-dependent impacts.

Our study was not fully factorial, which limits causal inference about the effect of predator hunting modes on prey metabolism. There are, however, existing theories for why ambush hunters would produce a metabolic response regardless of the prey antipredator defense, and why active hunters would not. Hunting modes are fundamentally defined by the activity level of the predator while hunting, which influences its interaction frequency with prey (Schoener 1971, Huey and Pianka 1981, Preisser et al. 2007). Active hunters are widely roaming, meaning their cues can be well-dispersed across their habitat domain and are therefore unreliable as an indicator of relative risk (Luttbeg and Schmitz 2000, Preisser et al. 2007, Schmitz 2008). When there is limited capacity to predict and evade an attack, it would be maladaptive for prey to invest energy into a primed response. Prey instead reduce the likelihood of an interaction with an active hunting predator by being reciprocally wide-roaming or settling into a reliable refuge. Unlike active hunters, ambush hunters stay in one location until prey pass within attack distance, meaning their cues are overall more reliable as an indicator of risk, and may additionally convey a high level of risk. Prey may be better able to predict an attack attempt, and can prepare a defense. Indeed, prey who experience chronic predation risk from ambush predators display long term signs of elevated stress (Creel et al. 2009).

Because constitutively defended prey do not have an antipredator defense that involves in-the-moment energy expenditure, we would not expect to see any kind of metabolic response to predation risk, regardless of the hunting mode of the predator. Our study systems did not permit this test, so we cannot rule out whether constitutively defended prey do not have a metabolic response as a product of their antipredator defense, or as product of the predator hunting mode.

We found freeze-flight prey under the same predator hunting mode have similar metabolic responses, mediated by the type of cue. When exposed to combined visual and olfactory cues from sit-and-wait predators, aquatic prey *R. sylvatica* suppressed its metabolism by 6%, while terrestrial prey *M. femurrubrum* under an olfactory-only cue suppressed
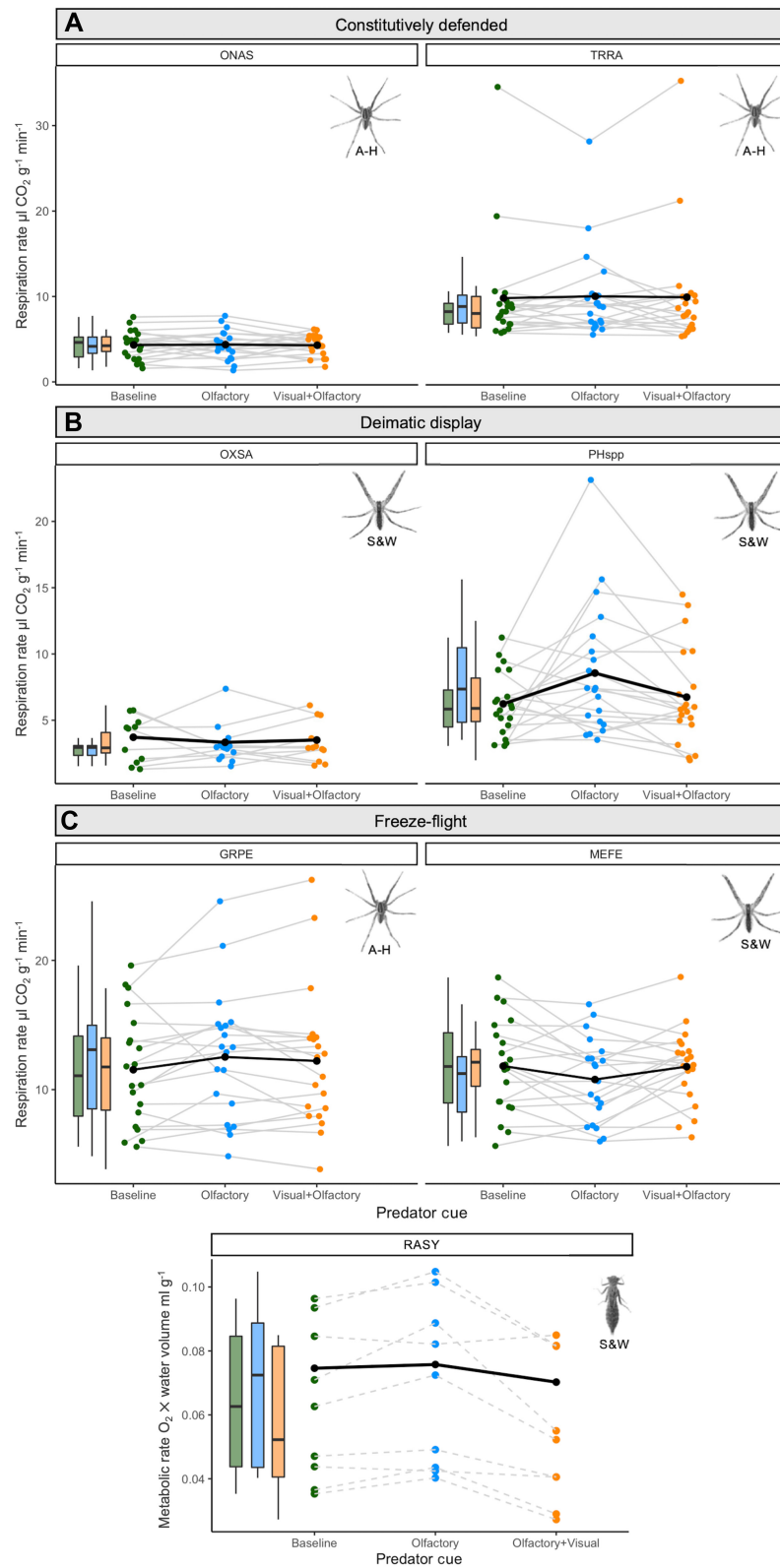
Figure 2. Respiration rate for all predator–prey pairs across cue types. Green points and boxplots show the baseline (no predation-cue) measurement, blue shows the olfactory-only and orange shows the visual + olfactory measurements. Each point shows the mean of the three measurements per individual taken in the predation context, while grey lines connecting points show the repeated measures for each individual. Black lines show the mean. For the aquatic predator–prey pairing (panel C), points represent the mean of the trial group, while dotted grey lines connecting points show the repeated measures for each trial group. Note the scale for respiration rate and metabolic rate is different between the figure panels. Predator abbreviations are A–H for active-hunting and S&W for sit-and-wait.

its metabolism by 8%. This result is consistent with an adaptive response to sit-and-wait predators – metabolic suppression and freezing behavior reduces the likelihood of an ambush. Notably, these different prey species responded to different cue types, which may be due to differences in their native habitat structure or life history (Wirsing et al. 2010, Wheatley et al. 2020).

Terrestrial prey *M. femurrubrum* roams within heavily structured grassland habitat; when prey can detect only an olfactory cue, they have information that the predator is present, but may not have a relative distance or direction. In this context, freezing reduces the likelihood of overlapping with the predator. Unlike terrestrial environments, structure within aquatic vernal ponds offers little protection for tadpoles; open water areas of vernal ponds represent the lowest risk of strike from a dragonfly larva predator (Formanowicz and Bobka 1989, Smith and Awan 2009, Kloskowski et al. 2020). And in contrast with the terrestrial component of our study using adult prey, we assessed juveniles for aquatic prey *R. sylvatica*. Under larval dragonfly olfactory cues, the adaptive strategy of juvenile *R. sylvatica* is to forage as much as possible and as quickly as possible (i.e. in the growth–predation tradeoff, Skelly and Werner 1990). However, when *Aeshna* can be both seen and smelled in an environment with poor visibility (Fischer et al. 2017) and few refugia, the adaptive response of *R. sylvatica* is to freeze. Previous experimental work indicates that freezing is a highly effective antipredator defense in *R. sylvatica* tadpoles (Skelly 1994). We conclude that the specific cue type of the predator may be less important than the predator identity and the coevolutionary history of the predator–prey pairing in explaining differences in metabolic responses.

Our results differed from other experiments in this same terrestrial system that examined metabolic responses to predation risk. Specifically, chronic exposure of prey *M. femurrubrum* to *P. mira* results in metabolic elevation (Hawlena and Schmitz 2010a, Schmitz et al. 2016) which contrasts with our finding of metabolic suppression under short-term exposure to olfactory cues (Fig. 2). The ecological reality is likely something in between these two conclusions, with *M. femurrubrum* experiencing variation in risk and exposure duration, and should be simulated in future work. Understanding nonconsumptive effects on metabolism across varied contexts of risk perception will give us a better grasp of both the demographic effects and the behavioral consequences of prey physiological shifts under predation (Sheriff et al. 2020, Wirsing et al. 2020).

We selected predators based on relative abundance and encounter probabilities from known habitat domains. A more comprehensive evaluation of predator hunting mode would have included a reciprocal treatment in which sit-and-wait predator cues were tested alongside the active hunting predator cues (Fig. 1). While our terrestrial system did not allow us to evaluate this combination, future work on metabolic responses to predation risk should identify predator–prey pairs where different hunting modes do overlap with prey of multiple antipredator types. One way to do this might be to use a predator whose hunting mode changes across development, while its habitat remains static.

A general limitation of any sensory ecology with arthropods is the inability to distinguish between when an animal did not detect a cue, and when an animal detected a cue and did not react. Without mapping the neural network or sensory receptors of an individual, it is currently impossible to identify the specific route of information transfer and the mechanistic response. Specifically to our study, we cannot know if the constitutively defended prey simply did not detect the cues, or if they did not react to the cues. However, this limitation does not invalidate our findings because the metabolic outcome and consequences for trait expression are the same, whether an individual does not react to cues or does not detect cues. The distinction can be important for other areas of sensory ecology, (e.g. when metabolic rate is used to infer a fear-based behavioral response, or lack thereof), but we do not make any such interpretations here.

We studied how both predator traits and prey defenses determine whether prey increase their metabolism in response to predation risk (Wirsing et al. 2020). While our findings are limited to short-term exposure, we found that prey antipredator types interacts with predator hunting mode to determine the prey metabolic responses. Most other studies on nonconsumptive predator–prey interactions employ behavioral traits such as foraging, vigilance and habitat use, or life history traits such as growth rate, survival and fecundity (Hermann and Landis 2017). These responses are then used to infer effects within individuals, and at the population and community level, which has been met with recent criticism (Peers et al. 2018, Sheriff et al. 2020). Our study instead used metabolism to understand the energetic cost of a nonconsumptive interaction with a predator. Energy is the common currency of ecological systems (Enquist et al. 2003, 2015, Brown et al. 2004), thus examining nonconsumptive interactions in terms of energetic costs for prey allows for scaling across levels of ecological organization in ways that behavior and life history alone cannot.

Our multi-system study also shows the potential of integrating multiple modalities, multiple taxa and systems, and multiple predator–prey pairings into one empirical study, and the conceptual benefits that arise from such designs. Attempts to synthesize predation risk studies into unifying theory have relied on interpreting independent studies of particular predator and prey combinations (Peacor et al. 2020), but have not yet succeeded in deducing generalizable effects and conceptual similarities across systems. While there are numerous benefits to conducting system-centric work, there is also a large benefit to performing empirical work across systems. Though we found responses in the mass-specific metabolic rates of prey under ambush predators, the effect size was low in comparison to the variance between individuals of a given prey species. This is an important point to consider when scaling inferences from individuals to the ecosystem level.

For future work that may adopt our approach, we first suggest expanding the number of species within each functional

group and creating a fully factorial design. We chose to prioritize realistic predator–prey pairings based on natural history observations over increasing the number of species. The results of our work should therefore be interpreted with high fidelity to the species and functional types in our two systems, but we caution against overinterpretation – additional work is needed to build a stronger foundation for widely applicable theory. To that end, our study offers an experimental approach for assessing nonconsumptive effects across taxa and systems, by using a ubiquitous response variable. We provide some initial hypotheses about the interaction between predator hunting modes, cue types and prey antipredator strategies that are worth further exploration.

We also see ample space to expand our cross-taxon metabolic approach into more nuanced questions around size-selective predation, multiple-predator effects, cue intensity and additional cue types. Animals in real communities experience both size-dependent predation risk and simultaneous risk from multiple predators. In combination with more cue types – particularly mechanical and hydraulic vibration – prey metabolic responses may be altogether different. At present, it is challenging to experimentally control the intensity and types of predator cues for terrestrial systems, at least in part due to the bulky machinery of respirometers. Creative methods are needed to circumvent this limitation, but aquatic systems may currently prove more conducive to pursuing cue types and intensity as an additional line of interrogation.

Overall, our results support a growing body of work suggesting that nonconsumptive effects of predators are contingent upon both the functional type of the prey and the predator (Preisser et al. 2007, Heithaus et al. 2009, Creel 2011, Schmitz and Trussell 2016, Dröge et al. 2017, Wheatley et al. 2020, Wirsing et al. 2020). With knowledge about the functional types of predators and prey for any given ecological community, we can use metabolic responses to make predictions about energetic flows due to nonconsumptive effects. Integrating this variation should provide rich conceptual understanding of how individual interactions can scale to affect ecosystem dynamics (Enquist et al. 2003, Hawlena and Schmitz 2010b, Humphries and Mccann 2014, Schmitz et al. 2015).

Author contributions

Nathalie R. Sommer and **Yara A. Alshwairikh** contributed equally to this publication. **Nathalie R. Sommer**: Conceptualization (equal); Formal analysis (lead); Investigation (lead); Methodology (equal); Visualization (lead); Writing – original draft (lead); Writing – review and editing (lead). **Yara A. Alshwairikh**: Formal analysis (supporting); Investigation (equal); Writing – original draft (equal); Writing – review and editing (equal). **A. Z. Andis Arietta**: Conceptualization (equal); Formal analysis (supporting); Methodology (equal); Writing – review and editing (supporting). **David K. Skelly**: Conceptualization (supporting); Funding acquisition (lead); Resources (lead); Writing – review and editing (supporting). **Robert W. Buchkowski**: Conceptualization (equal); Formal analysis (supporting); Methodology (equal); Writing – review and editing (supporting).

Data availability statement

Data and code for this paper are available on Zenodo : https://doi.org/10.5281/zenodo.7530529 (Sommer et al. 2023).

Supporting information

The Supporting information associated with this article is available with the online version.

References

Antoł, A., Kierat, J. and Czarnoleski, M. 2018. Sedentary prey facing an acute predation risk: testing the hypothesis of inducible metabolite emission suppression in zebra mussels, *Dreissena polymorpha*. – Hydrobiologia 810: 109–117.

Bates, D., Machler, M., Bolker, B. and Walker, S. 2015. Fitting linear mixed-effects models using lme4. – J. Stat. Softw. 67: 1–48.

Bouwma, P. and Hazlett, B. A. 2001. Integration of multiple predator cues by the crayfish *Orconectes propinquus*. – Anim. Behav. 61: 771–776.

Briceño, F. A., Polymeropoulos, E. T., Fitzgibbon, Q. P., Dambacher, J. M. and Pecl, G. T. 2018. Changes in metabolic rate of spiny lobster under predation risk. – Mar. Ecol. Prog. Ser. 598: 71–84.

Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M. and West, G. B. 2004. Towards a metabolic theory of ecology. – Ecology 85: 1771–1789.

Brown, J. S. 1999. Vigilance, patch use and habitat selection: foraging under predation risk. – Evol. Ecol. Res. 1: 49–71.

Caro, T. 2014. Antipredator deception in terrestrial vertebrates. – Curr. Zool. 60: 16–25.

Chovanec, A. 1992. The influence of tadpole swimming behaviour on predation by dragonfly nymphs. – Amphibia-Reptilia 13: 341–349.

Clark, T. D., Sandblom, E. and Jutfelt, F. 2013. Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. – J. Exp. Biol. 216: 2771–2782.

Creel, S. 2011. Toward a predictive theory of risk effects: hypotheses for prey attributes and compensatory mortality. – Ecology 92: 2190–2219.

Creel, S., Winnie, J. A. and Christianson, D. 2009. Glucocorticoid stress hormones and the effect of predation risk on elk reproduction. – Proc. Natl Acad. Sci. USA 106: 12388–12393.

Culler, L. E., McPeek, M. A. and Ayres, M. P. 2014. Predation risk shapes thermal physiology of a predaceous damselfly. – Oecologia 176: 653–660.

Dalton, C. M., Tracy, K. E., Hairston, N. G. and Flecker, A. S. 2018. Fasting or fear: disentangling the roles of predation risk and food deprivation in the nitrogen metabolism of consumers. – Ecology 99: 681–689.

Dröge, E., Creel, S., Becker, M. S. and M'Soka, J. 2017. Risky times and risky places interact to affect prey behaviour. – Nat. Ecol. Evol. 1: 1123–1128.

Enquist, B. J., Economo, E. P., Huxman, T. E., Allen, A. P., Ignace, D. D. and Gillooly, J. F. 2003. Scaling metabolism from organisms to ecosystems. – Nature 423: 639–642.

Feder, M. E. and Burggren, W. W. 1992. Environmental physiology of the amphibians. – Univ. of Chicago Press.

Ferrari, M. C. O., Sih, A. and Chivers, D. P. 2009. The paradox of risk allocation: a review and prospectus. – Anim. Behav. 78: 579–585.

Fischer, S., Oberhummer, E., Cunha-Saraiva, F., Gerber, N. and Taborsky, B. 2017. Smell or vision? The use of different sensory modalities in predator discrimination. – Behav. Ecol. Sociobiol. 71: 143.

Formanowicz, D. R. and Bobka, M. S. 1989. Predation risk and microhabitat preference: an experimental study of the behavioral responses of prey and predator. – Am. Midl. Nat. 121: 379–386.

Gjoni, V., Basset, A. and Glazier, D. S. 2020. Temperature and predator cues interactively affect ontogenetic metabolic scaling of aquatic amphipods: T and P effect on metabolic scaling. – Biol. Lett. 16: 12–15.

Glazier, D. S., Butler, E. M., Lombardi, S. A., Deptola, T. J., Reese, A. J. and Satterthwaite, E. V. 2011. Ecological effects on metabolic scaling: amphipod responses to fish predators in freshwater springs. – Ecol. Monogr. 81: 599–618.

Glazier, D. S., Borrelli, J. J. and Hoffman, C. L. 2020. Effects of fish predators on the mass-related energetics of a keystone freshwater crustacean. – Biology 9: 40.

Guiliano, S. M., Karr, C. M., Sommer, N. R. and Buchkowski, R. W. 2020. Woodlice change the habitat use of spiders in a different food chain. – PeerJ 8: e9184.

Hawlena, D. and Schmitz, O. J. 2010a. Herbivore physiological response to predation risk and implications for ecosystem nutrient dynamics. – Proc. Natl Acad. Sci. USA 107: 15503–15507.

Hawlena, D. and Schmitz, O. J. 2010b. Physiological stress as a fundamental mechanism linking predation to ecosystem functioning. – Am. Nat. 176: 537–556.

Hawlena, D., Kress, H., Dufresne, E. R. and Schmitz, O. J. 2011. Grasshoppers alter jumping biomechanics to enhance escape performance under chronic risk of spider predation. – Funct. Ecol. 25: 279–288.

Hawlena, D., Strickland, M. S., Bradford, M. A. and Schmitz, O. J. 2012. Fear of predation slows plant-litter decomposition. – Science 366: 1434–1438.

Heinen, J. T. 1995. Predator cues and prey responses: a test using eastern garter snakes *Thamnophis s. sirtalis* and American toads *Bufo a. americanus*. – Copeia 3: 738–741.

Heithaus, M. R., Wirsing, A. J., Burkholder, D., Thomson, J. and Dill, L. M. 2009. Towards a predictive framework for predator risk effects: the interaction of landscape features and prey escape tactics. – J. Anim. Ecol. 78: 556–562.

Hermann, S. L. and Landis, D. A. 2017. Scaling up our understanding of non-consumptive effects in insect systems. – Curr. Opin. Insect Sci. 20: 54–60.

Hettena, A. M., Munoz, N. and Blumstein, D. T. 2014. Prey responses to predator's sounds: a review and empirical study. – Ethology 120: 427–452.

Hirvonen, H. and Ranta, E. 1996. Prey to predator size ratio influences foraging efficiency of larval *Aeshna juncea* dragonflies. – Oecologia 106: 407–415.

Huey, R. and Pianka, E. 1981. Ecological consequences of foraging mode. – Ecology 62: 991–999.

Humphries, M. M. and Mccann, K. S. 2014. Metabolic ecology. – J. Anim. Ecol. 83: 7–19.

Iyengar, E. V. and Harvell, C. D. 2002. Specificity of cues inducing defensive spines in the bryozoan *Membranipora membranacea*. – Mar. Ecol. Prog. Ser. 225: 205–218.

Johansson, F. 1991. Foraging modes in an assemblage of odonate larvae – effects of prey and interference. – Hydrobiologia 209: 79–87.

Kats, L. B., Dill, L. M. and Taylor, P. 1998. The scent of death: chemosensory assessment of predation risk by prey animals. – Ecoscience 5: 361–394.

Kloskowski, J., Nieoczym, M. and Stryjecki, R. 2020. Between-habitat distributions of pond tadpoles and their insect predators in response to fish presence. – Hydrobiologia 847: 1343–1356.

Krams, I., Kivleniece, I., Kuusik, A., Krama, T., Freeberg, T. M., Mänd, R., Vrublevska, J., Rantala, M. J. and Mänd, M. 2013. Predation selects for low resting metabolic rate and consistent individual differences in anti-predator behavior in a beetle. – Acta Ethol. 16: 163–172.

Large, S., Torres, P. and Smee, D. 2012. Behavior and morphology of *Nucella lapillus* influenced by predator type and predator diet. – Aquat. Biol. 16: 189–196.

Lighton, J. R. B. 2008. Measuring metabolic rates: a manual for scientists. – Oxford Univ. Press.

Lima, S. L. and Dill, L. M. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. – Can. J. Zool. 68: 619–640.

Lima, S. L. and Bednekoff, P. A. 1999. Temporal variation in danger drives antipredator behavior: the predation risk allocation hypothesis. – Am. Nat. 153: 649–659.

Luttbeg, B. and Schmitz, O. J. 2000. Predator and prey models with flexible individual behavior and imperfect information. – Am. Nat. 155: 669–683.

Makowski, D., Ben-Shachar, M. S. and Lüdecke, D. 2019. Describe and understand your model's parameters. – R package. https://cran.r-project.org/web/packages/parameters/index.html

Maldonado, H. 1970. The deimatic reaction in the praying mantis *Stagmatoptera biocellata*. – Z. Vergleich. Physiol. 68: 60–71.

Manzur, T., Vidal, F., Pantoja, J. F., Fernández, M. and Navarrete, S. A. 2014. Behavioural and physiological responses of limpet prey to a seastar predator and their transmission to basal trophic levels. – J. Anim. Ecol. 83: 923–933.

Mathot, K. J., Abbey-Lee, R. N., Kempenaers, B. and Dingemanse, N. J. 2016. Do great tits *Parus major* suppress basal metabolic rate in response to increased perceived predation danger? A field experiment. – Physiol. Behav. 164: 400–406.

Mirza, R. S., Mathis, A. and Chivers, D. P. 2006. Does temporal variation in predation risk influence the intensity of antipredator responses? A test of the risk allocation hypothesis. – Ethology 112: 44–51.

Moore, T. Y. and Biewener, A. A. 2015. Outrun or outmaneuver: predator–prey interactions as a model system for integrating biomechanical studies in a broader ecological and evolutionary context. – Integr. Comp. Biol. 55: 1188–1197.

Mukherjee, S. and Heithaus, M. R. 2013. Dangerous prey and daring predators: a review. – Biol. Rev. 88: 550–563.

Munoz, N. E. and Blumstein, D. T. 2012. Multisensory perception in uncertain environments. – Behav. Ecol. 23: 457–462.

Murali, G., Merilaita, S. and Kodandaramaiah, U. 2018. Grab my tail: evolution of dazzle stripes and colourful tails in lizards. – J. Evol. Biol. 31: 1675–1688.

Okuyama, T. 2015. Metabolic responses to predation risk in a jumping spider. – J. Zool. 297: 9–14.

Peacor, S. D., Barton, B. T., Kimbro, D. L., Sih, A. and Sheriff, M. J. 2020. A framework and standardized terminology to facilitate the study of predation-risk effects. – Ecology 101: e03152.

Peckarsky, B. L., Abrams, P. A., Bolnick, D. I., Dill, L. M., Grabowski, J. H., Luttbeg, B., Orrock, J. L., Peacor, S. D., Preisser, E. L., Schmitz, O. J. and Trussell, G. C. 2008. Revisiting the classics: considering nonconsumptive effects in textbook examples of predator prey interactions. – Ecology 89: 2416–2425.

Peers, M. J. L., Majchrzak, Y. N., Neilson, E., Lamb, C. T., Hämäläinen, A., Haines, J. A., Garland, L., Doran-Myers, D., Broadley, K., Boonstra, R. and Boutin, S. 2018. Quantifying fear effects on prey demography in nature. – Ecology 99: 1716–1723.

Pettett, C. E., Johnson, P. J., Moorhouse, T. P., Hambly, C., Speakman, J. R. and MacDonald, D. W. 2017. Daily energy expenditure in the face of predation: hedgehog energetics in rural landscapes. – J. Exp. Biol. 220: 460–468.

Preisser, E. L., Bolnick, D. I. and Bernard, M. F. 2005. Scared to death? The effect of intimidation and consumption in predator prey interactions. – Ecology 86: 501–509.

Preisser, E. L., Orrock, J. L. and Schmitz, O. J. 2007. Predator hunting mode and habitat domain alter nonconsumptive effects in predator–prey interactions. – Ecology 88: 2744–2751.

Relyea, R. 2001. Morphological and behavioral plasticity of larval anurans in response to different predators. – Ecology 82: 523–540.

Relyea, R. A. 2003. How prey respond to combined predators: a review and an empirical test. – Ecology 84: 1827–1839.

Robison, A. L., Chapman, T. and Bidwell, J. R. 2018. Predation cues influence metabolic rate and sensitivity to other chemical stressors in fathead minnows *Pimephales promelas* and *Daphnia pulex*. – Ecotoxicology 27: 55–68.

Rodgers, G. G., Tenzing, P. and Clark, T. D. 2016. Experimental methods in aquatic respirometry: the importance of mixing devices and accounting for background respiration. – J. Fish Biol. 88: 65–80.

Rosenblatt, A. E., Crowley, B. T. and Schmitz, O. J. 2016. Linking trophic interactions to plasticity in thermal sensitivity of geographically separated populations of a herbivore. – Evol. Ecol. 30: 649–661.

Rosenblatt, A. E., Wyatt, K. S. and Schmitz, O. J. 2019. Will like replace like? Linking thermal performance to ecological function across predator and herbivore populations. – Ecology 1000: 1–12.

Rowe, R. J. 1987. Predatory versatility in a larval dragonfly, *Hemianax papuensis* (Odonata: Aeshnidae). – J. Zool. 211: 193–207.

Schmitz, O. J. 2007. Predator diversity and trophic interactions. – Ecology 88: 2415–2426.

Schmitz, O. J. 2008. Effects of predator hunting mode on grassland ecosystem function. – Science 319: 952–954.

Schmitz, O. J. and Price, J. R. 2011. Convergence of trophic interaction strengths in grassland food webs through metabolic scaling of herbivore biomass. – J. Anim. Ecol. 80: 1330–1336.

Schmitz, O. J. and Trussell, G. C. 2016. Multiple stressors, state-dependence and predation risk – foraging tradeoffs: toward a modern concept of trait-mediated indirect effects in communities and ecosystems . – Curr. Opin. Behav. Sci. 12: 6–11.

Schmitz, O. J., Hawlena, D. and Trussell, G. C. 2010. Predator control of ecosystem nutrient dynamics. – Ecol. Lett. 13: 1199–1209.

Schmitz, O. J., Buchkowski, R. W., Burghardt, K. T. and Donihue, C. M. 2015. Functional traits and trait-mediated interactions. Connecting community-level interactions with ecosystem functioning. – In: Pawar, S., Woodward, G. and Dell, A. I. (eds), Advances in ecological research, 1st edn., Vol. 52. Elsevier , pp. 319–343.

Schmitz, O. J., Rosenblatt, A. E. and Smylie, M. 2016. Temperature dependence of predation stress and the nutritional ecology of a generalist herbivore. – Ecology 97: 3119–3130.

Schoener, T. W. 1971. Theory of feeding strategies. – Annu. Rev. Ecol. Syst. 2: 369–404.

Schoeppner, N. M. and Relyea, R. A. 2005. Damage, digestion and defence: the roles of alarm cues and kairomones for inducing prey defences. – Ecol. Lett. 8: 505–512.

Sheriff, M. J., Peacor, S. D., Hawlena, D. and Thaker, M. 2020. Non-consumptive predator effects on prey population size: a dearth of evidence. – J. Anim. Ecol. 89: 1302–1316.

Sih, A. 1987. Prey refuges and predator–prey stability. – Theor. Popul. Biol. 31: 1–12.

Skelhorn, J., Holmes, G. G. and Rowe, C. 2016. Deimatic or aposematic? – Anim. Behav. 113: e1–e3.

Skelly, D. K. 1994. Activity level and the susceptibility of anuran larvae to predation. – Anim. Behav. 47: 465–468.

Skelly, D. K. and Werner, E. E. 1990. Behavioral and life-historical responses of larval American toads to an odonate predator. – Ecology 71: 2313–2322.

Smith, G. R. and Awan, A. R. 2009. The roles of predator identity and group size in the antipredator responses of American toad *Bufo americanus* and bullfrog *Rana catesbeiana* tadpoles. – Behaviour 146: 225–243.

Sommer, N. R. and Schmitz, O. J. 2020. Differences in prey personality mediate trophic cascades. – Ecol. Evol. 10: 9538–9551.

Sommer, N. R., Alshwairikh, Y. A., Arietta, A. A. Z., Skelly, D. K. and Buchkowski, R. W. 2023. Data and code from: Prey metabolic responses to predators depend on predator hunting mode and prey antipredator defenses. – Zenodo https://doi.org/10.5281/zenodo.7530529.

Stankowich, T. and Campbell, L. A. 2016. Living in the danger zone: exposure to predators and the evolution of spines and body armor in mammals. – Evolution 70: 1501–1511.

Steffensen, J. F. 1989. Some errors in respirometry of aquatic breathers: how to avoid and correct for them. – Fish Physiol. Biochem. 6: 49–59.

Steiner, U. K. and Van Buskirk, J. 2009. Predator-induced changes in metabolism cannot explain the growth/predation risk trade-off. – PLoS One 4: e6160.

Stibor, H. and Machacek, J. 1998. The influence of fish-exuded chemical signals on the carbon budget of *Daphnia*. – Limnol. Oceanogr. 43: 997–1000.

Storm, J. J. and Lima, S. L. 2008. Predator-naïve fall field crickets respond to the chemical cues of wolf spiders. – Can. J. Zool. 86: 1259–1263.

Thaler, J. S., Contreras, H. and Davidowitz, G. 2014. Effects of predation risk and plant resistance on *Manduca sexta* caterpillar feeding behaviour and physiology. – Ecol. Entomol. 39: 210–216.

Tollrian , R. and Harvell, C. D. 1999. The ecology and evolution of inducible defenses. – Princeton Univ. Press.

Toscano, B. J. and Monaco, C. J. 2015. Testing for relationships between individual crab behavior and metabolic rate across ecological contexts. – Behav. Ecol. Sociobiol. 69: 1343–1351.

Turner, A. M. 1996. Freshwater snails alter habitat use in response to predation. – Anim. Behav. 51: 747–756.

Van Buskirk, J. and Arioli, M. 2002. Dosage response of an induced defense: how sensitive are tadpoles to predation risk? – Ecology 83: 1580–1585.

Ward, J. F., MacDonald, D. W., Doncaster, C. P. and Mauget, C. 1996. Physiological response of the European hedgehog to predator and nonpredator odour. – Physiol. Behav. 60: 1469–1472.

Weissburg, M., Smee, D. L. and Ferner, M. C. 2014. The sensory ecology of nonconsumptive predator effects. – Am. Nat. 184: 141–157.

Werner, E. E. and Peacor, S. D. 2003. A review of trait-mediated indirect interactions in ecological communities. – Ecology 84: 1083–1100.

Wheatley, R., Pavlic, T. P., Levy, O. and Wilson, R. S. 2020. Habitat features and performance interact to determine the outcomes of terrestrial predator–prey pursuits. – J. Anim. Ecol. 89: 2958–2971.

Wirsing, A. J., Cameron, K. E. and Heithaus, M. R. 2010. Spatial responses to predators vary with prey escape mode. – Anim. Behav. 79: 531–537.

Wirsing, A. J., Heithaus, M. R., Brown, J. S., Kotler, B. P. and Schmitz, O. J. 2020. The context dependence of non-consumptive predator effects. – Ecol. Lett. 24: 113–129.

Zaguri, M., Zohar, Y. and Hawlena, D. 2018. Considerations used by desert isopods to assess scorpion predation risk. – Am. Nat. 192: 630–643.